REALIZING LIVELIHOOD AND ENVIRONMENTAL BENEFITS OF FORAGES IN TROPICAL CROP-TREE-LIVESTOCK SYSTEMS

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Editorial: Realizing livelihood and environmental benefits of forages in tropical crop-tree-livestock systems

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Editorial on the Research Topic
Realizing livelihood and environmental benefits of forages in tropical crop-tree-livestock systems

Ruminant livestock, such as cattle, can convert biomass into high-quality, nutrient-dense foods (Broderick, 2018). This ability enables livestock to play a critical role in increasing the productive utilization not only of fertile but also of marginal lands unsuitable for crop production (Wang et al., 2021). In the tropics, the sustainable intensification of livestock production systems plays a critical role in supporting rural livelihoods and meeting food security and environmental goals (Herrero et al., 2013; Rao et al., 2015). Despite its importance, less is known about the productivity and environmental impacts of tropical livestock systems compared to livestock production systems under other climatic regimes (i.e., temperate climate). This knowledge gap limits our ability to inform actions that lead to sustainable intensification in the tropics. However, it is unambiguous that the intensification of livestock systems in the tropics heavily depends on availability and access to quality feed since the limited previous studies have generally reported higher levels of animal production when feed supplements are included in livestock diets. Specifically, feed options such as cultivated forage legumes, crop residues and improved grasslands represent necessary feed resources, which can be accessible to tropical farmers with limited investments and better organization.

The papers in this collection, which explored livestock production systems in Latin America, Africa and Asia, all suggest the possibility of increasing livestock productivity by adopting innovative policies, technologies, and management practices.

The presented evidence suggests that the inclusion of legumes in grazed pastures has the potential to increase cattle production (Valencia et al.), reduce methane emissions (Quintero-Anzueta et al.) and increase the persistence of forage grasses (Valencia et al.). Including feed supplements such as Altoandina oat silage was reported to be an economically viable option for increasing the productivity of Colombia's High-Altitude Dairy Systems (Enciso, Castillo et al.). Management options that optimize rotational pasture grazing based on simple metrics such as sward height may increase livestock productivity and reduce enteric methane emissions from grazing cattle (Marín et al.). Besides the adoption of better pasture management systems, genetic innovations can be used to overcome challenges such as droughts (Carvajal-Tapia et al.), soil salinity (Liu et al.) and low biomass accumulation (Mwendia et al.).

An additional emerging use of tropical forages is their potential as a food source for edible insects (Bawa et al., 2020; Oonincx et al., 2020). Buitrago et al. share their perspectives on this aspect and suggest that integrating tropical forage-based diets in edible insect production systems represents low-cost feed sources for insects and supports transiting to circular economies. On the other hand, as Hernández et al. highlight, tropical forage production systems must be protected from harmful insects such as Spittlebugs. Narjes Sanchez et al. also provide critical insights into the possible role of tropical forage legumes in pollinator conservation efforts, income generation, and closing the forage legume seed bottleneck that still limits further advances in sustainable intensification efforts of the cattle sector as of today.

In addition to providing food, the livestock sector can generate ecosystem benefits such as increased on-farm agro-biodiversity, soil restoration, mitigation of GHG emissions and more efficient use of nutrients and water resources. Narjes Sanchez et al. showed that silvopastoral systems have the potential to support the provision of ecosystem services such as pollination. In a separate study, Notenbaert et al. used previous studies to demonstrate the multiple potential benefits of managed livestock production systems. They further demonstrate linkages between managed livestock production systems and agroecology and how the sustainable intensification of livestock production systems can contribute to the 13 principles of agroecology.

From this paper collection, it appears there is clarity on what needs to be done to sustainably intensify tropical livestock production systems to meet livelihood, food security and environmental goals. Nevertheless, the slow progress appears disproportionately attributable to non-technical aspects such as a disconnect between institutions and other actors along livestock value chains resulting in insufficient synchrony of efforts to support the adoption of critical innovations (Enciso, Triana et al.). While the need to sustainability intensify livestock production systems at the national and global levels is frequently well articulated, connections between policies and investments and, thus, actions on the ground largely remain weak (Lerma et al.). Chirinda et al. emphasize the need to create inclusive and creatively organized livestock value chains that improve stakeholder linkages, information flows and equity.

Author contributions

NC made the first draft of the Editorial. MP, SB, AN, and RV made edits and suggestions to improve the draft version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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ORIGINAL RESEARCH
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Nutritional Evaluation of Tropical Forage Grass Alone and Grass-Legume Diets to Reduce *in vitro* Methane Production

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Forage grass nutritional quality directly affects animal feed intake, productivity, and enteric methane (CH_4) emissions. This study evaluated the nutritional quality, *in vitro* enteric CH_4 emission potential, and optimization of diets based on two widely grown tropical forage grasses either alone or mixed with legumes. The grasses *Urochloa* hybrid cv. Cayman (UHC) and *U. brizantha* cv. Toledo (UBT), which typically have low concentrations of crude protein (CP), were incubated *in vitro* either alone or mixed with the legumes *Canavalia brasiliensis* (CB) and *Leucaena diversifolia* (LD), which have higher CP concentrations. Substitution of 30% of the grass dry matter (DM) with CB or LD did not affect gas production or DM degradability. After 96 h of incubation, accumulated CH_4 was 87.3 mg CH_4 g^{-1} DM and 107.7 mg CH_4 g^{-1} DM for the grasses alone (UHC and UBT, respectively), and 100.7 mg CH_4 g^{-1} DM and 113.2 mg CH_4 g^{-1} DM for combined diets (70% grass, 15% CB, and 15% LD). Diets that combined legumes (CB or LC) and grass (UHC or UBT) had higher CP contents, gross, and metabolizable energy (GE, ME, respectively) densities, as well as lower concentrations of neutral detergent fiber (NDF) and acid detergent lignin (ADL). The ME and nutritional variables such as NFD, tannins (T), and CP showed a positive correlation with *in vitro* net gas production, while ruminal digestibility was affected by CP, ADL, T, and GE. Optimal ratios of components for ruminant diets to reduce rumen net gas production and increase protein content were found with mixtures consisting of 60% grass (either UHC or UBT), 30% CB, and 10% LD. However, this ratio did not result in a decrease in CH_4 production.

Keywords: ***Canavalia brasiliensis*****,** ***in-vitro*** **fermentation,** ***Leucaena*** **sp., nutritional quality,** ***Urochloa brizantha*** **cv. Toledo,** ***Urochloa hybrid*** **cv. Cayman**

INTRODUCTION

Cattle and other ruminant livestock are a significant food source for the global human population and are good at converting fibrous species indigestible by humans into highly nutritious food (Wilkinson, 2011). This metabolic conversion is possible due to rumen-dwelling microorganisms that can break down low-quality fibrous plant material, with the formation of gases (methane [CH_4] and CO_2) that are expelled into the atmosphere, plus energy-rich compounds that are required to perform vital functions for both the population of rumen organisms and the host animal (Hyland et al., 2016; Cammack et al., 2018). However, this symbiosis between microorganisms and ruminants is negatively affected by the consumption of diets that are low in protein and high in insoluble fiber (Figueiras et al., 2010).

Therefore, in the search for suitable diets based on tropical forages that simultaneously meet the nutritional needs of livestock and decrease their impact on the environment, mixed production (i.e., agro-pastoral, silvopastoral, and agro-silvopastoral) systems are proposed as a viable option (Arango et al., 2020). In these systems, forage grasses and legumes are combined toward a process of sustainable intensification of livestock production, aiming at not only improving available feed for ruminants but also to restore degraded lands and increase system resilience to more frequent droughts and floods that are associated with climate change (Rao et al., 2015; Ku-Vera et al., 2020a). Furthermore, if properly managed, grass-legume tropical pastures can potentially accumulate large amounts of soil organic carbon; improve chemical, physical, and biological soil health characteristics; fix atmospheric nitrogen; inhibit soil nitrification; improve animal productivity and animal welfare; and reduce CH_4 emissions per unit of livestock product (Peters et al., 2012; Rao et al., 2015; Aynekulu et al., 2020; Ku-Vera et al., 2020a; Vazquez et al., 2020).

Despite the multiple benefits of silvopastoral systems (SPS), the use of grass-legume associations is limited in tropical agricultural systems by several factors. These include reduced plant growth associated with interspecies competition and shading, the potentially low palatability of legumes, the reluctance of farmers to adopt new species due to a general lack of awareness of the benefits of these systems, and the limited availability of legume seeds (Karsten and Carlassare, 2002). However, the specific effects of each association depend on the plant species involved.

A widely studied species in the tropics is the shrub legume *Leucaena* sp., which when planted in SPS provides multiple benefits to grazing livestock, including the provision of high quality protein throughout the year without the need for nitrogen inputs from synthetic fertilizers (Shelton and Dalzell, 2007; Cook et al., 2020), increased forage biomass (Naranjo et al., 2012; Gaviria et al., 2015), improved voluntary forage intake (Cuartas Cardona et al., 2015; Gaviria-Uribe et al., 2015), increased animal productivity (Cuartas Cardona et al., 2014), and reduced CH_4 emissions (Molina et al., 2015; Montoya-Flores et al., 2020). *Canavalia* sp. is a herbaceous legume that can grow in various Latin America locations by direct seeding, alone or in combination with tropical grasses, characterized by high concentrations of protein and high digestibility. However, the relationships between CH_4 emissions (*in vitro*) and nutritional quality of the legumes *Leucaena diversifolia* (more information is available on *Leucaena leucocephala*) and *Canavalia brasiliensis* have been little studied despite their potentials when associated with tropical grasses such as *Urochloa*, which is an important forage grass genus that is widely used in Latin America, Australia and parts of Asia (Low, 2011).

This work aimed to evaluate the effect of mixing different ratios of relevant tropical grasses (*Urochloa* sp. cv. Cayman and Toledo) and legumes (*Canavalia brasiliensis* and *Leucaena diversifolia*) on diet nutritional quality, rumen degradability, and net *in vitro* total gas and CH_4 production. In addition, using optimization analysis, we aimed to find out the ideal proportions of grass and legume(s) to not only reduce net gas production (as a possible indicator of CH_4) at the rumen level but also to increase crude protein (CP) content in the diet.

MATERIALS AND METHODS

Location

Forage samples were collected in the rainy season between April and May of 2016 from a silvopastoral experiment established at the International Center for Tropical Agriculture (CIAT), Palmira, Valle del Cauca, Colombia (3° 30′ 17″ N and 76° 21′ 24″ E) at an altitude of 965 meters above sea level. Soils are mollisols, with a pH of 7.2. During sample collection, average temperature was 25.4°C, average relative humidity was 65%, and total precipitation was 231 mm (5.5 mm day^{-1}) and these conditions allowed good regrowth of forage for 56 days.

Forage Samples and Mixed Diets

The tropical forage species evaluated were the two grasses *Urochloa* hybrid (CIAT BR02/1752) cv. Cayman (UHC) and *Urochloa brizantha* (CIAT 26110) cv. Toledo (UBT), the herbaceous legume *Canavalia brasiliensis* (CIAT 17009) (CB), and the shrub legume *Leucaena diversifolia* (ILRI 15551) (LD). Forage materials were planted 2 years before the start of the experiment (2014). The forage crops did not receive any fertilizers, pesticides or irrigation. One kilogram of each of UHC, UBT, and CB were collected at the vegetative stage of development before the beginning of flowering (after 6 weeks of regrowth), by cutting at 10 cm above soil level. Young leaf and stem samples (2:1 ratio) of LD were also manually collected. Two gas production experiments were conducted at two different times: one with UHC, CB, and LD forages, and the other with UBT, CB, and LD. In each experiment the individual forages were evaluated alone (100% UHC or 100% UBT, 100% CB, and 100% LD) and in mixtures with different proportions of DM of grasses and legumes. We used the order (UHC or UBT) - CB - LD, on a DM basis, with the treatment proportions of 0-50-50; 50-50-0 and 50-0-50 which correspond to a mixture in equal proportions (50%) between two species, either a grass with one of the two legumes or with both legumes without UHB or UBT. The treatment denoted 70-30-0 corresponds to the incubated mixture of 70% grass DM plus 30% CB DM, while 70-15-15 refers to the DM proportions 70% UHC or UBT plus 15% CB and 15% LD. Finally, the treatment: 33.3-33.3-33.3, indicates a mixture in equal DM proportions of 33% of the forages (UHC or UBT): CB: LB. A total of nine different treatments were evaluated in each of the two experiments. The proportions of the forages incubated were determined in order to perform a simplex-centroid mixture design.

Nutritional Quality

Samples were evaluated at the Forage Quality and Animal Nutrition Laboratory of CIAT. Samples were dried in a Memmert® UF 750 forced air oven at 60°C for 72 h and until constant weight was achieved. Samples were ground using a cutting mill (Retsch® SM 100, Haan, Germany) with a 1 mm sieve. The content of acid detergent fiber (ADF) and neutral detergent fiber (NDF) was determined using an Ankom 2000 fiber analyzer (Ankom Technology Corp., Macedon, NY, USA) following the method of Van Soest et al. (1991). The ash content was determined using the AOAC method (Association of Official Analytical Chemists, 1990); organic matter (OM) content was calculated as 1,000—ash concentration in g kg^{-1} DM. Gross energy (GE) density was determined using a Parr 6400 (Parr Instrument Company, Illinois, USA) isoperibol calorimeter in accordance with International Standardization Organization, 1998: ISO 9831:1998 specifications. Acid detergent lignin (ADL) content was determined using the method of ANKOM (2016). Total nitrogen content was determined using an autoanalyzer (Skalar Analytical B.V. Breda, Holland) after digestion with sulfuric acid and selenium (Krom, 1980; Searle, 1984). Crude protein (CP) content was estimated as 6.25 × total nitrogen content. Total phenol and tannin contents were determined using Folin-Ciocalteu's method (Makkar, 2003). The metabolizable energy (ME) was calculated according to Lindgren (1983) from the *in vitro* digestibility value obtained at 96 h.

In vitro Gas Production and Dry Matter Degradation

The methodology of Theodorou et al. (1994) was employed for *in vitro* gas production. Rumen fluid was drawn and mixed from two rumen-fistulated *Brahman* steers, grazing on a star grass (*Cynodon plectostachyus*)-dominated pasture with *ad libitum* access to mineralized salt. Briefly, ∼1.0 g of dried/ground samples were placed in individual Wheaton bottles and inoculated with a rumen fluid/buffer solution mixture. After inoculation, all bottles were depressurized (at time 0) and placed in a water bath set at 39°C. Thereafter, pressure and volume measurements were taken at 3, 6, 9, 12, 24, 36, 48, 60, 72, and 96 h of incubation. After each reading, the bottles were gently shaken and placed back in the water bath. Pressure measurements were taken using an 8,40,065 wide-range pressure gauge (Sper Scientific, Arizona, USA) and a PS100 2-bar pressure transducer (Lutron Electronic Enterprise Co. Ltd., Taipei, Taiwan) connected to a three-way valve. The first output was connected to a 1″ 22 G needle (25 mm × 0.7 mm), the second output to the transducer, the third to a 60 mL syringe, making it possible to record the gas volume removed at each time point required to reduce the bottle internal pressure to atmospheric pressure and to save gas samples for subsequent chromatographic analysis. Upon completion of the test, the contents of the bottles were filtered and dried in a forced-air oven at 105°C for 24 h to determine DM loss. Dry matter degradability (DMD, g kg^{-1}) was calculated for each sample as the change in sample DM weight following incubation, divided by the starting sample DM weight, multiplied by 1,000.

Accumulated gas production (AGP) curves were fitted to the Gompertz model, as proposed by Lavrenčič et al. (1997), using the CurveExpert Professional® software, version 2.4.0 (Hyams, 2016). This model was used to evaluate the gas production points using the following equation:

$$\mathrm{AGP}(\mathrm{mL\ g^{-1}OM}) = \mathrm{ae}^{-\mathrm{e}^{\mathrm{b-ct}}} \quad (1)$$

Where a, b, and c are the equation parameters [*a*, maximum gas production; *b*, the difference between initial and final gas at time x; *c*, specific gas accumulation rate; and *t*, time (hours; h)], the accumulated gas production results were expressed on an organic matter (OM) basis. Other biologically significant values were calculated based on parameters a, b, and c. These included the time at inflection point (TIP, h), gas volume at inflection point (GIP, mL), maximum gas production rate (MGPR, mL h^{-1}), and lag phase (LP or microbial settlement, h). These values were estimated using the following formulas:

$$\mathrm{TIP} = \mathrm{b} \times \mathrm{c}^{-1} \quad (2)$$

$$\mathrm{GIP} = \mathrm{a} \times \mathrm{e}^{-1} \quad (3)$$

$$\mathrm{MGPR} = (\mathrm{axc})\,\mathrm{xe}^{-1} \quad (4)$$

$$\mathrm{LP} = \left(\left(\mathrm{bxc}^{-1}\right) - \left(1\mathrm{xc}^{-1}\right)\right) \quad (5)$$

where "e" is Euler's number, ca. 2.72.

Methane Measurements

Methane concentration was quantified in all gas samples collected at the Greenhouse Gas Laboratory of CIAT using a gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. A three-meter long HayeSep N column was used and the mobile phase was high purity nitrogen at a flow rate of 35 mL min^{-1}. The oven, injector, and detector temperatures were 250, 100, and 325°C, respectively.

Experimental Design and Data Analysis

The nutritional quality, DMD, and CH_4 production data were analyzed using a randomized complete block design, where each treatment had three replicates at each time the readings were taken and three inoculums, the latter being the blocking factor. Mean comparisons were made using Tukey's test when a significant treatment effect ($P < 0.05$) was identified. To check for the normality of data distribution, the Shapiro-Wilk test was conducted on the original residuals using PROC GLM. To determine the correlations among the above variables, type II linear regressions were carried out using the bisector model linear functional relationship procedure of Genstat 18th Edition (VSN International Ltd., Hemel Hempstead, UK). All analyses were conducted using SAS® 9.4 Software (SAS Institute, 2012).

The completely randomized model was:

$$Yij = \mu i + eij \quad (6)$$

Where: *Yij*: observation of the *j*-th repetition of the *i*-th treatment; μi: mean value of the *i*-th treatment, *eij*: experimental error of unit *ij*

The linear regression model employed was:

$$Yi = \beta_0 + \beta_{1*}xi + ei \tag{7}$$

Where: *Yi:* observation of the *i*-th variable response, corresponding with the i-th value xi of the x predictive variable (dependent variable); β_0 *and* β_1 are the regression parameters; *xi* is the independent variable; and *e*: experimental error of unit *i*.

Regression analysis of nutritional quality data (NDF, CP, ADL, GE, and ME) against AGP and DMD parameters was carried out to identify an optimal mixed-diet in which the nutritional quality could be improved (specifically CP) while at the same time reducing gas production. A simplex-centroid mix design was run, using the special cubic model as a response adjustment model using the StatPoint Technologies Inc., 2010: Statgraphics® software (Centurion XVI, version 16.1.18).

The complete simplified special cubic model was:

$$\begin{aligned} y = {} & x_1G + x_2C + x_3L + x_{1,\,2}GC + x_{1,\,3}GL \\ & + x_{2,\,3}CL + x_{1,\,2,\,3}GCL \end{aligned} \tag{8}$$

Where (y) is the crude protein (CP g kg^{-1}) response variable or accumulated gas production (mL g^{-1} OM), x_1, $x_{1,2}$, $x_{1,2,3}$ are the regression coefficients for individual ingredients and mix interactions; G, C, and L are the relative ratios of forage components (grass, CB, and LD).

RESULTS

Nutritional Quality

The CP content of LD was 3.5 times greater than that of both grasses (**Tables 1**, **2** for UHC and UBT, respectively) and it was also greater than that of CB ($P \leq 0.05$). The NDF contents ranged from 492 (CB) to 700 g kg^{-1} DM (100% UBT) ($P < 0.0001$) while the concentrations of ADF were less variable, ranging between 344 and 399 g kg^{-1} DM. For the treatments where different proportions of legumes and grasses were mixed, in both experiments it was observed that the CP content decreased as the proportion of grasses increased, however, the opposite occurred with the NDF content. The lignin content of CB was similar to that of UBT, whereas the lignin content of LD was similar to that of UHC. Legumes, especially LD, have higher GE contents than that reported for 100% grasses treatments or when grasses are replaced up to 30% by legumes ($P = 0.001$), however, this trend is reversed when ME is calculated, since LD treatments or the combination of legumes in equal proportions (50% LD + 50% CB) obtain the lowest values of ME. Much higher concentrations of phenols and tannins were measured in LD compared to both grasses and CB, and the concentrations of both of secondary metabolites were also higher in CB than in both grasses.

Gas Production and Dry Matter Degradation

The total volume of gas produced during the fermentation process ranged from 150 to 255 mL g^{-1} OM (**Tables 3**, **4** for UHC and UBT, respectively). The diet combinations from both systems had a very fast fermentation rate, as evidenced by the low TIP and LP values. The lowest total accumulated gas production values at 96 h occurred with the LD-only treatment in both systems, a value that was almost 0.6 of that from the diet comprising UHC, LD, and CB in a ratio of 70:15:15 and UBT-only diet, respectively (**Figures 1**, **2**).

The highest (inverse) correlation was observed between the content of CP and the AGP values ($R^2 = 0.919$; **Table 5**). In contrast, ME content and gas production were positively related, i.e., the higher the ME content, the higher the gas production ($R^2 = 0.907$). Other strong inverse relationships were observed between the concentration of ADL and DMD g kg^{-1}, GE density and DMD g kg^{-1}, and between T and AGP, and T and DMD.

The correlation analysis results provided the basis for carrying out the optimization objective of selecting the best forage combination for increasing the CP concentration of a dietary mix while decreasing AGP. In the case of the UHC-based treatments, the percentage variance accounted for by these two parameters was 87.9% for CP and 84.3% for AGP. In the UBT-based treatments, the percentage variance accounted for CP and AGP was 87.8 and 87.9%, respectively. **Table 6** shows the restrictions used for obtaining a suitable inclusion of grasses and legumes, as well as the ratio of the best mix found (optimized) and the CP and AGP obtained with the specific mix.

Methane Production

When incubated alone, CH_4 production from CB started declining rapidly after 60 h in measurements of both grasses (**Tables 7**, **8** for UHC and UBT, respectively). The same CH_4 accumulation trend was observed with the other diets for 96 h. It is worth noting that the largest production of CH_4 in the UHC diets came from the 70% UHC: 15% CB: 15% LD diet. The incorporation of legumes into the UBT system contributed to decreased CH_4 production compared to the 100% of UBT diet.

DISCUSSION

Feeds intended for livestock are typically evaluated individually to determine their nutritional values and not integrated with a diet (Tang et al., 2008). Evaluations of individual forages does not allow us to determine interactions with other dietary components in the digestion process (Moss et al., 1992). Although the values of some nutritional parameters of diet components are additive (e.g., CP concentrations), there are possible interactions and synergies between different feeds in a diet and their nutritional values (e.g., energy yield and CP concentrations) that could not be evaluated independently (Tang et al., 2008). This situation can be explained at the rumen level, because depending on the type of diet, some synergy or antagonism may develop due to co-existence of nutrients and their interactions with different microorganisms (i.e., bacteria, protozoa, fungi, and methanogenic archaea) in the rumen (Cammack et al., 2018).

In this investigation, great variability in nutritional composition was found among the different forage diets. For example, the legumes contained twice as much CP as the two grasses evaluated, and the grasses had higher concentrations

TABLE 1 | Mean chemical composition of *Urochloa* hybrid grass cv. Cayman (UHC) and the two forage legumes, *C. brasiliensis* (CB) and *L. diversifolia* (LD), and their mixed proportions used in the study.

Mix UHC-CB-LD	DM	Ash	NDF	ADF	ADL	CP	GE, MJ kg^{-1} DM	ME, MJ kg^{-1} DO	TP	T
100-0-0	199de	122^{a}	642^{a}	365	157^{ab}	68^{i}	17.29^{c}	8.09^{ab}	24.6	1.07
0-100-0	189^{e}	117^{a}	492^{e}	346	98^{c}	195^{c}	17.90^{bc}	7.10^{d}	46.2	15.24
0-0-100	292^{a}	51^{e}	530^{d}	344	176^{a}	256^{a}	19.82^{a}	5.94^{e}	101.1	47.47
0-50-50	240^{bc}	84^{d}	529^{d}	330	132^{bc}	214^{b}	19.32^{a}	6.95^{d}	–	–
50-50-0	194de	117^{a}	565^{c}	368	99^{c}	125^{f}	17.66^{c}	8.02^{ab}	–	–
50-0-50	245^{b}	85^{d}	598^{b}	320	166^{a}	154^{e}	18.49^{b}	7.48^{cd}	–	–
1/3-1/3-1/3	224^{bcd}	96^{c}	566^{c}	317	108^{c}	164^{d}	18.55^{b}	7.66^{cb}	–	–
70-30-0	192de	119^{a}	608^{b}	354	96^{c}	101^{h}	17.76^{c}	8.22^{a}	–	–
70-15-15	206^{cde}	109^{b}	603^{b}	320	91^{c}	110^{g}	17.75^{c}	8.25^{a}	–	–
p-value	0.001	0.001	0.001	0.062	0.001	0.0001	0.001	0.001	–	–
EMS	23.435	1.85	4.68	20.58	15.01	33.8	5.789	0.18	–	–

Data presented as g kg^{-1} DM unless otherwise indicated.
[a,b,c,d,e,f,g,h,i]Mean values in a column with a different letter are statistically different ($P < 0.05$).
UHC, Cayman grass; CB, Canavalia brasiliensis; LD, Leucaena diversifolia; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; GE, gross energy; ME, metabolizable energy; TP, total phenols; T, tannins; EMS, error mean square.

TABLE 2 | Mean chemical composition of *Urochloa brizantha* cv. Toledo (UBT) and the two forage legumes, *C. brasiliensis* (CB) and *L. diversifolia* (LD), and their mixed proportions used in the study.

Mix UBT-CB-LD	DM	Ash	NDF	ADF	ADL	CP	GE, MJ kg^{-1} DM	ME, MJ kg^{-1} DO	TP	T
100-0-0	248.5^{a}	97.2^{d}	700^{a}	399^{a}	83^{cde}	77^{i}	17.69^{e}	8.57^{a}	21.2	0.68
0-100-0	188.7^{f}	117.0^{a}	492^{f}	346^{ab}	98^{cde}	195^{c}	17.78^{de}	7.42^{c}	46.2	15.24
0-0-100	292.0^{bc}	51.4^{i}	530^{e}	344^{ab}	176^{a}	256^{a}	20.06^{a}	6.17^{e}	101.1	47.47
0-50-50	240.3^{cd}	84.4^{g}	529^{e}	330^{b}	132^{b}	214^{b}	19.32^{bc}	6.85^{d}	–	–
50-50-0	218.6^{de}	107.5^{b}	595^{d}	363^{ab}	91^{cde}	129^{f}	17.76^{de}	8.30^{ab}	–	–
50-0-50	270.2^{ab}	73.5^{h}	623^{c}	335^{b}	113^{bc}	158^{e}	18.80^{c}	7.58^{c}	–	–
1/3-1/3-1/3	240.6^{cd}	87.4^{f}	583^{d}	338^{b}	105^{bcd}	167^{d}	18.26^{cd}	7.86^{bc}	–	–
70-30-0	206.6^{ef}	102.2^{c}	655^{b}	383^{ab}	79de	107^{h}	17.74^{de}	8.53^{a}	–	–
70-15-15	213.2^{ef}	92.5^{e}	654^{b}	368^{ab}	67^{e}	116^{g}	18.13^{de}	8.19^{ab}	–	–
p-value	0.001	0.001	0.001	0.0045	0.001	0.001	0.001	0.001	–	–
EMS	6.77	4.3	4.79	19.49	11.01	10.76	3.588	0.16	–	–

Values in g kg^{-1} DM unless otherwise indicated.
[a,b,c,d,e,f,g,h,i]Mean values in a column with a different letter are statistically different ($P < 0.05$).
UBT, Toledo grass; CB, Canavalia brasiliensis; LD, Leucaena diversifolia; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; GE, gross energy; ME, metabolizable energy; TP, total phenols; T, tannins; EMS, error mean square.

of NDF than the legumes. Similarly, concentrations of phenolic compounds were lower in the grasses than the legumes. These findings concur with data reported in the literature for these tropical species (Lee, 2018; Cook et al., 2020; Gaviria-Uribe et al., 2020), where CP values for grasses can range between 40 and 140 g kg^{-1} DM, and for both legumes studied here, shrub and herbaceous, ranged between 190 and 250 g CP kg^{-1} DM. However, the CP content obtained in the present study was slightly lower than that reported by Peters et al. (2002) for *U. brizantha cv*. Toledo who stated that under optimal conditions CP content ranges between 90 and 120 g kg^{-1} DM. Likewise, the NDF content was within the range of 600 and 800 g kg^{-1} DM reported for *U. brizantha* sp. (Cook et al., 2020; Gaviria-Uribe et al., 2020). However, forage quality has been shown to be closely related to pasture age (Vendramini et al., 2014; Gaviria-Uribe et al., 2020) and the time of the year (Demarchi et al., 2016; Abdalla et al., 2019). The ADL content of *Urochloa* grasses was 86 and 157 g/kg DM, both values were between the ranges reported by Wassie et al. (2018), according to these authors ADL content can vary between 91.2 and 186.9 g/kg depending on ecotype, regrowth age (60, 90 and 120 d) and altitude of the sowing site (1,230, 1,774, and 2,650 masl). It is noteworthy that little information is available on the ADL content of *Urochloa* hybrid cv. Cayman. The ME values found for the legume *L. diversifolia* are slightly lower (8.6 MJ ME kg^{-1} DM) than the results reported by Geleti et al. (2013), while the ME for grasses are above those obtained by Nguku (2015) for 9 grasses of the genus *Urochloa*, whose values ranged between 6.6 and 5.9 MJ ME kg^{-1} DM. However, this variable, as well as the rest of the nutritional components of the diet, can vary according to the

TABLE 3 | Accumulated gas production (AGP; mL g^{-1} OM), dry matter degradability (DMD), and profiles of the adjustment made using the Gompertz model for UHC, CB, LD, and their mixes.

Mix UHC-CB-LD	AGP (mL g^{-1} OM)	DMD (g kg^{-1})	Gompertz model						
			a	*b*	*c*	TIP (h)	GIP (mL)	MGPR (mL h^{-1})	LP (h)
100-0-0	231.5^{a}	712^{a}	215.05^{a}	1.11^{a}	0.06^{c}	17.09^{a}	79.09^{a}	5.12^{cd}	1.63^{a}
0-100-0	180.3^{cd}	638^{bc}	168.63^{bcd}	0.98^{ab}	0.11^{a}	8.66^{f}	62.02^{bcd}	7.05^{a}	−0.15^{b}
0-0-100	150.0^{de}	517^{d}	146.91^{d}	0.88^{b}	0.06^{c}	14.95^{abc}	54.04^{d}	3.18^{e}	−2.06^{h}
0-50-50	167.5^{d}	608^{c}	159.35^{cd}	0.88^{b}	0.08^{bc}	11.07^{e}	58.61^{cd}	4.64^{cd}	−1.56^{g}
50-50-0	210.0^{ab}	703^{a}	200.73^{ab}	0.96^{ab}	0.09^{b}	11.19^{ab}	73.83^{ab}	6.31^{ab}	−0.52^{c}
50-0-50	199.6^{bc}	641^{bc}	196.84^{ab}	0.95^{ab}	0.06^{c}	16.38^{e}	72.40^{ab}	4.21^{de}	−0.83^{e}
33.3-33.3-33.3	193.4^{bc}	662^{b}	185.91^{abc}	0.90^{b}	0.07^{bc}	12.32^{de}	68.38^{abc}	5.00^{cd}	−1.36^{f}
70-30-0	213.5^{ab}	718^{a}	205.75^{a}	0.95^{ab}	0.07^{bc}	12.87^{cde}	75.68^{a}	5.59^{bc}	−0.65^{cd}
70-15-15	234.8^{a}	713^{a}	218.88^{a}	0.95^{ab}	0.07^{bc}	14.02^{bcd}	80.51^{a}	5.45^{bc}	−0.75^{de}
p-value	0.001	0.001	0.001	0.015	0.001	0.001	0.001	0.001	0.001
EMS	8.824	12.01	12.575	0.066	0.007	0.839	4.626	0.402	0.600

[a,b,c,d,e,f,g]Mean values in a column with a different letter are statistically different ($P < 0.05$).
UHC, Cayman grass; CB, Canavalia brasiliensis; LD, Leucaena diversifolia; AGP, accumulated gas production; OM, organic matter; DMD, dry matter degradability; a, maximum gas production (mL); b, difference between initial gas and final gas at an × time; c, specific gas accumulation rate; TIP, time to the inflection point, h; GIP, gas at the inflection point, mL; MRGP, maximum rate of gas production, mL/h; LP, lag phase, h; EMS, error mean square.

TABLE 4 | Accumulated gas production (AGP; mL g^{-1} OM), dry matter degradability (DMD), and profiles of the adjustment made using the Gompertz model for UBT, CB, LD, and their mixes.

Mix UBT-CB-LD	AGP (mL g^{-1} OM)	DMD (g Kg^{-1})	Gompertz model						
			a	*b*	*c*	TIP (h)	GIP (mL)	MGPR (mL h^{-1})	LP (h)
100-0-0	252.9^{a}	726^{ab}	249.84^{a}	1.13^{a}	0.05^{cd}	20.93^{a}	91.89^{a}	4.96^{ab}	2.41^{a}
0-100-0	182.0^{de}	661^{d}	171.64^{cd}	0.98^{ab}	0.09^{a}	10.38^{e}	63.13^{cd}	5.96^{a}	−0.20^{c}
0-0-100	155.6^{f}	532^{f}	153.65^{d}	0.92^{b}	0.05^{cd}	16.87^{bc}	56.51^{d}	3.09^{d}	−1.39^{g}
0-50-50	175.7^{def}	598^{e}	167.20^{cd}	0.94^{ab}	0.07^{ab}	12.34^{de}	61.5^{bc}	4.68^{bc}	−0.79^{d}
50-50-0	225.7^{bc}	715^{ab}	214.91^{ab}	0.92^{b}	0.07^{bc}	13.17^{de}	79.04^{ab}	5.56^{ab}	−1.04^{ef}
50-0-50	202.4^{e}	640^{d}	200.69^{bc}	0.99^{ab}	0.05^{d}	18.71^{ab}	73.81^{cd}	3.91^{cd}	−0.17^{bc}
33.3-33.3-33.3	207.1^{cde}	669^{cd}	199.47^{bc}	0.92^{b}	0.06^{bcd}	14.59^{cd}	73.36^{bc}	4.67^{bc}	−1.11^{f}
70-30-0	230.6^{ab}	727^{a}	230.76^{ab}	0.94^{ab}	0.06^{bcd}	15.16^{cd}	84.87^{ab}	5.28^{ab}	−0.90^{de}
70-15-15	233.7^{ab}	695^{bc}	228.05^{ab}	1.00^{ab}	0.05^{bcd}	17.07^{bc}	83.88^{ab}	4.92^{bc}	0.03^{b}
P	0.001	0.001	0.001	0.028	0.001	0.001	0.001	0.001	0.001
EMS	7.119	1.098	14.784	0.066	0.006	1.045	5.437	0.355	0.076

[a,b,c,d,e,f,g]Mean values in a column with a different letter are statistically different ($P < 0.05$).
UBT, Toledo grass; CB, Canavalia brasiliensis; LD, Leucaena diversifolia; AGP, accumulated gas production; OM, organic matter; DMD, dry matter degradability; a, maximum gas production (mL); b, difference between initial gas and final gas at an × time; c, specific gas accumulation rate; TIP, time to the inflection point, h; GIP, gas at the inflection point, mL; MRGP, maximum rate of gas production, mL/h; LP, lag phase, h; EMS, error mean square.

age of the species and time of year (Givens et al., 1993, Nguku, 2015). In the present investigation, there were differences in ME content between legumes and grasses, contrary to what was reported by Evitayani et al. (2004), who found average values of 7.6 ± 0.14 and 7.3 ± 0.12 MJ ME kg^{-1} DM for grasses and legumes, respectively. Likewise, the highest ME concentrations were for the treatments: 100-0-0, 70-30-0 or 70-15-15, this may favor the synthesis of microbial proteins at the rumen level (Krizsan et al., 2020).

For the *in vitro* analysis, the highest gas production and degradability rates were obtained for samples of both grasses that were individually incubated and when 30% legumes were added to these grasses. Despite this, there was a clear pattern and as the level of inclusion of legumes increased, gas production and degradability decreased. Blümmel et al. (1997) suggested that a feed consisting of a mix of different kinds of ingredients can result in asynchrony in releasing nutrients, thus changing both the biomass of microorganisms produced and gas produced by them. In addition, one factor that can affect the fermentation and gas production of feeds is the configuration of their cell wall polysaccharides (Molina-Botero et al., 2020, Valencia-Salazar et al., 2021). Therefore, the digestibility values depend upon their composition of structural carbohydrates, including the concentration of lignin (Barahona and Sánchez, 2005) and

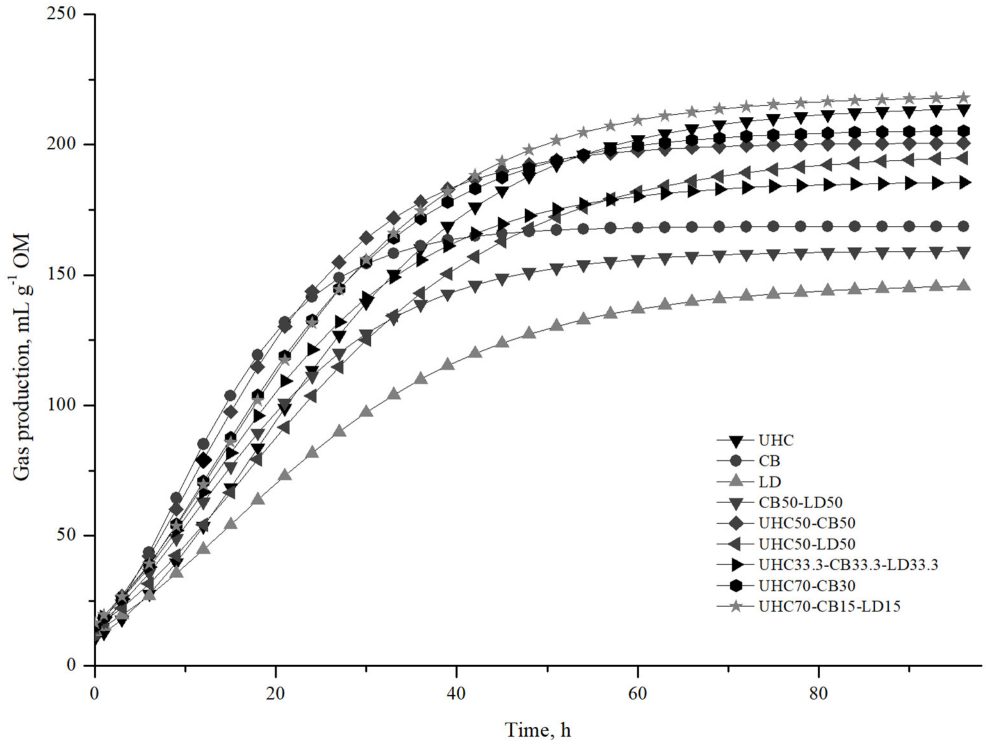

FIGURE 1 | Modeled mean accumulated gas production (mL g^{-1} OM) for UHC, CB, LD, and 6 dietary mixtures. UHC, *Cayman* 100%; CB, *Canavalia* 100%; LD, *Leucaena* 100%; CB50LD50, *Canavalia* 50% + *Leucaena* 50%; UHC50CB50, *Cayman* 50% + *Canavalia* 50%; UHC50LD50, *Cayman* 50% + *Leucaena* 50%; UHC33.3CB33.3LD33.3, *Cayman* 33.3% + *Canavalia* 33.3% + *Leucaena* 33.3%; UHC70CB30, *Cayman* 70% + *Canavalia* 30%; UHC70CB15LD15, *Cayman* 70% + *Canavalia* 15% + *Leucaena* 15%.

the protein included in the diet or treatment evaluated. This postulate agrees with the high correlation values obtained in this study between nutritional compounds such as CP or ADL and variables such as DMD or AGP. Similar results were reported by Lee (2018) where 136 forage plant species or hybrid cultivars grown in 30 countries were evaluated, finding that parameters such as ADF, NDF, ADL content had a correlation >0.7 with DMD or OMD. Although Lee (2018) affirmed that there is a positive correlation (0.62, respectively) between CP and DMD, in the current study there was an inverse correlation between both parameters, perhaps due to the concomitant increase of the content of anti-nutritional compounds associated with the inclusion of CB and/or LD, which could potentially mask the full expression of a diet rich in CP and GE, as was also reported by Jayanegara et al. (2011).

It is clear that to increase our understanding of the nutritive value of forage mixtures composed of tropical forages, the action of the various secondary metabolites (i.e., tannins, saponins) that are present in some legumes must be taken into consideration (Tiemann et al., 2008a,b; Lascano and Cárdenas, 2010). The effect of secondary metabolites depends on their concentration or proportion to the substrate with which they interact. For example, tannins can be found both in the cell wall and inside the cytoplasmic vacuoles of some legumes, primarily in the form of condensed tannins (McAllister et al., 1994; Patra et al., 2017) and their effect depends on their concentration or ratio with the substrate with which they interact. High concentrations of tannins, such as the ones found in diets containing legumes (CB and LD) can delay the digestion of forages by reducing the activity of fibrolytic enzymes (Archimède et al., 2016; Henke et al., 2017; Ku-Vera et al., 2020b). This phenomenon is related to the microbial degradation of structural polysaccharides, and the rate and extent of forage degradation (Archimède et al., 2016; Henke et al., 2017). Likewise, a negative effect has been shown on protein degradation when tannins encapsulate it at low rumen pH (Hess et al., 2003; Archimède et al., 2016). The described tannin effect could explain our results obtained in this study, as in the treatments with an inclusion between 50 and/or 100% of some of these two containing-tannin- legumes (15.2 and 47.5 g kg^{-1} DM for *Canavalia* and *Leucaena*, respectively) and total phenols (46.2 and 101.1 g kg^{-1} DM) a reduction in digestibility variables and therefore in gas production was observed. These results are in contrast to the 100% grass treatments where the values of tannins and total phenols did not exceed 1.07 g T kg^{-1} DM and 24.2 g TP kg^{-1} DM. This observation is consistent with the study of Seresinhe et al. (2012), where a strong inverse relationship was found between tannin concentration and gas production. Tolera et al. (1998) reported condensed tannins content ranging from 7.1 to 13.5% in LD. This concentration of tannins could have bacteriostatic effects on some populations,

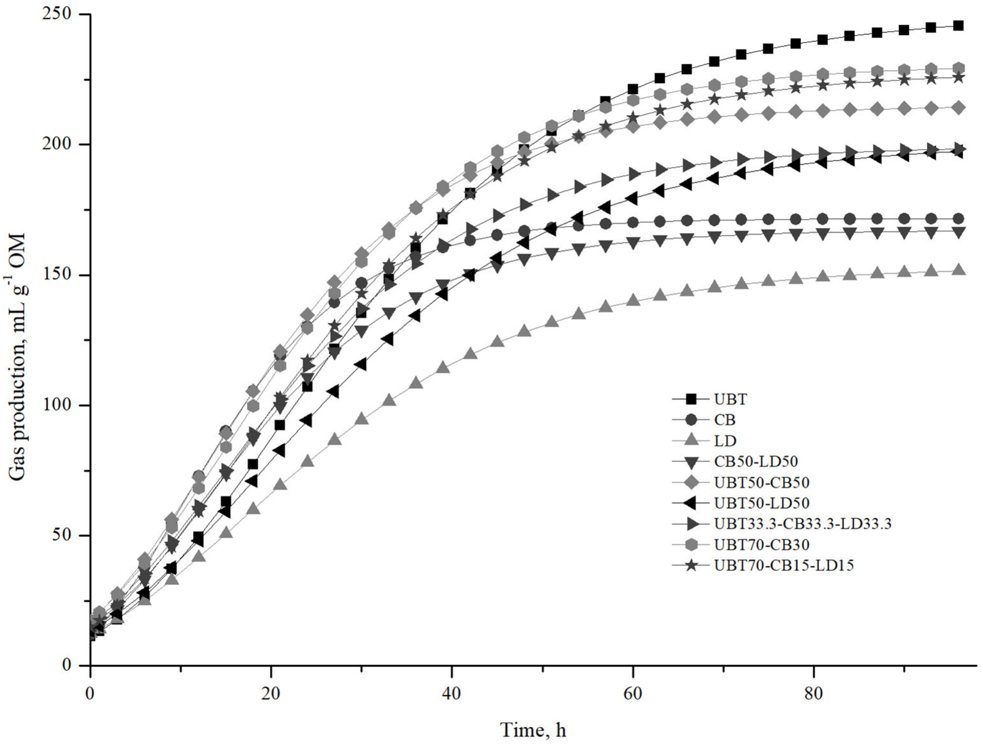

FIGURE 2 | Accumulated gas production (AGP; mL g^{-1} OM) for UBT, CB, LD, mixed diets. UBT, *Toledo* 100%; CB, *Canavalia* 100%; LD, *Leucaena* 100%; CB50LD50, *Canavalia* 50% + *Leucaena* 50%; UBT50CB50, *Toledo* 50% + *Canavalia* 50%; UBT50LD50, *Toledo* 50% + *Leucaena* 50%; UBT33.3CB33.3LD33.3, *Toledo* 33.3% + *Canavalia* 33.3% + *Leucaena* 33.3%; UBT70CB30, *Toledo* 70% + *Canavalia* 30%; UBT70CB15LD15, *Toledo* 70% + *Canavalia* 15% + *Leucaena* 15%.

TABLE 5 | Correlations obtained by type II linear regression analysis.

Correlation	Equation	R^2	SE slope	SE constant
NDF g kg^{-1} (*x*) on AGP mL g^{-1} OM (*y*)	$y = 0.46x - 63.8$	0.743	0.064	37.55
NDF g kg^{-1} (*x*) on DMD g kg^{-1} (*y*)	$y = 1.12x + 7.47$	0.434	0.247	148.7
CP g kg^{-1} (*x*) on AGP mL g^{-1} OM (*y*)	$y = -0.51x + 281.6$	0.919	0.037	6.145
CP g kg^{-1} (*x*) on DMD g kg^{-1} (*y*)	$y = -1.12x + 834.9$	0.876	0.102	16.86
ADL g kg^{-1} (*x*) on AGP mL g^{-1} OM (*y*)	$y = -0.70x + 280.0$	0.622	0.142	16.55
ADL g kg^{-1} (*x*) on DMD g kg^{-1} (*y*)	$y = -1.70x + 847.3$	0.769	0.240	28.07
GE MJ kg^{-1} (*x*) on AGP mL g^{-1} OM (*y*)	$y = -44.9x + 1,025$	0.654	16.56	303.7
GE MJ kg^{-1} (*x*) on DMD g kg^{-1} (*y*)	$y = -83.9x + 2,198$	0.864	26.93	494.1
ME MJ kg^{-1} (*x*) on AGP mL g^{-1} OM (*y*)	$y = -36.8x + 77.7$	0.907	2.936	22.94
T g kg^{-1} (*x*) on AGP mL g^{-1} OM (*y*)	$y = -2.20x + 243.2$	0.791	0.279	6.185
T g kg^{-1} (*x*) on DMD g kg^{-1} (*y*)	$y = -4.56x + 742.5$	0.948	0.266	6.019

NDF, neutral detergent fiber; CP, crude protein; ADL, acid detergent lignin; GE, gross energy; ME, metabolizable energy; T, tannins; AGP, accumulated gas production; OM, organic matter; DMD, dry matter degradation; R^2, determination coefficient; SE, standard error.

leading to lower digestibility of the fermented material (Tavendale et al., 2005).

Evaluation of the AGP and CP content in a mix of the three dietary components (grass, CB, and LD) yielded an optimal diet ratio of 60% grass (UHC or UBT), 30% CB, and 10% LD. It should be clarified that although a reduction in gas production was pursued as a measure to reduce CH_4 production and emission, it was never intended to be zero. This expectation is because gas production is of great importance to maintain ideal conditions inside the rumen. For example, in the case of cattle it is important that the formation and utilization of metabolic hydrogen is synchronized (Calsamiglia et al., 2005) in the metabolic pathway that is responsible for glucose oxidation (glycolysis). This is required to regenerate the reducing power

TABLE 6 | Optimization of the crude protein (CP; maximize) and accumulated gas production (AGP; minimize) response variables in the UHC and UBT forage systems.

Systems	Factor	Restrictions		Calculated optimal value	Optimal value (desirability)	Optimized CP (g kg^{-1} DM)	Optimized AGP (mL g^{-1} OM)
		Minimum	Maximum				
UHC	UHC (%)	60	100	60.0	0.398	147	200
	CB (%)	0	40	30.0			
	LD (%)	10	40	10.0			
UBT	UBT (%)	60	100	60.0	0.420	151	215
	CB (%)	0	40	30.0			
	LD (%)	10	40	10.0			

UHC, Cayman 100%; UBT, Toledo 100%; CB, Canavalia 100%; LD, Leucaena 100%; AGP, accumulated gas production; CP, crude protein; DM, dry matter; OM, organic matter.

TABLE 7 | Methane yield from UHC, CB, LD, and their mixed diets.

Treatment	Methane yield (g CH_4 kg^{-1} DM) at different post-incubation times										g CH_4 kg^{-1} DMD
	3 h	6 h	9 h	12 h	24 h	36 h	48 h	60 h	72 h	96 h	96 h
UHC	0.07^{c}	0.98^{b}	1.99^{c}	3.05^{c}	8.98^{a}	11.11^{ab}	13.19^{a}	14.27^{a}	15.55^{a}	18.08^{a}	24.36^{ab}
CB	0.50^{a}	1.99^{a}	4.43^{a}	6.85^{a}	10.42^{a}	13.72^{a}	14.99^{a}	16.34^{a}	11.72^{b}	11.44^{b}	18.52^{b}
LD	0.25^{b}	0.93^{b}	1.77^{c}	2.87^{c}	7.68^{a}	8.90^{b}	9.72^{b}	10.77^{b}	11.16^{b}	12.68^{b}	23.28^{ab}
UHC70CB30	0.49^{a}	1.55^{a}	3.01^{b}	4.65^{b}	10.31^{a}	12.58^{a}	14.68^{a}	15.19^{a}	15.76^{a}	17.84^{a}	23.62^{ab}
UHC70CB15LD15	0.56^{a}	1.66^{a}	3.02^{b}	4.68^{b}	10.68^{a}	12.65^{a}	14.81^{a}	15.97^{a}	17.00^{a}	19.66^{a}	26.29^{a}
p-vale	0.001	0.001	0.001	0.001	0.001	0.0072	0.0013	0.001	0.001	0.001	0.012
EMS	0.054	0.163	0.356	0.546	1.431	1.255	1.184	1.260	0.979	1.232	1.817

[a,b,c]*Mean values in a column with a different letter are statistically different ($P < 0.05$).*
DM, dry matter; CH_4, methane; DMD, dry matter degradation; UHC, Cayman 100%; CB, Canavalia 100%; LD, Leucaena 100%; UHC70CB30: Cayman 70% + Canavalia 30%; UHC70CB15LD15: Cayman 70% + Canavalia 15% + Leucaena 15%.

TABLE 8 | Methane yield from UBT, CB, LD, and their mixes.

Treatment	Methane yield (g CH_4 kg^{-1} DM) at different post-incubation times										g CH_4 kg^{-1} DMD
	3 h	6 h	9 h	12 h	24 h	36 h	48 h	60 h	72 h	96 h	96 h
UBT	0.07^{b}	0.46^{c}	1.41^{d}	3.27^{b}	10.71^{a}	14.64^{a}	16.65^{ab}	18.18^{ab}	19.84^{ab}	22.44^{a}	31.57^{a}
CB	0.14^{ab}	0.85^{a}	2.90^{bc}	6.18^{a}	11.85^{a}	13.87^{a}	16.03^{b}	17.13^{b}	16.26^{b}	11.29^{c}	17.69^{c}
LD	0.08^{ab}	0.55^{bc}	1.87^{cd}	3.30^{b}	7.07^{b}	8.93^{b}	10.55^{c}	11.42^{c}	11.32^{c}	12.71^{b}	25.22^{b}
UBT70CB30	0.14^{a}	0.83^{a}	4.00^{a}	5.69^{a}	13.28^{a}	15.71^{a}	18.16^{a}	19.70^{a}	20.81^{a}	21.85^{a}	30.83^{a}
UBT70CB15LD15	0.12^{ab}	0.74^{ab}	3.17^{ab}	5.25^{a}	12.43^{a}	15.04^{a}	17.24^{ab}	18.54^{ab}	19.24^{ab}	21.42^{a}	31.69^{a}
p-vale	0.016	0.0015	0.001	0.001	0.0008	0.001	0.001	0.001	0.001	0.001	0.001
EMS	0.027	0.092	0.405	0.709	1.207	0.741	0.703	0.847	1.350	0.953	1.714

[a,b,c,d]*Mean values in a column with a different letter are statistically different ($P < 0.05$).*
DM, dry matter; CH_4, methane; DMD, dry matter degradation; UBT, Toledo 100%; CB, Canavalia 100%; LD, Leucaena 100%; UBT70CB30, Toledo 70% + Canavalia 30%; UBT70CB15LC15, Toledo 70% + Canavalia 15% + Leucaena 15%.

of cofactors such as NAD+ and FAD+, while increasing the synthesis the synthesis of adenosine triphosphate, promoting the growth of other microbial species (e.g., fibrolytic) and helps to regulate the osmotic pressure inside the rumen (Yokoyama and Johnson, 1993; Calsamiglia et al., 2005). Regarding the proportions established in the evaluated diets, this is consistent with the observations of Rojas et al. (2005), who suggested that the percentage of legumes should range from 30 to 40% in mixtures of grasses and legumes to improve the quality of the diet and have an optimal protein:energy balance at the rumen level. Moreover, these proportions coincide with those found in experiments with ruminants fed with tropical legumes that are rich in tannins and whose results affirm that DM intake was reduced when the amount of CT exceeds 50 g kg^{-1} DM (Patra and Saxena, 2011). Likewise, cattle systems where the diet is composed of 100% low quality grasses, have low productive indexes due to the low CP concentration, required by ruminal microorganisms for the breakdown of carbohydrates, in addition to a reduction in DM intake due to the high content of structural carbohydrates (Krizsan et al., 2010).

Enteric CH_4 emission rates are associated with the physicochemical characteristics of the diet (e.g., CP and NDF contents), which have a direct impact on diet intake (Gaviria-Uribe et al., 2020) and eating frequency (Grant et al., 2015). Several studies have evaluated the effect of adding a legume to a grass on CH_4 production both *in vitro* (Tope et al., 2013; Molina-Botero et al., 2020) and *in vivo* (Molina-Botero et al., 2019a,b; Gaviria-Uribe et al., 2020; Montoya-Flores et al., 2020). Nevertheless, the conclusions drawn from these studies are unclear, as in some cases the addition of a legume increased *in vitro* CH_4 production (Carulla et al., 2005; Molina-Botero et al., 2020), but in others, it had the opposite effect (Lee et al., 2004). In our case, net CH_4 production per kg of DM did not differ between treatments containing legumes (up to 30% inclusion) and grasses alone, but less gas was produced when 100% legumes were incubated. A similar trend was observed for CH_4 production per unit DMD, being most noticeable for the treatment of 100% CB. When comparing both legumes, we observed that CB was characterized by containing less NDF and ADL than LD, contributing to improved digestibility and therefore higher gas production. This finding coincides with the conclusion reached by Hess et al. (2003), who stated that the difference in *in vitro* CH_4 production among various kinds of forages could be accounted for by the differences between the ratios of digestible carbohydrates and cellulose. Likewise, Patra and Saxena (2010) proposed that the presence of secondary metabolites can affect methanogenesis. However, this was not observed in the present study, because the inclusion of up to 30% of legumes did not reduce *in vitro* CH_4 production. In addition, a greater reduction would be expected with the LD treatment alone, since it contained a greater amount of total phenols and tannins compared to CB alone. These results can be explained by indirect effects of other secondary compounds present in these species, such as mimosine, alkaloids, saponins, steroids, among others that were not evaluated (Hu et al., 2005; Oseni et al., 2011). With our results, it should not be ignored that the *in vitro* technique, despite being an artificial system, is a viable option to initially simulate possible dietary combinations of forages (Danielsson et al., 2017) that can then be validated using ruminants. This is why we highlight the importance of including legumes in cattle diets as a strategy to reduce CH_4 emissions.

Although it was not the primary aim of this study, the use of herbaceous and shrub legumes was shown to have potential positive environmental benefits besides improving nutritive values of diets for ruminants. Vazquez et al. (2020) showed how combining the three types of forages tested here clearly improved chemical, physical and biological soil health characteristics. In addition, the use of shrubs and trees in silvopastoral systems have shown the capacity to sequester greater amounts of carbon at a system level (Aynekulu et al., 2020).

CONCLUSIONS

Diets that combined legumes (CB or LC) with grass (UHC or UBT) had higher protein contents and gross and metabolizable energy densities, as well as decreased concentrations of NDF and lignin. Metabolizable energy and nutritional compounds such as NDF, T, and CP had a high correlation with net gas production, while ruminal digestibility was affected by CP, ADL, GE, T, and other unidentified compounds provided by CB and/or LD.

Optimal ratios of dietary components in both systems were found with mixtures consisting of 60% grass (either UHC or UBT), 30% CB, and 10% LD. The system containing UHC yielded the best combination in terms of an increase in CP and a decrease in AGP. However, this ratio did not result in a decrease in methane production. Therefore, further characterization of the content and activity of other secondary metabolites, perhaps present in both legumes, is required to better explain the behavior response resulting from grass-legume interactions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Colombian law No. 84/1989 and the Ethics Committee of the International Center for Tropical Agriculture.

AUTHOR CONTRIBUTIONS

SQ-A, IM-B, JR-N, and RB-R: conceptualization. SQ-A and IM-B: methodology. SQ-A, IM-B, and JM: formal analysis. SQ-A: writing—original draft preparation. SQ-A, IM-B, IR, RB-R, NC, JA, and JM: writing—review and editing. RB-R and JA: supervision. JA: project administration. NC, JM, and JA: funding acquisition. All authors contributed to the article and approved the submitted version.

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Ex-Ante Evaluation of Economic Impacts of Adopting Improved Forages in the Colombian Orinoquía

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Forage-based cattle systems play a key role in rural economies of developing countries in terms of food security and poverty alleviation. However, they can generate negative environmental impacts by contributing to increased greenhouse gas emissions, land degradation, and reduction of biodiversity. As a result of that, large amounts of resources have been allocated to research and development (R&D) in forage material improvement and a broad range of improved materials were released showing superior characteristics in terms of productivity and environmental impacts compared to native or naturalized materials. However, data are still scarce on both the economic and environmental "yields" of investments in R&D activities around improved forage materials. Through an ex-ante evaluation, this study aims at estimating the potential "yields" of the investment in R&D and diffusion activities of the improved forage variety *Brachiaria brizantha* 26,124 cv. Agrosavia Caporal in the Colombian Orinoquía region. The analysis used two evaluation methodologies: 1) a combined discounted free cash flow model and Monte Carlo simulation using the simulation software @Risk to determine the impact on individual welfare, and 2) an economic surplus model an risk analysis to determine the potential social benefits of the technologies and their distribution among producers and consumers, considering changes in adoption rates, productivity levels and probability of success. The results suggest that the evaluated material presents important economic benefits for the study region and results in a positive return on the investments made in R&D activities. The results are a key input for decision making processes among public and private institutions involved in funding and executing the development of improved forage materials and will help to set research priorities and resource allocation.

Keywords: agricultural research and development, priority setting, technological change, economic surplus analysis, decision making, funding allocation for research, sustainable intensification (SI), conservation

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INTRODUCTION

The Cattle Sector in the Colombian Orinoquía

Cattle production is one of the main agricultural activity in Colombia and plays a major role in the achievement of the Sustainable Development Goals in the region, as it holds a large potential for economic, social and environmental improvements. The Colombian cattle sector contributes with 21.8% of the agricultural Gross Domestic Product of the country and generates approximately 6% of the national and 19% of agricultural employment, respectively (FEDEGAN, 2018). Its importance also lies in its impact on a social level. Cattle farming is mainly carried out by small-scale farmers (81% of the cattle farms in Colombia possess less than 50 animals, with an average of 18 animals per farm) (ICA, 2020). Additionally, it is estimated that 44% of the cattle producer households live in conditions of poverty (DANE-CNA, 2014; UPRA, 2019, 2020). According to the Food and Agriculture Organization of the United Nations (FAO, 2018), the sector has the potential to contribute to the goals of income and poverty reduction, reducing the environmental footprint, enhancing the provision of ecosystem services and promoting peace and social stability, among others. Over 20% of the total agricultural production from developing countries comes from this sector, and the increasing demand for animal source foods, coupled with changing diets and decreased availability of suitable land, pose major pressures on increasing the efficiency of the sector in ways that are inclusive, environmentally responsible and improve food security. In Colombia, its environmental relevance is primary, as cattle production generates 16% of the greenhouse gas emissions of the Colombian Agriculture, Forestry and Other Land Use sector (AFOLU), and is also one of the principal activities associated with deforestation and the expansion of the agricultural Frontier (IDEAM and MADS, 2016).

The Orinoquía region is of special importance for the country's cattle sector, as it holds approximately 20% of the total national cattle inventory (ICA, 2020), with nearly 55% of its agricultural land destined to cattle grazing (UPRA, 2015a; UPRA, 2015b; UPRA, 2015c). Although the average farm size in the region is rather large (534 ha), this is biased by a small number of large-scale farmers while the region is dominated by small-scale cattle farms with an inventory of less than 50 animals (ICA, 2020). The sector faces important challenges, as the expansion of cattle production threatens biodiversity and strategic ecosystems in the region, such as natural savannas, gallery forests, foothills or flooded forests. Additionally, forage supply is highly dependent on the marked water seasonality of the region (excessive rainfall and drought), directly affecting cattle production and making the sector more vulnerable to climate change. Investments in more intensive cattle production systems, considering the specific environmental conditions, water dynamics and presence of strategic ecosystems in the region, therefore, have been the main approach for achieving a sustainable development of the regional cattle sector (CIAT and CORMACARENA, 2018).

To advance towards sustainable intensification of cattle farming in the Orinoquía, institutions such as the Colombian Agricultural Research Corporation (AGROSAVIA, before Corpoica) and the International Center for Tropical Agriculture (CIAT) have been commissioned to carry out research on new forage materials. Government and research institutions consider the region as strategic for forage research and development (R&D), due to high soil acidity and low fertility - both key for carrying out adaptation and productivity trials with new and promising materials (Peters et al., 2013; Rao et al., 2015). Research has been aimed at identifying new forage materials with better productive characteristics, a greater range of adaptation to extreme conditions and higher resistance to local pests and diseases. Among the released materials, the grasses *Brachiaria humidicola* CIAT 679 cv. Humidicola, *Brachiaria brizantha* CIAT 26110 cv. Toledo and, more recently, *Brachiaria brizantha* CIAT 26124 cv. Agrosavia Caporal stand out as superior alternatives to the traditional *Brachiaria decumbens* cultivars mainly used in the Orinoquía (Miles et al., 1996).

Processes of identification and release of new forage materials represent the first step towards sustainable intensification (improving efficiency without the need to further expand pasture areas), increasing food security and decreasing environmental trade-offs (including greenhouse gas emission intensities of the cattle sector). Under the right enabling conditions (e.g., subsidized credit, technical assistance, protective tariffs and land tenure security), sustainable intensification can help in achieving the objective of liberating areas with potential for crop cultivation, reforestation, conservation or landscape recovery.

Research on new varieties for the agricultural sector is recognized as a powerful instrument to accelerate economic growth and development (The World Bank, 2008; Stads and Beintema, 2009), but this process requires steady financing to maintain and enhance the necessary scientific, technical and technological capacities and infrastructure. In particular, most resources for agricultural research come from public funds, making it of special importance that the technologies derived from R&D processes are profitable and viable. Ex-ante impact evaluations allow estimating the possible benefits of R&D investments, providing information for prioritization and more strategic decision-making (Maredia et al., 2014).

Studies on the evaluation of impacts generated by the development of new forage materials in Colombia are scarce and date back to the 1990s and early 2000s. They focus on new *Brachiaria* hybrids and accessions adapted to different regions of the country (e.g., Vera et al., 1989; Seré et al., 1993; Rivas and Holmann, 2004a, 2004b), providing consistent results on the positive economic impacts derived from the adoption in cattle systems. No recent studies, however, evaluate the potential benefits of new forage materials. New grasses and legumes --including cv. Agrosavia Caporal, the most recent technology to be delivered to Colombia's cattle producers–lack economic evaluation. *B. brizantha* cv. Agrosavia Caporal will be the third *Brachiaria brizantha* material released in the country, after the La Libertad (CIAT 26646) and the Toledo varieties released in 1987 and 2002, respectively. This material has been evaluated since 1986 and was identified as a promising alternative to improve cattle production in well-drained soils of the Orinoquía. In this sense, the objective of this study is to evaluate the impact of R&D and adoption of the new variety *Brachiaria brizantha* 26,124 cv. Agrosavia Caporal (Agrosavia Caporal from here on) in the Colombian Orinoquía region, with emphasis on the beef raising and fattening production system. For this purpose, we applied models at two aggregation scales - the micro and macro level. At the micro or farm level, a cost-benefit analysis was performed using a discounted free cash flow model and a Monte Carlo simulation analysis. This model was used to evaluate and analyze potential impacts on the primary producer and to determine if the adoption of the technology is economically feasible. At the macro level or the regional scale, an economic surplus model was used in order to estimate and analyze the potential added benefit for the society and its distribution among two different social groups: producers and consumers. The economic surplus model is the most widely used model for measuring ex-ante impacts of technological innovations, providing a consistent theoretical basis with minimum data requirements. Although there are other more precise models (e.g., the IMPACT model), we aimed at maximizing the precision of our estimates, considering budget limitations, time constraints and access to available data.

Agrosavia Caporal has already been developed, but it is not yet available to producers (planned year of release: 2022). One of the aims of this study is, therefore, to not only guide the decision-making process of investing in the development of future varieties, but to also provide evidence on the potential benefits of other endeavors with similar contexts. This study also attempts to highlight some of the minimal conditions in terms of adoption levels and expected benefits, necessary to make such investments profitable both at the individual and social levels. The article is structured as follows: First, we present the theoretical framework on adoption processes at the micro and macro level, a literature review on previous studies on the subject and the empirical methodology we applied. In Section *Results*, we present our results. Section *Discussion* discusses these results considering previous studies on the subject and on-going adoption processes in the region. The final section presents the conclusions of the article.

Review on Economic Evaluations of *Brachiaria* in Latin America

In the context of adopting improved forages, impact evaluation studies were conducted mainly at the end of last century, and especially regarding *Brachiaria* hybrids and accessions in different regions of Latin America (e.g., Seré and Estrada, 1982; Rivas and Holmann, 2004a; Rivas and Holmann, 2004b). Seré and Estrada (1982) evaluated the profitability of cattle fattening under different feeding scenarios (with improved forages) in various locations of the Orinoquía, finding Internal Rates of Return (IRR) of between 10.7 and 30.4% (Vera et al., 1989). calculated that the use of *Antropogon gayanus* (Carimagua I) is 33% more profitable than traditional (naturalized) forages in the Orinoquía region and 78% the northern Caribbean of Colombia, respectively. Seré et al. (1993) examined the profitability of tropical forages released by CIAT and its local partners in Latin America, identifying an IRR of between 20 and 100%. Rivas and Holmann (2004a) evaluated the potential impact of new *Brachiaria* hybrids resistant to spittlebug in the eastern Orinoquía region and the Caribbean coast of Colombia, and estimated benefits for 2004 of US$960 million, which was equivalent to 43% of the country's meat and milk production value in 2003 (direct impact on the livestock sector). More recent studies on the subject were found for the African continent, where the impact of higher-yielding *Brachiaria* varieties was estimated. Elbasha et al. (1999), for example, evaluated the impact of different planted forages in West Africa during the period from 1977 to 1997 and estimated economic benefits of approximately US$11.8 million, which represents an internal social rate of return on investments of 38% over a 20-year period. Schiek et al. (2018) evaluated the potential economic impact of the development and release of improved *Brachiaria* varieties in six East African countries, using an economic surplus model. According to their results, investment in a forage research program is a low risk endeavor with a high probability of obtaining positive results at a minimum adoption rate of 10%.

Most of the described studies used the economic surplus method as main approach for impact evaluation. In general, across all reviewed studies, positive results were found regarding the benefits of research on forage alternatives with better productive characteristics as strategy for intensifying cattle production. Although some of the past studies focused on the impacts of improved forages in different regions of Colombia, neither more recent ex-ante evaluations were found, nor particular studies regarding the species *Brachiaria brizantha* or micro-level studies that include quantitative risk assessments, which give more robust results and improve decision-making at the primary producer level. This document is intended to be a contribution to the literature in that sense, and provides useful information to donors and decision-makers regarding the potential yields of investing in forage research for the Colombian Orinoquía.

MATERIALS AND METHODS

Data Sources

Productivity data for the Agrosavia Caporal variety were obtained from field trials carried out by AGROSAVIA and CIAT in the Colombian Orinoquía region. Evaluations were carried out at the Taluma experimental station and the Carimagua Research Center under well-drained soil conditions. The average temperature at the site is 26°C and the average annual rainfall 2,500 mm. Productivity was calculated as the average of the accumulated live weight gain over a year in a cattle raising and fattening system. These measurements were carried out on a monthly basis between 2011 and 2015, with six groups of young crossbred bulls in a rotational grazing design, with 14 days of occupation and 28 days of recuperation. Information on the traditional technology (reference technology) used in the region was obtained through interviews with AGROSAVIA researchers and from past field evaluations conducted in the region. The ex-ante impact analysis seeks to compare a novel technology with a technology traditionally used in the study region. In our case, *Brachiaria decumbens* as monoculture is the technology with the largest area in the Colombian Orinoquía, with important characteristics in terms of productivity and adaptability to well-drained soils in the region (Rincón et al., 2010). The grass *Brachiaria decumbens*, was introduced and used massively in the country in the 1970s. The scenario assumes adequate management practices in terms of fertilization and rotation, to avoid overestimating the benefits associated with the adoption of the new variety.

Information related to economic and technological assumptions, as well as the R&D costs used in the economic surplus model, was obtained through expert consultation and literature review. Section 2.6.1 shows the data sources corresponding to each parameter used. The establishment and management costs of the evaluated technologies were calculated based on the economic information collected during the trials, which was adjusted with the help of forage and livestock experts according to the conditions of a typical beef cattle raising and fattening farm in the Orinoquía region. Prices were updated to 2018 according to the price bulletins of the Colombian Price Information System of the Agricultural Sector SIPSA/DANE (2020) and databases of the Colombian Cattle Farmer Federation, FEDEGAN, (2019a).

Characteristics of the New Technology

B. brizantha cv. Agrosavia Caporal is a new forage alternative coming directly from the species *Brachiaria brizantha*, which was collected in Karuzi (Burundi, Africa) in 1985. CIAT researchers collected this material in collaboration with the Burundian national agricultural research institution (ISABU) (Rincón et al., 2021). Agrosavia Caporal is a perennial grass that grows in clumps, with decumbent stems of a height of 60–150 cm, capable of rooting in the ground and favoring soil coverage, persistence and lateral displacement of the grass. Its leaves are lanceolate with little pubescence, reaching up to 60 cm in length and 2.5 cm in width. It grows well in tropical conditions up to altitudes of 1,800 m above sea level. It develops best at temperatures between 20 and 35°C, with the highest forage production occurring during rainy season and in conditions with annual rainfall between 1,600 and 3,500 mm (Rincón et al., 2021). Although the variety was targeted to the Orinoquía region, it holds the potential for broader adoption in other regions of Colombia, given its adaptation potential to different climates (humid and sub-humid tropics) and soils (medium to good fertility) (M. Sotelo, personal communication, May 17, 2020).

The first evaluation records of *B. brizantha* cv. Agrosavia Caporal in Colombia date back to 1986, when antibiotic resistance to spittlebug was evaluated among 400 accessions of *Brachiaria*. Accession 26,124 was part of a group of 27 materials which were selected for presenting greater resistance compared to the commercial material *Brachiaria brizantha* cv. Marandú (CIAT, 1991). In 1997, it was one of the materials selected for presenting better drought resistance in trials established at the Carimagua research station in the Colombian Orinoquía (CIAT, 1997). In 1999, it was introduced for agronomic evaluation in different locations across Colombia (CIAT, 1999), and in 2000, in the Orinoquía (CIAT, 2001). In a participatory evaluation exercise, Agrosavia Caporal was selected by producers as a promising material for cattle production in the Orinoquían savannas, due to its good stem-leaf ratio, soft leaves, rooting behavior and rapid recovery after grazing (CIAT, 2001).

In 2011, in an inter-institutional agreement between AGROSAVIA and CIAT, forage germplasm evaluations under well-drained soils were started in the Orinoquía with the establishment of 58 materials and the aim of selecting the five most promising ones. The Agrosavia Caporal accession was identified as one of these materials, and was included in animal feeding trials carried out at two locations in the Orinoquía (Taluma experimental station and Carimagua Research Center), where it was compared with *Brachiaria decumbens* - the control material predominant in the region. The main characteristics that made Agrosavia Caporal an outstanding alternative for animal feeding, and especially compared to other evaluated accessions such as Toledo (*Brachiaria brizantha* CIAT 26110), are its high forage productivity and quality, drought resistance (i.e., avoiding cattle weight losses during dry season) and grazing persistence (Rincón et al., 2021). *B. brizantha* cv. Agrosavia Caporal also shows good tolerance to water stress during the rainy season, as well as to different spittlebug species (*Aeneolamia varia* and *Zulia pubescens*) present in the region (Rincón et al., 2021).

Table 1 provides a summary of the main productive indicators of cv. Orinoquía, as well as the reference technology (*Brachiaria decumbens*) for comparison. The adoption of Agrosavia Caporal increases the total available forage biomass by 23% and the protein content by 28% compared to the reference technology, reflected also in the animal response, with average annual live weight gains per hectare of 226 kg for Agrosavia Caporal versus 198 kg for *Brachiaria decumbens*. According to the daily live weight gain data, the raising and fattening cycle until reaching the final sales weight (from 200 kg to 450 kg) is 19 months for Agrosavia Caporal and 24 months for *Brachiaria decumbens*.

Methodological Approach: Cost-Benefit Analysis

Through a cost-benefit analysis, we estimated the impact of investing in the establishment of Agrosavia Caporal in a cattle raising and fattening system at the micro level (from a primary producer's point of view) in the Colombian Orinoquía. This methodology was used as it allows to analyze the market viability of an investment project in a reliable way, considering all the relevant costs and benefits in a process of technology adoption at the farm level, the lifespan of the technology, productivity flows and relevant market prices. Such analysis is being applied when a comparison has to be made between a traditional technology and a new one, in order to determine the changes in costs and income associated with the new technology. In our case, the comparison is made with the reference technology–a monoculture pasture of *Brachiaria decumbens* (A. Rincón, personal communication, February 12, 2021).

The cost-benefit analysis is based on a discounted free cash flow model to estimate financial profitability indicators and to determine the viability of the different investment options. Profitability indicators include the Internal Rate of Return (IRR), Net Present Value (NPV), Benefit/Cost ratio (B/C) and investment payback period (PRI). The model includes a systematic categorization of the variable costs and benefits associated with the two evaluated options. Specifically, the following per hectare cost categories have been considered: establishment costs, renovation and maintenance costs, opportunity costs of capital during the establishment period (3 months, from establishment until first grazing), and operating costs (e.g., purchase of animals, animal health,

TABLE 1 | Dry matter production, nutritional quality and animal response of the evaluated grasses.

Parameter	Variable	*Brachiaria brizantha* 26,124 cv. Agrosavia caporal	*Brachiaria decumbens* (reference technology)
Biomass production	DM (ton ha^{-1} y^{-1})	7.1	5.8
Nutritional quality	Crude protein (% DM)	9.6	7–8
	IVDMD (%)	65	62
Animal response	Animal carrying capacity (AU)	1.4	1.2
	Live weight gain (g AU^{-1} d^{-1})	418	345
	Animal productivity (kg ha^{-1} y^{-1})	226	198
	Raising and fattening period (months)[1]	19	24

IVDMD = In Vitro Dry Matter Digestibility; 1 AU (Animal Unit) = 400 kg/animal; DM = Dry Matter; 1 Period of time required to bring an animal of 200 kg average weight to a sales weight of 450 kg.

TABLE 2 | Variables simulated with the Monte Carlo model.

#	Variable	Distribution	Most likely value	Minimum value	Maximum value
1	Meat price (US$ kg^{-1})	Triangular[1]	1.26	1.21	1.31
2	Live weight gain Agrosavia Caporal (g AU^{-1} d^{-1})	PERT[2]	226	199	262
3	Live weight gain References technology (g AU^{-1} d^{-1})	PERT[2]	198	128	227
4	Establishment costs Agrosavia Caporal (US$ ha^{-1})	Triangular	341	273	409
5	Establishment costs References technology (US$ ha^{-1})	Triangular	306	245	368

[a]Prices in US$–/US$/COP XRT: Average 2020; [1]This triangular distribution is an average of the three values and is recommended to specify situations that involve costs and investments; [2]A PERT distribution is a weighted average of the three values with greater emphasis on the center of the distribution and was selected by judgment of the researchers according to data availability.

supplementation, permanent and occasional labor). On the other hand, the benefits are derived from beef production in a cattle raising and fattening system, according to the obtained animal response indicators (**Table 1**). For the construction of the cash flow we assumed constant prices and an evaluation horizon of 10 years according to the estimated lifespan of pastures (Riesco and Seré, 1985). The cost of financing is chosen as the discount rate according to the rural credit lines of the Colombian Fund for the Financing of the Agricultural Sector (FINAGRO), and considered as the opportunity cost of capital, associated with a risk factor present in the activities of the rural sector. The following discount rate was, therefore, established: Fixed-term deposit rate (DTF) + 5% effective annual interest rate. The investment is assumed to happen in year 0, and from year one to year 10, the income and expenses associated with each technology are generated. It is important to mention that, although data were obtained at an experimental level, we expect the differences to the real conditions of the region to be insignificant, if the producers follow the technical recommendations for pasture management (e.g., fertilization plans, periods of pasture occupation and recovery) and if the material is established under agroecological conditions similar to those recommended (e.g., altitude, soil type, precipitation regime). In addition, at a methodological level, different scenarios are applied for the returns of each of the evaluated technologies (**Table 2**).

To include risk and uncertainty levels and consider different scenarios, a quantitative risk analysis was performed using a Monte Carlo simulation with the software @Risk (Paladise Corporation). In this simulation, values of the variables identified as critical (meat price, live weight gain, establishment costs) are randomly assigned, according to their probability distribution functions, to later calculate the determined profitability indicators (model outputs). This process is repeated numerous times to obtain the probability distributions of said outputs (Park, 2007). In our study, 5,000 simulations or iterations were carried out, where the variables live weight gain (per animal and day), investment costs, and sales price (per kg live weight) were randomly combined. The simulation used a 95% confidence interval. The probability distributions for the input variables are presented in **Table 2**.

The decision criteria are the mean values and the variations of the profitability indicators resulting from the simulation, as well as the probability of success (NPV>0). The use of the mean value criterion is based on the law of large numbers, which states that if many repetitions of an experiment are carried out, the average result will tend towards the expected value (Park, 2007). Additionally, a sensitivity analysis was performed using a tornado diagram, which displays each variable according to its impact on the variance of the model result. The diagram identifies the variables defined as critical and those with greater effects on the profitability indicators.

Methodological Approach: Economic Surplus Model

The equation system for the economic surplus model is based on Alston et al. (1995) (**Figure 1**). It proposes to model and measure the economic effects of technological changes induced by research in market environments, through parallel and linear shifts of the supply and demand curves. In this case, the product in question (beef) is a perishable good that is not closely linked to international markets and therefore, equations for a closed economy are used.

The annual change in total surplus is defined as:

$$\Delta ET = K_t P_0 Q_0 \left(1 + \frac{1}{2} Z_t n\right) \quad (1)$$

where P_0 and Q_0 are the equilibrium prices and quantities, respectively; Z_t is the proportional price decrease in year t, defined as:

$$Z_t = \frac{K_t \varepsilon}{\varepsilon + n} \quad (2)$$

and K_t is the supply displacement factor associated with technological change, and its value is variable over time, depending on the dynamics of the adoption process; n is the absolute value of demand elasticity and ε the supply elasticity:

$$K_t = \left[\frac{E(Y)}{\varepsilon} - \frac{E(C)}{1 + E(Y)}\right] p A_t \delta_t \quad (3)$$

where $E(Y)$ is the average proportional yield increase per hectare, with ε being the supply elasticity used to convert the gross output effect of R&D-induced performance changes into a gross unit production cost effect; $E(C)$ is the average proportional change in variable costs per hectare required to achieve the increased yield; p is the probability of success in the technology adoption process; δ_t is the depreciation factor of the technology; A_t is the adoption rate in year t, and is determined by a logistic curve:

$$A_t = \frac{A_{MAX}}{1 + e^{-(\alpha + \beta t)}} \quad (4)$$

Amax is the maximum adoption rate, and the parameters α and β control displacement and slope, respectively and are determined by both the duration of research and adoption.

The annual change in consumer surplus is defined as:

$$\Delta EC_t = Z_t P_0 Q_0 \left(1 + \frac{1}{2} Z_t n\right) \quad (5)$$

The change in producer surplus is defined as:

$$\Delta EP_t = \Delta ET_t - \Delta EC_t \quad (6)$$

The economic benefits associated with the change in surpluses are expressed as annual flows of net benefits and the NPV is estimated. The NPV of the new R&D technology is calculated as:

$$NPV = \sum_t^T \frac{\Delta ET_t - k_t}{(1 + r)^t} \quad (7)$$

The aggregate IRR was calculated as the discount rate that equates the aggregate NPV to zero as follows:

$$\sum_t^T \frac{\Delta ET_t - k_t}{(1 + TIR)^t} = 0 \quad (8)$$

Additionally, for the estimation of the ex-ante evaluation model, the following assumptions are considered (Alston et al., 1995): 1) There are no policy distortions such as subsidies, production quotas, or others; 2) markets are competitive; 3) the supply equals the demand for the good, since prices are adjusted to reach equilibrium quantities, 4) the change in total surplus is a measure of the change in social welfare; and 5) the shift in the supply curve is only the result of technological change.

Model Parameters

To estimate the social benefits of forage varieties by means of the surplus model, it is necessary to consider different technical and economic parameters. Technical parameters allow identifying the magnitude of the shift in the supply function and the behavior of the adoption curve over time and are related to: 1) changes in productivity levels, 2) year of technology launch and duration of the diffusion period, 3) speed and intensity of the adoption process, and 4) R&D levels. The economic parameters define the markets under analysis in terms of: 1) type of economy, 2) initial equilibrium quantities and prices, and 3) price elasticities of supply and demand.

Table 3 presents a summary of the parameters related to both the market and the technology used to estimate the model in the basic scenario, as well as the respective data sources. The impact calculations at the national level were made assuming values of productivity increases and a potential area determined by the current rate of adoption of the *Brachiaria brizantha* species at the national level, given its high adaptation potential. Technology adoption behavior and the estimation of R&D costs are further explained in the subsequent sections. R&D costs occur from the initial year of research until the release of the new technology (2011–2022). After its release, the technology is acquired by the private sector (in this case a seed production and marketing company from Brazil) who assumes the subsequent costs associated with seed production, marketing and distribution. As these costs do not correspond to public research institutions or governmental institutions, they are excluded from the calculations in our study.

In order to examine the sensitivity of the model results, three analysis scenarios have been considered: basic (B), optimistic (O) and pessimistic (P). The parameters that vary between scenarios are productivity, maximum expected adoption rate, and probability of success (**Table 4**). The probability of success is defined as the success of developing a technology for commercial use, as well as the annual adoption rate being met at a defined percentage. Although Agrosavia Caporal has already been developed, it is not yet commercially available to producers. According to preliminary agreements with seed producing companies, it will be commercialized in 2022. Additionally, heat maps were elaborated to analyze the effect of the variation simultaneal of the first two variables on the IRR indicator.

Cost of Research and Development

The R&D costs for the evaluation and selection of the new Agrosavia Caporal variety were estimated according to the requirements of scientific personnel in a process of improvement by selection, and the annual budgets approved under the macroproject *Evaluación y desarrollo de materiales*

TABLE 3 | Description of the key parameters and data sources for the analysis of economic surpluses in the basic scenario.

Parameter	Value	Description	Source
Economic assumptions			
Economy type	Closed	Beef from the Orinoquía region is destined for the local and extra-regional market (mainly Bogotá, Cúcuta and Bucaramanga). At the national level, 93% of the beef produced is destined for internal consumption	Own estimate based on data from DANE (2021)
Supply elasticity	0.7	The offered quantities vary less than proportionally to price changes	Rivas and Holmann (2004a)
Demand elasticity	−1.17	According to Ramirez (2012), the long-term elasticity of the beef demand is relatively elastic (>1). The estimates of cross elasticity with the other types of meat (chicken, pork) show a high substitution effect regarding price changes	Ramirez (2012)
Regional initial production (tons)	200,560		Own estimate based on data from FEDEGAN (2019a) and ICA (2020)
National initial production (tons)	932,813		FEDEGAN (2019a)
Initial price (US$/ton)	2,376		Own calculations based on data from FEDEGAN and Bogota (2019b)
R&D costs (US$)	563,243		Expert estimation based on R&D budgets involved in the selection process of a new forage variety
Technical assumptions			
R&D period (years)	5 (2011–2015)	Evaluations for the selection of promising materials under the agreement AGROSAVIA-CIAT.	
Diffusion period (years)	27	The diffusion period can vary between 25 and 30 years, depending on the agro-ecosystem and the production system	Rivas and Holmann (2004b)
Year of release	2022	The initial year of introducing *Brachiaria brizantha* 26,124 cv. Agrosavia Caporal has been set for 2022, since AGROSAVIA is currently in the process of producing basic seed and in negotiations with seed companies in Brazil for seed production at a commercial level	(A. Rincón, personal communication, February 12, 2021)
Effects on productivity (%)	+14	Better animal response associated with the best characteristics in terms of nutritional quality and biomass production of the new variety compared to traditional technologies in the region	Estimates according to agronomic and animal response trial data
Changes in costs (%)	0	There are no changes in production costs associated with the new material	Information provided by livestock and forages experts
Probability of success of research (%)	80	As a basic scenario, the assumptions used in the model are expected to be fulfilled by 80%	Judgment of the researchers according to expert opinion regarding the success of other research programs in other countries and regions
Discount rate (%)	12	Social rate recommended by the National Planning Department for public investment projects in Colombia	DNP (2013)
Adoption profile	Logistic adoption curve	Behavior of the adoption-diffusion process of agricultural technologies	Alston et al. (1995)
Initial adoption rate (%)	0.001	A logistical distribution is assumed	Alston et al. (1995)
Maximum expected adoption rate (%) - Regional	2.22	Percentage of area grown with *Brachiaria brizantha* in the Colombian Orinoquía region	Labarta et al. (2017)
Maximum expected adoption rate (%) - National	2.8	Percentage of area grown with *Brachiaria brizantha* in Colombia	Labarta et al. (2017)

TABLE 4 | Scenarios for the sensitivity analysis of the economic surplus model for *Brachiaria brizantha* 26,124 cv. Agrosavia Caporal.

Scenario	Regional			National		
	P	**B**	**O**	**P**	**B**	**O**
Changes in productivity (%)	10	14	20	10	14	20
Probability of success (%)	70	80	100	70	80	100
Expected final adoption rate (%)	1.11	2.22	3.33	1.4	2.8	4.2

P: pessimistic scenario; B: basic scenario; O: optimistic scenario.

forrajeros para integrarlos a los sistemas de producción ganaderos de la Orinoquía, financed by the Colombian Ministry of Agriculture and Rural Development (MADR), and executed by AGROSAVIA and the International Center for Tropical Agriculture (CIAT). In this project, 58 forage accessions were evaluated in the Orinoquía region in order to identify five promising varieties adapted to the local edaphoclimatic conditions. The R&D period was 5 years, from 2011 to 2015. The project had an annual budget of US$65,000, where 30% was allocated for the evaluation of Agrosavia Caporal. This included

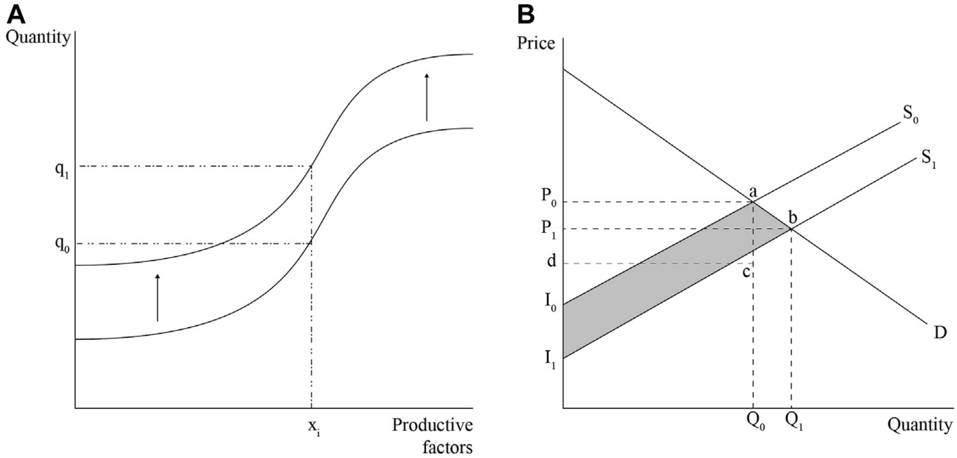

FIGURE 1 | Effects of technological change at different scales: a) Production function (micro level); b) Producer and consumer surplus (macro level). Source: Adapted from Alston et al. (1995, 206).

operational expenses for the establishment, maintenance and evaluation of the trials, such as agricultural inputs, agricultural services (e.g., labor for field work), equipment and machinery, transportation, travel expenses, and laboratory analysis.

Personnel requirements were estimated from the percentages of time devoted by scientists, researchers, technicians and workers in a process of improvement by selection. This process consists of five main stages: 1) evaluation of the visual characteristics of the materials (height, coverage, dynamometer, vigor, pests and diseases); 2) evaluation of visual characteristics, dry matter production (DM) and nutritional quality (e.g., protein content, digestibility, neutral detergent fiber) of the pre-selected materials in (i); 3) evaluation of plant-animal interaction of the materials identified in (ii), which are established on a larger scale to determine palatability, material persistence and animal productivity (meat or milk); 4) evaluation of the plant-animal interaction of the materials identified in (iii); and 5) establishment of the selected materials in different locations depending on whether they are for release at the regional or national level. Prior to these stages, the costs associated with processes of application, reception, and field establishment of the seed for multiplication, as well as institutional costs and equipment depreciation were also included. The total duration of the evaluation process was five consecutive years (2011–2015). Since 2016, some evaluations have continued, mainly at the Taluma experimental station, with an approximate annual budget of US$2,708. This includes the costs associated with the maintenance of the trials and administrative expenses. In the years 2014–2016, multiplication of basic seed was carried out CIAT's facilities in Palmira, Colombia, and the associated costs were also included. The total estimated R&D cost for the variety was estimated with US$563,243.

Technology Adoption and Diffusion

Before any economic impact associated with technical change can occur, a process of adoption and diffusion of the new technology needs to happen. By adoption we mean, in the context of technological innovations, the individual decision-making process about the acceptance of a previously unknown innovation, which implies learning through the acquisition of information and its incorporation into the production function. On the other hand, diffusion refers to the process of acceptance of a technology by a set of individuals in time and for a given region (Rogers, 2003).

Empirical evidence on adoption/diffusion processes of new agricultural technologies shows that it normally follows a logistic or sigmoid pattern (Mansfield, 1961; Mahajan and Peterson, 1985). On the subject of pastures, although literature is scarce, the studies of Jarvis (1981) confirm that adoption adjusts to a logistic model, meaning that the adoption curve is characterized by three stages: 1) early adoption, 2) exponential growth, and 3) the transition phase. In the first stage, the technology has a low adoption rate since only the least risk averse producers, or in other words, those who are more innovative, decide to invest in a new technology (in our case a new forage variety). After that, the benefits of the new technology begin to be known and a stage of rapid growth starts, characterized in turn by two sub-stages (2a) an early majority and (2b) a late majority. In the latter stage, adoption continues to grow, but each time at lower rates, as the process approaches its upper limit.

To estimate the adoption curve, we make use of ex-post data on the adoption of varieties similar to the new Agrosavia Caporal. Data were obtained from a nationally representative adoption study carried out by Labarta et al. (2017) in Colombia. Their results indicate that 2.2 and 2.8% of the total area, respectively at regional and national levels, are planted with the variety *Brachiaria brizantha* cv. La Libertad. Considering that this grass was introduced to the country 50 years ago, it is plausible to assume that the adoption-diffusion process is already in a maturation stage. This rate is considered, therefore, as the maximum level of adoption for the basic scenario. For the pessimistic and optimistic scenarios, we expect the adoption rate to be 50% below/above the maximum adoption rate expected for the basic scenario, indicating a minimum rate of 1.11% and a maximum rate of

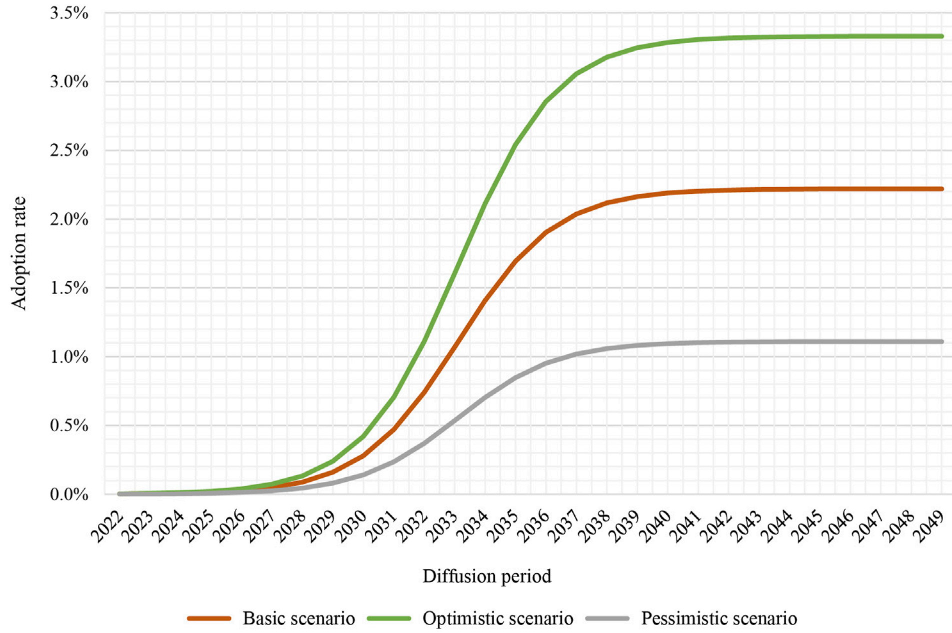

FIGURE 2 | Adoption curves at the regional level for the basic, optimistic and pessimistic scenarios of the economic surplus model.

TABLE 5 | Costs and income for cattle raising and fattening under both evaluated technologies.

Parameter	***Brachiaria brizantha* 26,124 cv. Agrosavia caporal**	***Brachiaria decumbens* (reference technology)**
Investment costs		
Establishment (US$ ha^{-1})	341	306
purchase of animals (US$ ha^{-1} cycle^{-1})	284	244
Operational costs		
Maintenance costs (US$ ha^{-1})[1]	182	182
Permanent labor (US$ ha^{-1} yr^{-1})[2]	89	84
Animal health (US$ ha^{-1} yr^{-1})	6.51	5.56
Supplementation (US$ ha^{-1} yr^{-1})[3]	14.1	12.03
Other costs	8.60	7.93
Gross income (average US$ ha^{-1} yr^{-1})	583	456
Unit cost of production (average US$ kg^{-1})[4]	1.027	1.029
Net income (average US$ ha^{-1} yr^{-1})[5]	112	94

[1]Maintenance is carried out every 2 years and includes weed control, fertilizing with half the dose used for establishment; [2]Estimated: 2.5 permanent jobs required for every 100 animals in a cattle raising and fattening system (FEDEGAN, 2003), and a legal minimum wage in force plus benefits in 2020 of US$375. [3]Supplementation with mineralized salt at a rate of 50 g ha^{-1} d^{-1}. [4]Unit cost of production: dividing total cost of the product by total production. [5]Net income: total income (sales price x yield) minus total costs.

TABLE 6 | Profitability indicators of the simulation model.

Decision criterion	Indicator	***Brachiaria brizantha* 26,124 cv. Agrosavia caporal**	***Brachiaria decumbens* (reference technology)**
NPV (US$)	Mean[a]	328	182
	SD[b]	95	134
	IC (95%)[c]	(30)-622	(223)-509
IRR (%)	Mean	21%	18%
Payback period (years)	Mean	5	5

[a]Mean value of the NPV obtained in the simulation (5,000 iterations).
[b]SD: Standard deviation of the NPV with respect to the mean value.
[c]IC: Minimum and maximum values with a 95% confidence interval.

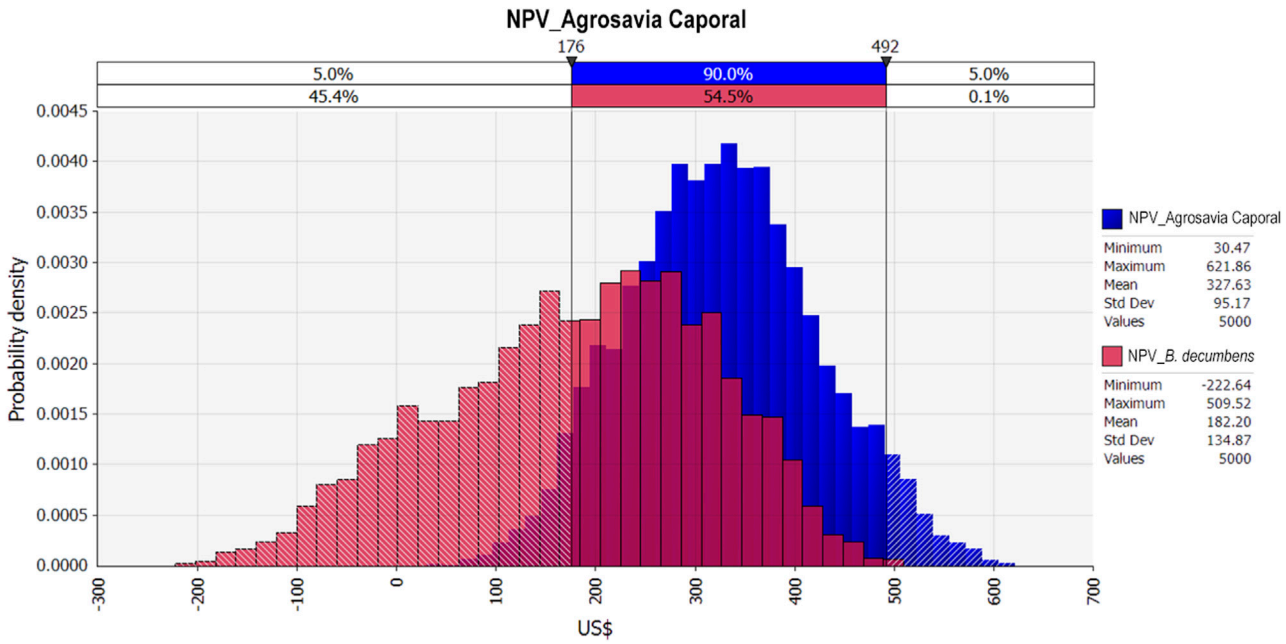

FIGURE 3 | Probability and accumulative density distributions of the NPV.

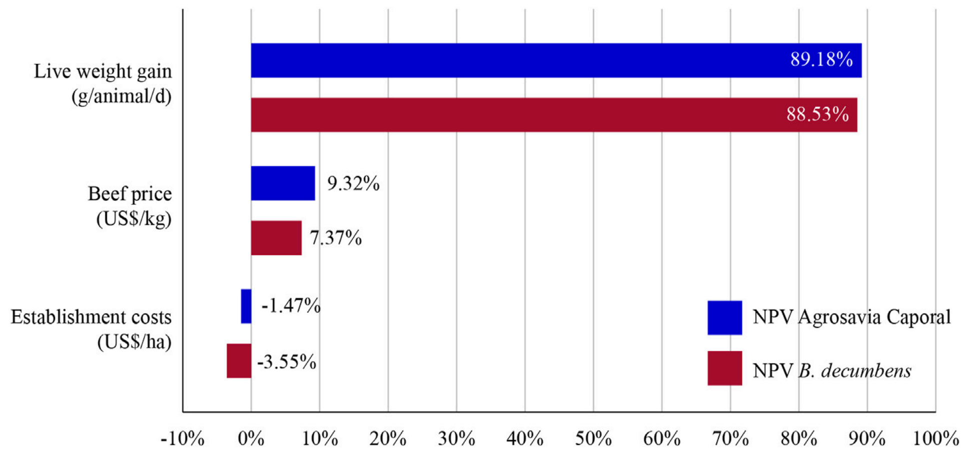

FIGURE 4 | Tornado diagram showing contributions of random input variables to the variance of the NPV.

3.3% at the regional level, and a minimum rate of 1.4% and a maximum rate of 4.2% at the national level, respectively (**Figure 2**). In both cases, the aim is to examine the changes in the net social benefits when a successful dissemination process is assumed or when a process with serious difficulties is considered. However, much higher rates could be expected in an optimistic scenario, given adoption rates for other *Brachiaria* species, such as *Brachiaria dictyoneura* cv. Llanero and *Brachiaria decumbens*, which register adoption levels of 10.7 and 12.87%, respectively (Labarta et al., 2017). Nevertheless, in order to avoid, as far as possible, the overestimation of potential benefits coming along with adopting the new Agrosavia Caporal variety, we preferred to make more conservative estimates. The total period of diffusion and adoption is 27 years (2022–2048), the maximum adoption rate will be reached in year 20 (2041), and from there on, a constant behavior is assumed.

RESULTS

Cost-Benefit Analysis

Table 5 provides an overview on the per hectare costs and income for both the Agrosavia Caporal and the reference technology. Regarding the direct production costs, the purchase of animals, pasture establishment and labor make up the highest shares.

TABLE 7 | Economic surplus model results (values in thousand US$).

Level	Scenario	Change CS	Change PS	Change TS	NPV	IRR (%)	B/C
Regional	B	1,184	1,979	3,165	903	19	8
	O	2,540	4,246	6,786	1,573	20	18
	P	444	742	1,186	-36	11	3
National	B	7,115	11,893	19,008	5,087	26	50.3
	O	15,261	25,508	40,768	11,342	30	108
	P	1,905	3,184	5,089	1,085	19	13.5

CS: Consumer Surplus, PS: Producer Surplus, TS: Total Surplus.

These three items participate with more than 80% of the total value. The unit cost per kilogram of beef produced was US$1.027 for the Agrosavia Caporal variety and US$1.029 for the reference technology. As a result of the better animal response indicators of the Agrosavia Caporal, the average gross income per year increased by 28% and the net profit by 19%.

The summary of the main financial results of the simulation is presented in **Table 6**. Under the assumptions used in this model, Agrosavia Caporal proves to be financially profitable and allows the improvement of all risk and performance indicators when compared to the reference technology. For Agrosavia Caporal, the model estimates an average NPV of US$328 and an IRR to equity of 21% per hectare. Regarding the probability of not obtaining financial feasibility of the evaluated technologies, **Figure 3** shows the NPV indicator distributions, which reflect the amplitude of its variation. For the reference technology, the indicator could range between US$-90 and US$540, with a probability of obtaining negative values of 13%. For Agrosavia Caporal, the improvement in productivity allows a shift to the right of the distribution curve, reducing the probability of losses to 0%, with values ranging from US$52 to 708.

The contribution of the input variables to the NPV variance is shown in the tornado diagram in **Figure 4**. The correlation coefficients calculated between the input values and the NPV variance show that profitability is affected mainly by two variables: liveweight gain and beef sales price. Increases in these variables have a positive effect on the variability of the indicator as follows: changes in the animal productivity variable modify the variance of the indicator by 89 and 90% for the new variety and the reference technology, respectively. Similarly, changes in the beef sales price lead to changes in the variance of 9 and 6%, respectively. Under the reference price of US$1.24 kg^{-1}, animal productivity below 0.174 tons ha^{-1} year^{-1} (equivalent to a live weight gain of 126 kg AU^{-1} year^{-1}) are not profitable for Agrosavia Caporal. Under the same reference price, the threshold for the base technology is a productivity level of 0.155 tons ha^{-1} year^{-1} (equivalent to a live weight gain of 129 kg AU^{-1} year^{-1}).

Economic Surplus Model

The results of the economic surplus model are presented in **Table 7**. At both the regional and national levels, the potential benefits of Agrosavia Caporal are positive in the three analyzed scenarios. Under the basic scenario, at the regional level, a total benefit of US$3,165,000 is estimated, which represents an internal social rate of return on investments of 19%. At the national level, the results are similar to the ones at regional level, except that their magnitude is greater as a result of the increase in the expected adoption rate and affected production volume. The distribution of benefits is concentrated on the producers, who would receive 62.5% of the surplus. In the absence of international trade, the surplus production generated by the use of the new variety must be absorbed by the domestic market. Given that the demand curve is elastic (E_D =1.17), the new equilibrium point is reached through small price variations, increasing beef sales and producer incomes significantly while reducing consumer prices. The increase in production and reduction in consumer prices, in particular, favor low-income consumers who are more sensitive to price changes and thus contribute to improving food and nutritional security of the population.

Under the optimistic scenario, the new variety could achieve productivity increases of 16%, and cover 3.33% of the total Orinoquía region, respectively 4.2% of the national territory, leading to expected benefits of US$6,786,000 and US$40, 768, 000, respectively. Under this scenario, the investments in the development of Agrosavia Caporal would be very profitable, since the IRR would be >30% and the benefit/cost ratio would indicate that around US$108 are generated from every US$ invested. Under the pessimistic scenario, changes in yields of 12%, a regional adoption rate of 1.11% and a probability of success of 70% were considered, which would yield total benefits of US$1,186,000 for the Orinoquía region. Likewise, the estimated profitability would be 11% and thus lower than the social discount rate of 12%, meaning that the total surpluses generated at the regional level would not be sufficient to compensate the spent R&D costs. These results show a latent risk that the R&D investment spent for developing the material might not exceed the additional benefits and, therefore, in such scenario, an investment would not be recommended. For an investment to become socially and economically profitable, a series of requirements must be met that go beyond the R&D phase and the release of a material with outstanding characteristics, such as the development of efficient technology promotion and dissemination strategies (including the availability of commercial seed, distribution networks, communication strategies and competitive costs) that lead to both higher adoption levels than the projected 1.11% and productivity changes superior than 12%. In addition, since a probability of success of >70% is necessary, it is important that the developed technologies, in addition to their differentiating technical characteristics, are cost efficient and provide sufficient

TABLE 8 | Heat map for the sensitivity of the IRR (total surplus basis) with respect to changes in the adoption rate and productivity level.

		Adoption rate (regional level)									
Change in productivity	18.8%	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
	5%	8.9%	12.5%	14.7%	16.3%	17.5%	18.6%	19.5%	20.3%	21.0%	21.7%
	10%	12.5%	16.3%	18.6%	20.3%	21.7%	22.8%	23.8%	24.7%	25.5%	26.2%
	15%	14.7%	18.6%	21.0%	22.8%	24.3%	25.5%	26.5%	27.4%	28.2%	28.9%
	20%	16.3%	20.3%	22.8%	24.7%	26.2%	27.4%	28.5%	29.4%	30.2%	31.0%
		Adoption rate (National level)									
Change in productivity	26%	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
	5%	14.9%	18.3%	20.4%	22.0%	23.2%	24.2%	25.1%	25.8%	26.5%	27.1%
	10%	18.3%	22.0%	24.2%	25.8%	27.1%	28.2%	29.1%	29.9%	30.7%	31.3%
	15%	20.4%	24.2%	26.5%	28.2%	29.5%	30.7%	31.6%	32.5%	33.2%	33.9%
	20%	22.0%	25.8%	28.2%	29.9%	31.3%	32.5%	33.4%	34.3%	35.1%	35.8%

seed for multiplication. At the national level however, the IRR would be 19% given the higher overall adoption and total production affected by potential yield increases, suggesting that the R&D investment would be profitable at the national level–even under the pessimistic scenario.

To verify the robustness of the estimates for impacts and return on investment estimates, a sensitivity analysis was carried out with respect to the reference scenario. In particular, the variables of maximum expected adoption rate and productivity level were examined. **Table 8** shows heat maps corresponding to the changes of these variables and their effects on the IRR under basic scenario assumptions (probability of success of 80%, 2.8% adoption rate at regional and 2.2% at national level, respectively). The results suggest that, at the regional level, the technology is profitable when productivity increases greater than 5% occur and with an adoption rate of 1%. Although the results of the analysis are clearly sensitive to these two variables, investing in this alternative is highly profitable under most of the assigned values.

DISCUSSION

The material *Brachiaria brizantha* 26,124 cv. Agrosavia Caporal was identified as a promising variety for release, given its good characteristics in terms of nutritional quality, biomass production and persistence during dry season. Planting the variety leads to beef yield increases of around 14% when compared to *Brachiaria decumbens* (reference technology). This is consistent with the findings of Pardo and Pérez (2010), and Lascano et al. (2002), who have shown the potential of integrating new *Brachiaria brizantha* accessions in different areas of the Colombian Orinoquía to increase cattle productivity. These studies conclude that, compared to traditional technologies, the new accessions allow increasing meat production per hectare between 9 and 100%. According to our results, the higher productivity can improve the net returns of beef cattle production at a farm level by an average of 19%, as consequence of higher daily live weight gains, which reduce the length of the fattening cycle and generate faster and more frequent income flows. This translates into better financial indicators compared to the reference technology, with a 79% increase of the NPV and a 16% increase of the IRR, respectively. With an average NPV of approximately US$328 and an IRR of 21%, the technology appears as a viable alternative to improve both efficiency and profitability of the region's cattle farms.

Agrosavia Caporal also presents a reduction in the probability of obtaining economic losses (0 versus 13% for the reference technology), resulting from its higher productivity and lower yield variability (between 199 and 262 $kg\ ha^{-1}\ year^{-1}$). This is essential for regions such as the Orinoquía, where high water seasonality affects cattle production and the general availability of food. The region is projected to experience important difficulties due to climate change, with reductions in annual precipitation as well as increases in maximum temperatures (IDEAM et al., 2015). These increasing risks, coupled with changes in market conditions (e.g., sales and input price variations), substantially affect long-term investment decisions at the producer level, such as the adoption of new technologies. In this sense, forages that can guarantee a lower risk–such as Agrosavia Caporal–provide additional incentives for adoption (Marra et al., 2003). It is important to note that for both evaluated technologies, the productivity parameters used assume adequate pasture management. Inadequate management will inevitably translate into pasture degradation and affect the feasibility of the system, undermining the technology's potential as a promising material and affecting the environment by increasing carbon dioxide (CO_2) emissions. According to Rincón (2006), degraded pastures in the region cause a reduction in beef and dairy production of more than 50%, directly associated with a loss of biomass production, soil compaction, weed invasion and erosion, among others, making it essential to provide training to the primary producer through specific extension and technology transfer programs, focusing i.e., on establishing and maintaining the pasture.

Despite the previously mentioned benefits, pastures under monoculture remain significantly exposed to changes in production and quality throughout the year (Tedonkeng et al., 2007). The association of improved grass varieties with trees and legumes should be promoted as a technological package, since they can reduce heat stress in animals, contribute to increasing pasture persistence (due to nitrogen fixation) and improve the provision of ecosystem services (e.g., contribution of organic matter to pastures, improvement of soil quality and soil carbon accumulation, temperature regulation) (Harrison et al., 2015; Reckling et al., 2016; Dubeux et al., 2017). Cohn et al. (2014) found that policy instruments, such as taxes on cattle from conventional systems or subsidies for production in diversified, more sustainable systems, might be effective methods to promote such technological and cultural changes among farmers and strengthen the long-term sustainability, while reducing greenhouse gas emissions.

At a macro level, the results from the economic surplus model show that, on average, investing in the development of more productive forages, such as Agrosavia Caporal, can be highly profitable from a social point of view, given the significant performance gains and the particular conditions of the cattle sector in both the Orinoquía and Colombia. We found that, if adopted, the forecasted productivity increases obtained with Agrosavia Caporal could generate a shift in beef supply, associated with significant economic benefits. The estimated NPV of the social benefits for the period from 2022 to 2048 would be approximately US$903,000 and US$11.3 million at the regional and national levels, respectively. These results are consistent with other studies that evaluated the impact of improved forage varieties in the country and identified internal social rates of return on investments of up to 100% (Vera et al., 1989; Rivas and Holmann, 2004a, 2004b). The results of the economic surplus model depend mainly on the variables *maximum expected adoption rate* and *productivity*. Under the pessimistic scenario, with an adoption rate of <1.11% (equivalent to 144,000 ha in the Orinoquía) and yield increases of <10%, the R&D investment would become unfeasible at a regional level. This has important implications both the R&D and dissemination processes. The use of new forage varieties that do not provide sufficient benefits at a social level may be economically feasible at a farm level but not justify a new R&D process. Even if reasonably larger productivity and risk reduction gains were to be expected, a strong dissemination process should be ensured so that the expected adoption levels can be reached. This includes a strong seed system that also focuses on communication, information and training. Success in that regard will depend entirely on the capacity of and coordination among institutions, which include actors from the public, private and mixed sectors. To ensure adoption, other barriers that need to be addressed include the access to credit and inputs, land tenure insecurity, market instability and inadequate infrastructure (e.g., Lapar and Ehui, 2004; Wunscher et al., 2004; Dill et al., 2015).

Regarding the social distribution of potential benefits, our study shows that they are mostly concentrated in the primary sector (supply side). Within the primary sector, it is not clear, however, how these benefits will be distributed among or concentrated within different segments (e.g., small, medium or large producers). Given that the micro level analysis reveals that the investment can be feasible even at minimum scales (1 ha), and considering the producer typology in the Orinoquía (53.4% of the producers have <50 animals (ICA, 2020)), we assume a large share of the potential beneficiaries will be small producers. These results, however, may be ambiguous: Labarta et al. (2017) describe a direct relationship between the adoption of improved forages in the region and the access to resources (e.g., credit, labor, level of wealth), making resource-rich producers the main group of potential adopters. Yet at the same time, when it comes to actual adoption, large producers are described as less likely to adopt, presumably due to scale limitations, security concerns, and lack of infrastructure. To the above-mentioned considerations, a series of structural factors can be added, such as land prices or local wage levels, that may or may not encourage the adoption of improved forages in the region.

Regarding environmental aspects, greenhouse gas emissions and deforestation are the main concerns for the Orinoquía cattle sector, with widespread adoption of improved forages potentially contributing to generating positive outcomes. But these improved forages also pose additional challenges and risks. Cattle production is one of the main sources of greenhouse gas emissions, resulting from the ruminant digestion process that generates methane (CH_4) and nitrous oxide emissions (CIAT and CORMACARENA, 2018). Higher quality forages allow increasing animal productivity and feeding efficiency (conversion of forage to animal protein), reducing CH_4 emissions per unit of product (Knapp et al., 2014; Zubieta et al., 2021). Cardoso et al. (2016) estimate that increased quality and quantity of forage can potentially decrease greenhouse gas emissions per kg carcass weight by 50%, principally resulting from a reduction of CH_4 emissions. The expansion of areas for cattle production is one of the main drivers of deforestation, a process that also generates high amounts of greenhouse gas emissions and is particularly problematic in the Orinoquía region, which holds various key ecosystems, such as natural savannas, flooded forests, humid forests or foothills (CIAT and CORMACARENA, 2018). In this regard, the effects of increasing productivity of agricultural systems on forest conservation can be ambiguous: it can incentivize the expansion of production in the agricultural Frontier through the clearing of forest areas, but it can also be used as an indirect tool to reduce the pressure of expanding the agricultural Frontier, an idea known as the Borlaug effect.

In the Orinoquía, the introduction of *Brachiaria* grasses since the end of the 1960s (*Brachiaria decumbens*, Rincón et al., 2010) has been a subject of debate, mainly in environmental terms. The adoption of these varieties occurred spontaneously and massively by the producers and was associated with several desirable traits that increased productivity, such as a high biomass production and nutritional quality, adaptation to marginal lands and low fertility soils (Rao et al., 1998). Different studies for the region have reported that the adoption of *Brachiaria* varieties resulted in productivity increases from 18 to 37 kg ha^{-1} $year^{-1}$ (no adoption) to 294–402 kg ha^{-1} $year^{-1}$ (with *Brachiaria*), resulting in important impacts at the productive, economic, environmental and social levels (Pérez and Vargas, 2001; Rincón et al., 2010). Positive impacts include the reduction of land degradation and pressure on the native savanna, methane emissions reductions due to increased feeding efficiency, greenhouse gas emissions reductions associated with native savanna burning (Smith et al., 1997), better soil cover and improved soil quality parameters (better water infiltration and reduced soil erosion), and higher nitrogen and carbon fixation to the soil (Boddey et al., 1998). These positive impacts are, however, often conditioned to the (proper) management of the pastures. Negative impacts are mainly associated with the degradation of native savannas, threats to biodiversity, soil erosion, deforestation for expanding grazing areas and increased greenhouse gas emissions (Peñuela et al., 2011; Peñuela et al., 2014; CIAT and CORMACARENA, 2018). Various studies evaluated the conditions in which both scenarios are more likely to occur. In Brazil (Cohn et al., 2014), and De Oliveira Silva et al. (2016) have estimated a large greenhouse gas mitigation potential through cattle ranching intensification when coupled with no deforestation scenarios, taxes on conventional pastures and subsidies for semi-intensive systems. Some studies have found that land use changes derived from agricultural intensification are strongly linked to the characteristics of a particular area and the land tenure conditions. Decreasing deforestation patterns were found when intensification occurs in consolidated agricultural regions, and increasing deforestation when it occurs on marginal lands (Maertens et al., 2006; Barretto et al., 2013) and land with unclear land tenure (Kubitza et al., 2018). A meta-study of 60 cases conducted by Rasmussen et al. (2018) found that there are scant cases where agricultural intensification has had simultaneously a positive effect on well-being and ecosystem services. These studies suggest that holding the sustainability claims of cattle ranching intensification would likely require a combination of various policy and market mechanisms, such as effective monitoring and control, law enforcement, taxes, subsidies and land tenure rights, among others. In areas where land is not a constraining factor, as is the case of the Colombian Orinoquía, there is a greater pressure to expand, making this a major threat and topic to consider. While there are initiatives in the country seeking to prevent deforestation derived from the cattle sector (such as the National Zero Deforestation Agreements), it is still too early to provide evidence that can support their effectiveness, and further research is advised.

As mentioned in the methodology section, our evaluation is based on a partial equilibrium model and does therefore neither include potential impacts on other economic sectors nor on natural resources. Our study demonstrates, however, the importance of new pasture technologies, their high potential to produce social benefits, and the need to develop mechanisms to take advantage of this potential. Both our study and other previously conducted ex-ante studies (reviewed at the beginning of this document), were carried out after the investments in R&D have already happened and just before the release of the particular technology. It is recommended, however, to conduct such studies before making decisions on R&D investments, so that the results can serve in the decision-making process and for the allocation of ever scarce funds. Despite this, our results still provide insights into the potential benefits at the regional level and serve for justifying future R&D processes of new forage varieties for other regions of the country. When interpreting our results, it is important to bear in mind that the economic surplus model used is a minimum data approach that simplifies reality. Given data limitations, production estimates affected by technical change are based on average yields at the regional and national levels. Likewise, the model assumes that yield increases are the same for all producers, without considering existing heterogeneities among them, e.g., in technological terms. Transaction costs that occur once the variety is released, i.e., related to its adoption, dissemination and promotion, and that are assumed by the private seed sector were ignored in our study, since they are not part of the publicly-funded R&D process. These simplifications can lead to an overestimation of the estimated net benefits. To mitigate such limitations, we made conservative estimations based on expert consultations. Our model does not consider additional benefits that could derive from, e.g., an increase in milk production (since we evaluated the technologies in a dual-purpose system) and other technical parameters in the region (e.g., interval between births, birth rates). Nevertheless, these could substantially increase the benefits of the new variety for the region. Hence, research should be conducted to quantify such additional benefits.

As mentioned before, the variety Agrosavia Caporal is the third *Brachiaria brizantha* variety released in the area after Toledo and La Libertad. These cultivars, together with the new variety, are materials with characteristics superior to the traditional technology predominantly used in the area (*Brachiaria decumbens*). There are, however, differences between them in both desirable forage characteristics and limitations. Toledo, for example, has shown to present better dry matter yields compared to Agrosavia Caporal (Lascano et al., 2002), and better characteristics in terms of tolerance to humidity, recovery after grazing, and vigor of the plant compared to La Libertad (Lascano et al., 2002). Agrosavia Caporal, on the other hand, has shown resistance to different species of spittlebug, while Toledo and La Libertad are more susceptible (Lascano et al., 2002), and has better palatability and drought tolerance in the dry season (A. Rincón, personal communication, August 06, 2021). In this sense, they are materials with differentiating characteristics that could also have different economic impacts associated with their adoption. It is recommended, therefore, to evaluate each of these technologies to determine their viability in terms of R&D and to identify the forage attributes that could have the greatest economic impact.

CONCLUSION

Our study shows the economic feasibility both at the primary producer level and at the social level of adopting a new forage technology with superior productive characteristics. The new Agrosavia Caporal variety, which will be released in 2022, shows very good animal response parameters that increase the economic viability of cattle raising and fattening systems in the Colombian Orinoquía region. At the social level, technology adoption could generate an outward shift in the supply of meat, which would be associated with important benefits at both the regional and national levels. However, the potential success of Agrosavia Caporal, as well as of other potential new varieties with superior characteristics, is highly conditioned to the adoption level and to proper technology management that allows maintaining expected productivity levels. Therefore, it is essential to develop adequate support mechanisms during the release and adoption process, in order to provide farmers with solid extension strategies and training programs that focus, for example, on planting and cultivar management. Likewise, it is crucial that commercial seed availability of the material is guaranteed in the release, adoption and diffusion processes.

The cattle sector in the Colombian Orinoquía region is not only important at an economic or social level but also plays a significant role at an environmental level. It is recognized for being one of the main contributors to the country's greenhouse gas emissions, and one of the main drivers of deforestation, affecting the different strategic ecosystems present in the Orinoquía. The sector is also highly dependent on and affected by water seasonality, a situation that could further aggravate under the forecasted climate change scenarios for the region. Sustainable intensification of the cattle sector is considered to be the route to reducing negative environmental impacts while improving per area productivity, and forages with superior characteristics play an important role in this sense. The inclusion of trees and legumes in cattle systems, which improve the provision of ecosystem services and animal welfare, however, should be considered as add-on in order to move towards more sustainability and away from grass monocultures. The superior nutritional characteristics of Agrosavia Caporal can have positive effects on the environmental impacts of the local cattle systems. Reduced CH_4 emissions and the release of areas can be expected, given the higher intensification and better digestibility. In order to achieve the economic, social and, above all, the environmental benefits of this new technology, coordinated efforts of the involved actors will be required. Extension campaigns need to provide information on the importance of sustainable intensification (focused on liberating areas for conservation) and conserving strategic ecosystems present in the region. Public policies and monitoring systems are needed in order to prevent an unwanted spread of the new technology (and any other new technology in the future) to protected areas or ecosystems of the region.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data is from another project/institution and still restricted. Requests to access these datasets should be directed to SB, s.burkart@cgiar.org

AUTHOR CONTRIBUTIONS

SB, KE, AC, and ARC: Conceptualization. KE, AC, ARC, and SB: Methodology. KE, AC, and ARC: Formal analysis. KE, AC, ARC, and SB: Writing the original draft and review and editing. AC, KE, ARC, and SB: Resources. SB: Supervision and funding acquisition. SB: Project administration. All authors contributed to the article and approved the submitted version.

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Promoting Forage Legume–Pollinator Interactions: Integrating Crop Pollination Management, Native Beekeeping and Silvopastoral Systems in Tropical Latin America

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Major declines of insect pollinators are a worldwide concern. Such losses threaten human food supplies and ecosystem functions. Monocultures of pastures used to feed cattle are among the drivers of insect pollinator declines in Tropical Latin America. Plants of the legume family (*fabaceae*) are mostly pollinated by insects, in particular by bees. The inclusion of legumes in pastures (grass-legume system), as forage banks or the development of silvo-pastoral systems (SPS) with tree legumes, has been widely promoted to improve livestock production and soil fertility, but not to enhance ecosystem services from pollinators. Shortages of seed for the establishment of legumes as forage banks or within pastures or SPS remain a bottleneck for the improvement of ecosystem services brought about by pollinators within these systems and beyond. In this perspective paper, we provide an overview of forage legumes, their interplay with pollinators, and the ecological and socio-economic benefits of pollinator–forage legume interactions, at different scales (farm and landscape level). We further discuss the challenges and opportunities of scaling sustainably intensified cattle production systems that integrate legume forage-seed production with principles of pollinator ecology and native beekeeping. Finally, we provide interested stakeholders, policy-and decision-makers with a perspective on how such agroecosystems may be designed and scaled into multifunctional landscapes.

Keywords: sustainable intensification, silvo-pastoral systems, cattle, forage legumes, meliponiculture, ecosystem services, pollinators, nature-based solutions

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INTRODUCTION

There is a growing demand for livestock products (Bernabucci, 2019). Intensification of cattle production systems (i.e., increase in production per unit of available resource) is proposed to meet market requirements (Sakamoto et al., 2020), increase economic returns and reduce environmental impacts (Cassman and Grassini, 2020) including land use (Martha et al., 2012) and greenhouse gas (GHG) emissions (Eckard et al., 2010; Herrero et al., 2013; Ruviaro et al., 2015; Cardoso et al., 2016). Traditionally, cattle production systems in Tropical Latin America rely on grazing animals that feed upon planted or naturalized pastures. For intensification in this region, pastures tend to be dominated by a single species of a high yielding grass (da Silva et al., 2020), and subject to practices aimed to improve their productivity and nutritional quality. This includes aspects such as grazing management and the application of fertilizers, herbicides and pesticides (Gerssen-Gondelach et al., 2017). However, evidence indicates an association between intensified pastures and biodiversity loss (Bobbink et al., 2010; Fontana et al., 2016), including the decline of insect pollinators (Potts et al., 2010).

Insect pollinator decline is a major concern. Overcoming this declination is essential for global food security and ecosystem functioning (Van der Sluijs and Vaage, 2016; Van der Sluijs, 2020). The inclusion of legumes (*fabacea*) is a nature positive action to increase plant diversity within a pasture. Most legumes are pollinated by insects (Suso et al., 2016), suggesting that legume inclusion in pastures might provide ecosystem services coming from pollination. Orford et al. (2016) showed that modest enhancements to pasture diversity can improve the provision of pollination services to surrounding habitats. Furthermore, the inclusion of legumes brings other benefits to improve the efficiency and sustainability of cattle production systems (*see* sustainable intensification of livestock production systems, Rao et al., 2015). Benefits of legumes introduction include: (1) increases in quantity and quality of livestock feed and (2) soil improvement as a result of biological nitrogen fixation, soil stabilization and nutrient recycling (Schultze-Kraft et al., 2018). There is a wide variety of legumes available for cattle production (i.e., forage legumes). Forage legumes can be annual or perennial plants with different growth habits and various forms (i.e., herbaceous, shrub and tree legumes). The use of legumes in cattle production systems is not restricted to their inclusion in pastures as a grass-legume system. They are also used as forage banks (i.e., plant material used to supplement animal diets) or within silvopastoral systems (SPS).

SPS consist of diverse agroforestry arrangements that combine herbaceous plants, shrubs and trees for animal nutrition and complementary uses like timber or fruit production (Murgueitio et al., 2011). In particular, SPS with tree legumes are a promising nature-based solution to reduce the environmental impact of cattle production, while increasing its productivity, especially in Latin America (Dubeux et al., 2017; Chará et al., 2018; Landholm et al., 2019; Arango et al., 2020; Lira Junior et al., 2020). SPS arrangements might be in the form of scattered trees in pastures, pastures within tree alleys, living fences and windbreaks surrounding a pasture, to name a few (Murgueitio and Ibrahim, 2001; Murgueitio et al., 2011; Chará et al., 2018). SPS promote biodiversity by creating complex habitats that support a diverse above-ground flora and fauna, harbor a richer soil biota and improve connectivity between forest fragments (Ibrahim et al., 2006; Cubillos et al., 2016). At a landscape level, they provide more ecosystem services than open pastures (Calle et al., 2009; Murgueitio et al., 2011). In Brazil, the conversion from pasture monocultures to SPS has increased the abundance, richness and diversity of insects, including pollinators (Auad et al., 2015; Paiva et al., 2020).

Through its Global Action on Pollination Services for Sustainable Agriculture, the FAO has joined efforts with governments, research institutions and academia to coordinate the global implementation of the International Pollinator Initiative (IPI) (FAO, 2021). The IPI's plan of action offers guidelines for the improvement and development of practices that promote the conservation and sustainable use of pollinator diversity, restoring pollinator habitats in agriculture and related ecosystems (Byrne and Fitzpatrick, 2009; CBD, 2018). Since its launch at the 5th COP of the Convention on Biological Diversity in 2000, the IPI has catalyzed the development and implementation of several other initiatives both at the regional (e.g., the African Pollinator Initiative) and national (e.g., the Brazilian and Colombian Pollinator Initiatives) levels. For instance, the Colombian Pollinator Initiative (CPI) recognizes the contribution of pollination services to food security through the role pollinators play in the production of both crops and livestock, also identifying the expansion of cattle ranching as a major threat to pollinator habitats (Nates-Parra, 2016), building on a national strategy for the conservation and sustainable use of pollinators. Currently, Colombia's National Congress is considering a bill that establishes mechanisms for the conservation of pollinators and fosters the husbandry of native bee species. Although not explicitly stated in the CPI, its roadmap presents an opportunity for pursuing synergies with Colombia's COP21 Nationally Appropriate Mitigation Actions (NAMA) for the cattle sector.

Aligning national and/or regional pollinator initiatives with national efforts to reduce GHG emissions from cattle production may contribute to the 2030 Agenda for Sustainable Development. Pollinators can indeed be protected (i.e., UN-SDG 15: Life on land), by taking climate protection and adaptation concepts into account (i.e., UN-SDG 13: Climate action), while generating opportunities for employment and additional income in rural areas (i.e., UN-SDG 8: Decent work and economic growth) and pursuing other sustainable development goals. Costa Rica's Cattle NAMA, for example, seeks to achieve an eco-competitive sector that reconciles the goals of employment generation, biodiversity conservation and gender equality (UN-SDG 5) through the implementation of SPS (Ministerio de Agricultura y Ganadería, 2019). It recently completed its pilot phase, which preceded a first scaling effort aimed at reaching 5% of Costa Rica's cattle farms. By 2030, Costa Rica expects up-scaling to 27% of its farms (Climate Clean Air Coalition, 2020). NAMAs are one of several public policies that have seen advances promoting SPS as a silver-bullet solution for the sustainable intensification of the cattle sector, such as has been the case of Colombia (Ministerio de Agricultura y Desarrollo Rural, 2019, 2020), Argentina (Presidencia de la Nación Argentina, 2018) and Costa Rica (Ministerio de Agricultura y Ganadería, 2011). A limited availability of legume seed, which depends on animal-mediated pollination for its production, may nevertheless hamper scaling efforts for sustainably intensified cattle systems and thus limit their potential to deliver ecological, environmental and socioeconomic benefits at larger scales (Rao et al., 2015; Rubyogo et al., 2019; Arango et al., 2020).

This perspective paper provides an overview of forage legumes and agroecosystem management tools, available to cattle systems for the conservation of insect pollinators, optimization of crop-pollination services and tackling legume forage-seed bottlenecks. We discuss the opportunities and challenges of integrating principles of pollinator ecology and native beekeeping into SPS and artisanal and large-scale propagation of legume forage-seeds. Finally, we provide interested stakeholders, policy- and decision-makers with a perspective on how such agroecosystems may be designed as mosaics or scaled into multifunctional landscapes. This article is structured as follows: The Forage Legume Seed Bottleneck section provides an overview on the limitation that a forage seed bottleneck currently imposes on the widespread adoption of SPS and grass-legume systems, and the role that pollinators can play in tackling this challenge. In Benefits of Bee Pollination on Legume Seed Production section, we present a list of interventions that can be implemented at the farm and landscape levels. We continue in the Proposed Interventions Section discussing macro-level conditions required to enable the implementation and guarantee the sustainability of the proposed interventions. Finally, the Required Enabling (Macro) Conditions Section offers concluding remarks and recommendations.

THE FORAGE LEGUME SEED BOTTLENECK

The benefits of introducing forage legumes into cattle production systems have been highlighted in numerous occasions (Schultze-Kraft et al., 2018 and references therein). However, widespread adoption of forage legumes in Tropical Latin America is very low (see Muir et al., 2017). Seed scarcity is one of the reasons limiting a wider use of forage legumes into cattle production systems in Tropical Latin America. This hinders the implementation of more sustainable, yet intensified, cattle production systems in the region. Several projects, such as the Sustainable Colombian Cattle Project, support and promote the use of SPS through establishing pilot/reference farms for scaling, and although these projects have made significant advances, e.g., the establishment of 35,500 hectares of SPS in Colombia (Ganadería Colombiana Sostenible, 2018), once they end, a widespread adoption of these systems may be limited by legume seed scarcity.

Even though there is a strong private tropical forage seed sector in Brazil and Mexico, its focus is set on *Gramineae* seed production, which leaves legume seeds largely neglected. This bottleneck could thus be tackled by having these companies add legume seeds to their portfolio. Alternatively, artisanal on-farm legume seed production could be integrated into the overall design of sustainably intensified systems (Peters et al., 2003; Chakoma and Chummun, 2019; Philp et al., 2019; Rubyogo et al., 2019), taking advantage of the numerous possible interactions between legume cultivars and local plant-pollinator networks (Palmer et al., 2009; Boelt et al., 2015; Suso et al., 2016; Cong et al., 2020). This approach offers the potential added benefit of income diversification and employment creation among smallholders.

BENEFITS OF BEE POLLINATION ON LEGUME SEED PRODUCTION

The role of pollination in legume seed formation and yield depends on these plants' species-specific reproductive systems. While many forage legumes require insects (i.e., bees) for their pollination (i.e., out-crossing plants), others, including most tropical forage legumes, are self-pollinating (Kumar et al., 2020). Many self-pollinating legumes, however, exhibit an increased seed formation when their flowers are visited by bees (Palmer et al., 2009).

With the exceptions of trees in SPS, both forage banks and grass-legume systems are intensively managed to minimize flowering of plants (i.e., no pollination service). In contrast, the set-up of legume seed production sites allows the creation of gardens for wild and managed bees (i.e., both introduced, such as *Apis mellifera,* and native). Tropical forage legumes are numerous and highly diverse (see www.tropicalforages.info; Cook et al., 2020). The large diversity of tropical forage legumes allows the design of diverse garden blends that can provide a rich source of nectar and pollen for bees. The inclusion of several forage legumes for seed production can also support differences in flowering times, thereby offering foraging sites throughout the year for a higher bee diversity. Pollination gardens are a doublee win, since they (i) enhance the abundance, diversity, and community composition of bees and other pollinators, whose populations are threatened to decline due to agricultural intensification (Kovács-Hostyánszki et al., 2017) and climate change, especially in the tropics (Forrest, 2017); and (ii) increase pollinator visitation rates of bees to legume flowers, resulting in higher seed yields (Suso et al., 2016). **Table 1** offers a list of herbaceous and tree legumes known to be self-pollinated but with increased out-crossing when visited by different bee species.

PROPOSED INTERVENTIONS

Table 2 presents various potential interventions at different levels (farm to landscape) and sectors (private and public) with the aim to promote the use of legumes as a nature-based solution that facilitate pollination services from insects, whilst allowing sustainable intensification of cattle production systems. Furthermore, these interventions allow the creation of seed production enterprises and different revenue avenues (e.g. meliponiculture).

REQUIRED ENABLING (MACRO) CONDITIONS

National Development Plans and other policies, e.g., in Colombia, Argentina or Costa Rica, increasingly outline the need for establishing SPS and other legume-based options as strategies for sustainable intensification of cattle farming, creating a demand for forage legume seed production (Ministerio de Agricultura y Ganadería, 2011; Presidencia de la Nación Argentina, 2018; Ministerio de Agricultura y Desarrollo Rural, 2019, 2020). Such demand is crucial for establishing large-

TABLE 1 | List of herbaceous and tree legumes known to be self-pollinated but with increased out-crossing when visited by different bee species.

Species	Plant growth habit	References for out-crossing legumes	Pollinating bee species; bee species relevant for meliponiculture? (Yes/No)	Interaction type
Cajanus cajan	Herbaceous	Saxena et al. (1994)	Pollinating bee spp. unknown to the authors	
Centrosema spp.	Herbaceous	Spears (1987), Miles et al. (1990), Maass and Torres (1992, 1998)	*Centris (Centris) aenea, Centris (Hemisiella) trigonoides, Centris (Centris) flavifrons, Centris (Trachina) sp.* (No)	Flower visitations with no reference to specific floral resource
Chamaecrista rotundifolia	Herbaceous	Maass and Torres (1998)	*Xylocopa frontalis*; (N)	Foraging for pollen
Desmodium spp.	Herbaceous	Hutton (1960), Rotar and Urata (1967), Quesenberry et al. (1989)	*Centris (Hemisiella) tarsata, Thygater aethiops;*(No)	Flower visitations with no reference to specific floral resource
Gliricidia sepium	*Tree*	Dawson et al. (1997), Srinivasa Rao et al. (2011)	*Xylocopa frontalis;* (No)	Foraging for pollen
			Bombus pullatus; (No)	Flower visitations with no reference to specific floral resource
			Melipona favosa, Tetragonisca angustula; (Yes)	
Codariocalix gyroides	Herbaceous	Maass and Torres (1998)	Pollinating bee spp. unknown to the authors	
Dicorynia guianensis	Tree	Latouche-Hallé et al. (2004)		
Dinizia excelsa	Tree	Dick et al. (2003)		
Galactia striata	Herbaceous	Nogueira Couto et al. (1997), Maass and Torres (1998)		
Indigofera spacitata	Herbaceous	Hutton (1960)		
Lablab purpureus	Herbaceous	Kukade and Tidke (2014)		
Leucaena leucocephala	Tree	Hutton (1981)		
Neonotonia wightii	Herbaceous	Hutton (1970)		
Platypodium elegans	Tree	Murawski and Hamrick (1991)		
Platypodium elegans	Tree	Hufford and Hamrick (2003)		
African *Trifolium* spp.	Herbaceous	Pritchard and t' Mannetje (1967)		
Senna multijuga	Tree	Ribeiro and Lovato (2004)		
Stylosanthes spp.	Herbaceous	Miles (1985), Santos-Garcia et al. (2011)		
Tachigalia versicolor	Tree	Loveless et al. (1998)		
Tachigalia versicolor	Tree	Murawski and Hamrick (1991)		
Vouacapoua americana	Tree	Dutech et al. (2002)		

The names of the bee species and the corresponding interaction types that are listed on this table were obtained from Nates-Parra (2016).

or small-scale seed production systems that integrate local plant-pollinator networks. These policies, however, lack the inclusion of pollinators and the ecosystem services they provide. Likewise, payment schemes for ecosystem services, such as for the establishment of SPS (e.g., Diaz et al., 2019a,b), do not include forage legume seed production models and pollinator ecosystem services.

Sustainable intensification strategies are a subject of algid debate. Despite the positive impacts of incorporation of forage legumes on cattle production systems (e.g., GHG emission reductions, animal welfare, biodiversity or land sparing) (Jansen et al., 1997; Rivas and Holmann, 2000; Peters et al., 2001; Valentim and Andrade, 2005; Enciso et al., 2019), an increased profitability of the system could be a driver for further expansion of the agricultural frontier at the expense of forests or protected ecosystems (Kaimowitz and Angelsen, 2008; Peñuela et al., 2011, 2014; CIAT and Cormacarena, 2017). This is likely to happen on marginal lands (Maertens et al., 2006; Barretto et al., 2013), cheap lands (White et al., 2001) or where land tenure is unclear (Kubitza et al., 2018). To counteract such developments, public policies (e.g., the Zero-Deforestation Agreements in Colombia and Brazil or the Brazilian Forest Code) (Presidência da República, 2012; Gibbs et al., 2015; FAO, 2016; Alianza Colombia TFA, 2021), safeguards and comprehensive monitoring/control mechanisms are required. Other instruments such as taxes, subsidies and land tenure rights are also needed (Cohn et al., 2014; de Oliveira Silva et al., 2016).

Investing in sustainable intensification strategies, smallholder legume seed production systems and meliponiculture require access to credit and inputs. Some advances stand out, such as credit lines destined to the establishment of SPS in Colombia (Ministerio de Agricultura y Desarrollo Rural, 2020). However, more access to credit is still missing for the establishment of seed multiplication plots and integrated meliponiculture. Resolving this bottleneck is crucial for assuring continuous seed supply, ecosystem services and the scaling up of SPS. Supporting the

TABLE 2 | List of potential interventions considering legume-pollinator interactions.

Intervention	Description	Potential benefits
Farm-level interventions		
Smallholder on-farm legume seed production	For own intensification purposes or as a business model to supply other producers who are intensifying or renewing their systems. Small-holder on-farm legume seed production should take account of local knowledge (i.e., the use of already present legumes in a particular area combined with local knowledge of the given species). Seed production systems should also consider the processes of selection, conservation and exchanging of locally adapted legumes by local farmers	Income diversification and additional income (seed sales), support of sustainable intensification (scaling), provision of habitats for pollinators (ecosystem services), employment creation and opportunities for women and rural youth (preventing migration to cities)
Integrated crop pollination (ICP)	Organizing framework that structures the development and evaluation of efficient and flexible crop pollination strategies around the use of managed pollinator species in combination with farm management practices. It focuses on integrating and diversifying pollinators, after balancing the pros and cons of using a single managed bee species, or mixtures of managed bee species and/or wild pollinators. In addition to the use of wild and managed bee species, ICP encompasses various strategies that enhance the farm environment for pollinators, including directed habitat management and pesticide stewardship. These strategies can be combined and adapted to the economic constraints of each specific farm by using decision support tools that consider crop value, yield benefits and the costs of adopting each alternative ICP component and practice Garibaldi et al., 2017; Isaacs et al., 2017	Maximization of economic returns from pollinator-dependent crops, resilience to crop-pollination threats, additional income from hive product revenues, benefits from other enhanced farmland ecosystem services, reduced health risks from occupational and dietary exposure to pesticides
Meliponiculture and other forms of traditional beekeeping	In addition to the introduced European honeybee (*A. mellifera*), other bees that can be managed for their hive products and crop pollination include many stingless bee species (Hymenoptera: Apidae: Meliponini), which constitute the most diverse group of eusocial tropical bees, the Asian honeybee (*A. cerana*) and a few Bombus species that are only reared for their crop pollination services. The integration of meliponiculture (i.e., keeping and managing native stingless bee species) in legume seed production systems can benefit farmers directly, through revenues from selling hive products, and indirectly due to an improved crop pollination, including that of legume forages. Beekeeping may also help raise the awareness of farmers with regard to the importance of adopting pollinator-friendly farm practices (e.g., sowing annual flowering plant strips to offer floral resources for pollinators throughout the year, integrated pest management, reduced insecticide application and minimizing pollinator poisoning by limiting insecticide applications to periods of low pollinator activity)	Income diversification and additional income (hive products, legume seed sales and increased yields of other pollinator dependent crops), home production and consumption of honey and propolis with characteristic physicochemical properties linked to traditional medicine, preservation of traditional knowledge and practices, employment creation (including the establishment of a local industry of handcrafted wooden beehives and the commercialization of other beekeeping supplies), benefits from other enhanced farmland ecosystem services and opportunities for women and youth in rural communities, which can help preventing rural exodus
Silvo-pastoral systems	The versatility of SPS allows matching plant functional groups–including multiple leguminous herb, shrub and tree species–with pollinator functional groups Fontaine et al., 2006; Woodcock et al., 2014	Promote biodiversity and enhance ecosystem services beyond carbon sequestration Phelan et al., 2015; Suso et al., 2016; Wu et al., 2017; Otieno et al., 2020
Public and private sector interventions		
Landscape restoration approach	The interventions presented above can be implemented at the farm level, yet pollinators are mobile organisms with foraging behaviors that cover distances between a few hundred meters to several kilometers. They are thus affected by the availability of resources and nesting sites at the landscape scale Pufal et al., 2017. The ecological effectiveness of the proposed interventions can therefore be maximized by integrating them into SPS that are planned, co-designed, coordinated and implemented at the landscape scale with the participation of local communities, local administrations, ecological restoration experts and environmental authorities. The versatility of legume-based SPS systems (e.g., with a high densities of tree legumes in combination with herbaceous legumes and with improved grasses) makes them especially suitable to restore the connectivity of fragmented landscapes, as their components (e.g., live fences, scattered trees and riparian buffers) can be arranged to provide ecologically important structural elements, such as connectivity corridors and hedgerows, thereby creating complex habitats for other wild animals and plants Murgueitio et al., 2011; Chará et al., 2019	Biodiversity conservation, supply of multiple ecosystem services that include improved local climate regulation and protection, water availability and a diverse cultural landscape with potential touristic attractiveness.
Large scale legume seed production through the private seed sector	As a company business model or through the integration of smallholder seed producers	Support of sustainable intensification (scaling), standardization of seed quality, provision of habitats for pollinators (ecosystem services), employment creation, opportunities for women and rural youth (preventing migration to cities)

organization of both cattle and seed producers could help in facilitating credit access and coordination of investment efforts. Likewise, the development of payment schemes for ecosystem services, incentives or new value chains with differentiated products (e.g., sustainable beef, honey) (Charry et al., 2019) could contribute to financing such investments at the farm level. Another financing model could be a cooperation amongst seed producers/beekeepers and companies who wish to green their image and are willing to finance the establishment of local seed production plots with integrated meliponiculture.

The establishment of seed multiplication plots, seed marketing and beekeeping also require access to different knowledge sets, such as legume seed production, treatment and marketing, beekeeping, and honey production, or product differentiation. Already existing knowledge should be integrated into the rural extension system, which also needs to be strengthened in reach and content (i.e., harmonization of different approaches to assure homogeneity of concepts and avoid confusion among producers) (Bravo et al., 2018; Charry et al., 2018; Enciso et al., 2018). Knowledge that helps to put the innovations into practice and facilitates scaling processes should be generated through research, i.e., regarding the adaptation to and selection of legumes for specific agro-ecological conditions and seed production, bee species for integrated meliponiculture, the ecology of plant-pollinator interactions, or pollinator diseases and invasiveness. Likewise, research should focus on the additional environmental and productive benefits of legume seed production with integrated meliponiculture, e.g., regarding GHG emissions, biodiversity, soil health, profitability or risk.

There is a vast diversity of forage legumes, of which a sample is safeguarded in the CGIAR gene banks (i.e., over 22,000 accessions of 72 species). Although the CGIAR gene banks hold the world's largest collection of tropical forage species (Alliance of Bioversity International-CIAT, 2020), this remains as a largely unexplored source of genetic material, key for the evaluation of legumes for sustainable intensification scenarios, seed production and integrated meliponiculture.

Regarding meliponiculture, legislation and codes of practice, such as those established by Colombia's Corporation for the Sustainable Development of the Southern Amazon (Corpoamazonia, 2016), must be set in place and enforced in order to avoid the overexploitation of native stingless bees, while promoting their sustainable use and propagation by smallholders and beekeepers. This is important considering the threat that the extraction and relocation of stingless bee colonies from their habitats imposes to their wild populations, not least because of the spatiotemporal dynamics of the parasites and diseases they carry. Additionally, research efforts need to be directed at harmonizing quality standards and export requirement specifications for the diversity of stingless bee honeys, in order to meet their increasing global demand as food and/or medicine, which could be seen as an additional opportunity for improved and diversified rural livelihoods.

Compared to grass monoculture pastures, which when largely expanded are associated with a homogenized vegetation and the application of insecticides and herbicides, silvo-pastoral systems improve biodiversity and offer promising results regarding the restoration of habitats and pollinator populations in agroecosystems, especially if combined with integrated crop pollination and native beekeeping. Nevertheless, research and adaptive farm management efforts should be considered for each agroecological context in order to leverage the potential pollinator conservation synergies from the interaction between traditional management practices and the natural regeneration processes of legume populations in legume-based silvo-pastoral systems.

CONCLUDING REMARKS AND LOOK FORWARD

The development of pollinator friendly environments, based on forage-legumes and SPS and their introduction into cattle systems, brings several benefits, including the (i) provision of habitats for pollinators on decline, and (ii) promotion of legume seed yield considered as barrier to the wider adoption of grass-legume, forage banks, or tree legume systems such as SPS. Higher seed yield makes it easier for seed producers to establish a business model to supply others to intensify or renew their forage-based cattle systems. It also allows the creation of different revenues such as those coming from bee farming (i.e., meliponiculture). The benefits from the interplay of pollinators and forage legumes can be further extended to the landscape level, affecting positively the yield of nearby pollinator-dependent crops. Furthermore, benefits of pollinators from cattle production systems can extend upon nearby ecosystems that might be fragmented or under decline due to several factors. For these pollination-based benefits to occur, enabling conditions, including policies, payment schemes for ecosystem services, incentives or new value chains, must be in place.

Seed availability is a bottleneck for the inclusion of legumes in cattle production systems at scale. It is noteworthy, however, that small scale cattle producers in Tropical Latin America often use and conserve native legumes in their production systems. These small-scale producers can be considered guardians of legume diversity and related knowledge (e.g., management and synergies/antagonism between grasses and legumes). Sadly, this knowledge is often neglected by top-down approaches driven by researchers or business interests. To counteract this shortcoming, approaches are needed that recognize small cattle producers' knowledge, and that foster their strategies for integrating legumes into their local farming systems in a sustainable and profitable manner. Likewise, increasing the forage legume seed availability might not result in impacts at scale unless measures are introduced and disseminated among farmers to ensure pasture management that favors the inclusion of legumes. In this sense, research and incentives are needed regarding, for example, rotational grazing and grazing pressure, weeding, burning, the use of agrochemicals, and the selection of *Gramineae* compatible with legume species.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MN, JC, and SB: conceptualization, methodology, formal analysis, writing the original draft and review and editing, and resources. SB and JC: supervision and funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

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Risk Reduction and Productivity Increase Through Integrating *Arachis pintoi* in Cattle Production Systems in the Colombian Orinoquía

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In many parts of the foothills of the Orinoquía region of Colombia, cattle production takes place on poorly drained soils. The region is dominated by extensive grazing systems of *Brachiaira humidicola* cv. Humidicola, a grass with high adaptation potential under temporal waterlogging conditions. Inadequate management practices and low soil fertility result in degradation, however, with important negative effects on pasture productivity and the quality and provision of (soil) ecosystem services–a situation that is likely to worsen in the near future due to climate change. Against this background, AGROSAVIA (Corporación Colombiana de Investigación Agropecuaria) selected *Arachis pintoi* CIAT 22160 cv. Centauro (Centauro) as a promising alternative for the sustainable intensification of livestock production and rehabilitation of degraded areas. This study assesses dual-purpose milk production in the foothills of the Colombian Orinoquía from an economic perspective. We compare two production systems: the Centauro–*Brachiaira humidicola* cv. Humidicola association (new system) and *Brachiaira humidicola* cv. Humidicola as a monoculture (traditional system). We used cashflow and risk assessment models to estimate economic indicators. The projections for economic returns consider changes in forage characteristics under regional climate change scenarios RCP (2.6, 8.5). The LIFE-SIM model was used to simulate dairy production. Results show that the inclusion of Centauro has the potential to increase animal productivity and profitability under different market scenarios. The impact of climatic variables on forage production is considerable in both climate change scenarios. Both total area and potential distribution of Centauro could change, and biomass production could decline. *Brachiaira humidicola* cv. Humidicola showed better persistence due to higher nitrogen levels in soil when grown in association with Centauro. The legume also provides a number of ecosystem services, such as improving soil structure and composition, and also contributes to reducing greenhouse gas emissions. This helps to improve the adaptation and mitigation capacity of the system.

Keywords: climate change, forage legumes, adoption, economic evaluation, risk analysis (RA), land-use change (LUC)

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INTRODUCTION

Context of Improved Forages in the Orinoquía

In many parts of the foothills of the Orinoquía region of Colombia, cattle production takes place on poorly drained soils. Consequently, extensive grazing systems characterize the region and *Brachiaira humidicola* cv. Humidicola is the most common feed option to be found. It was introduced in the 1970s in order to improve the production of the region, due to its high adaptation potential under temporal waterlogging conditions and good forage production (ICA, 1987). As a result of inappropriate management practices and low soil fertility in the region, however, most of these pastures are, today, in some state of degradation (Rincón et al., 2018). This has led to a significant reduction in pasture productivity, as well as negative effects on the quality and provision of soil ecosystem services (Fonte et al., 2014; Galdino et al., 2015), generating important economic and ecological implications for the region. Cattle and dairy production are considered to be one of the main sources of greenhouse gas emissions, derived from the digestion process of the animals (methane and nitrous oxide emissions), the use of nitrogen fertilizers, as well as the expansion of productive areas through deforestation or the invasion of protected areas (CIAT CORMACARENA, 2018). The cattle sector is therefore considered one of the key sectors for interventions with great potential for climate change mitigation. In this sense, achieving intensive livestock farming in a sustainable way has become one of the main approaches for sector development in the Orinoquía.

In short, this means more efficient cattle farms which consider, protect and sustainably use existing water and environmental resources, allowing for a reduction of greenhouse gas emissions and deforestation levels. This focus is particularly important for the Orinoquía, which is recognized for its strategic environmental importance with ecosystems of high conversation value, such as natural savannas, flooded forests, humid forests, foothills, estuaries and wetlands. The need to achieve more efficient cattle farming without affecting natural ecosystems is enshrined in the most recent Regional Climate Change Plan for the Orinoquía (CIAT CORMACARENA, 2018), which is aligned with the national approaches defined in the Strategic Plan for the Colombian Cattle Sector from 2019 (FEDEGAN, 2018)[1].

This context is likely to aggravate under a climate change scenario that would accelerate soil degradation processes (Olsson et al., 2019), especially when combined with unsustainable land use or poor management practices (Sattler et al., 2018). According to the climate projections for the Orinoquía to 2100, annual precipitation will decrease and maximum temperatures will increase, leading to periods of more extreme heat and heat stress (IDEAM, 2015). These forecasts would affect livestock production mainly through (i) changes in biomass production and quality of forages, which translates into a decrease in milk and meat production, and (ii) heat stress in animals, which leads to significant losses in production, growth, development and reproduction (CIAT CORMACARENA, 2018). Furthermore, not only projected mean changes can have an impact, but also changes in the variability and strength of extreme weather events, leading to significant consequences for livestock production (e.g., increased frequency of heat stress, drought events and floods; Thornton et al., 2009). The effect of these climatic phenomena is also reflected at the macroeconomic level via prices, because when faced with climatic events, food prices tend to vary, generating transitory inflationary pressures (Melo et al., 2017). As a result of reductions in precipitation levels caused by the El Niño phenomenon, for example, a reduction in agricultural supply is generated, which in turn leads to temporary price increases (Melo et al., 2017).

Apart from the climatic impacts on local production systems, the increasing demand for animal source food (OECD/FAO, 2020) creates pressure on livestock producers to extend production areas. In the case of the Orinoquía, this can cause increasing rates of deforestation and a penetration of important local ecosystems (such as native savannas), leading to irreversible changes within, and losses of, local ecosystems, biodiversity and cultural heritage, aggravating climate change even further.

In this sense, there is an increasingly pressing need to implement sustainable production systems with greater capacity for adaptation and mitigation to climate change, systems that contribute to maintaining, improving and protecting local ecosystems. One of the most promising alternatives to achieve the previous objectives, as well as to restore degraded areas, is the use of forage legumes in livestock systems (Fisher et al., 1994; Shelton et al., 2005; Murgueitio et al., 2011; Schultze-Kraft et al., 2018). Their high protein content improves nutritional values and the efficiency of animal feed, which in turn reduces enteric methane emissions (Dickie et al., 2014). Legumes also contribute Nitrogen (N) to the soil through symbiotic N fixation that improves both soil fertility and forage persistence (Rao et al., 2014; Villegas et al., 2020). According to Fisher et al. (1994), the association of deep-rooted grasses with nitrogen-fixing legumes has three important effects, namely (i) increased nutrient cycling, (ii) improved animal production, and (iii) increased soil biological activity, and thus play a key role in restoration, stabilizing the global carbon cycle and reducing greenhouse gas emissions. In addition to that, they provide many other ecosystem services, such as improved soil structure, water infiltration, increased carbon accumulation, favored biological activity, and contributions to weed control and soil conservation (Jensen et al., 2012; Schultze-Kraft et al., 2018).

As part of the research efforts to identify forage legumes adapted to the specific conditions of temporary water saturation in the Orinoquía foothills, AGROSAVIA started evaluating 22 promising legumes in 2013. After 3 years of agronomic evaluations, *Arachis pintoi* CIAT 22160 cv. Centauro (Centauro) was selected as the most promising material for release. It presents desirable characteristics in both productive terms (e.g., good nutritional quality, less weed presence, greater foliar area, absence of pests and diseases) and environmental terms (e.g., better soil coverage and, consequently, less susceptibility to soil erosion) and has high potential for integration in silvo-pastoral systems (shade tolerance) (Rincón et al., 2020). These characteristics make Centauro a good alternative for

[1]FEDEGAN: Federación Colombiana de Ganaderos, Colombian Cattle Federation.

the purposes of sustainable intensification and restoration of degraded pastures in the region. When it comes to new technologies, however, land-use and adoption decisions by the livestock producer are mainly based on the profitability promises that the technology can generate (Pannell et al., 2006). Profitability is a fundamental attribute to incentivize or generate adoption, information which, in many cases, is not available to the livestock producer or the extension agents supporting decision-making processes. Profitability is not, however, the only measure since other factors exist that contribute to incentivizing or discouraging the adoption of new technologies, such as cultural, behavioral or environmental factors.

Regarding economic studies on the inclusion of *Arachis pintoi* in livestock systems, limited advances have been made so far. Most of them were carried out by the International Center for Tropical Agriculture (CIAT) in Latin America more than two decades ago. These studies mainly dealt with measuring the effects on different economic indicators of the inclusion of *Arachis pintoi* CIAT 17434 in grazing systems. Rivas and Holmann (2000) evaluated changes that occurred between 1986 and 1997 in productive and economic indicators in farms in the Colombian Caquetá Department that were early adopters of the *Arachis pintoi* CIAT 17434 variety. According to their results, production levels of both meat and milk more than doubled with the inclusion of the legume, reflected in higher gross yields per hectare (6%) and animal (20%). Based on these results, the same authors carried out an ex-ante evaluation estimating an Internal Rate of Return (IRR) of between 19.3 and 21.1% resulting from the inclusion of the legume—which equates to an increase compared to the traditional production system (IRR = 12%). Evaluations in Costa Rica estimated a 30% reduction in production costs per kilogram of milk associated with the inclusion of *Arachis pintoi* and *Cratylia* (Peters et al., 2001). Also in Costa Rica, Jansen et al. (1997) estimated an IRR of 122% in a well-managed grass-legume association of *Brachiaria brizantha* and *Arachis pintoi*. For the Amazon region of Brazil, Valentim and Andrade (2005) estimated a gross profit per year of US$ 4,000 generated by the adoption of *Arachis pintoi* by ∼1,000 cattle producers. According to our literature review, neither more recent economic analyses nor any quantitative risk assessments or climate change impact estimates were found for *Arachis pintoi,* nor the new CIAT 22160 Centauro variety. Our study therefore contributes to closing an important knowledge gap and provides updated information on the new Centauro variety, released in 2020, in order to facilitate dissemination and adoption processes for the actors involved (e.g., cattle producers, extension agents, development agencies or donors).

In this sense, the objective of our study is to evaluate the economic viability of milk production in a dual-purpose cattle system in the foothills region of the Colombian Orinoquía under a grass-legume association with *Brachiaria humidicola* cv. Humidicola and *Arachis pintoi* CIAT 22160 cv. Centauro (grass-legume association). We compare these results with a traditional production system under a *Brachiaria humidicola* cv. Humidicola monoculture (grass monoculture). In order to estimate economic indicators, we used a cashflow model and conducted a risk assessment using a Monte Carlo simulation model. The projection of economic returns is carried out considering changes in forage characteristics (dry matter production) for both production systems as a response to changes in projected climatic variables, according to the climate change scenarios for the Representative Concentration Pathways of the region (RCP 2.6 and 8.5; IDEAM, 2015). It also includes potential effects on price variations as a consequence of recurring climatic events (El Niño and La Niña). With this information, profitability indicators for each system (e.g., Net Present Value, Internal Rate of Return) are calculated and help in the identification of the treatment with better adaptability under climate change scenarios.

Research on *Arachis pintoi* in the Neotropics

Historical Review: Technical Evaluation Processes of *Arachis pintoi* in Colombia

The evaluation of *Arachis* genotypes in Colombia began in 1978 with the introduction of 45 accessions from germplasm collections in the U.S. [i.e., from the University of Florida and the United States Department of Agriculture (USDA)] by CIAT to its Carimagua Research Center in the Orinoquía region (Rincón et al., 1992). These accessions have been wild-collected since 1981 by USDA, EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) and CIAT (Valls and Pizarro, 1995). *Arachis* species are present in countries such as Brazil (more than 60 wild species), Bolivia (15), Paraguay (14), Argentina (6), and Uruguay (2) (Valls and Pizarro, 1995).

From 1987 to 1990, CIAT (in collaboration with other institutions) worked on the selection of *Arachis pintoi* germplasm with potential for adaptation to acid soils and to restore large areas of degraded pastures in the Colombian Caquetá Department. The grass-legume association of *Arachis pintoi* with various species of *Brachiaria* was identified as the most promising solution (Lascano et al., 2005), and after several years of research, the variety *Arachis pintoi* CIAT 17434 (perennial forage peanut) was released in 1992. The variety is characterized by its good adaptation to climate and soil conditions in the Colombia Orinoquía but also has some important limitations such as slow establishment, low forage production during the first 2 years and high defoliation rate in the dry season (Rincón, 2001). In an attempt to solve these problems, the evaluation of new *Arachis pintoi* accessions was taken up in 1994 in various South American countries (Colombia, Brazil, Ecuador, Peru and Bolivia). The accession CIAT 22160 was among the evaluated materials. This accession is native to Brazil, was found in the eastern Andes, between the Amazon and La Plata rivers, and was collected in 1992 by the researcher Wantuil Werneck and delivered to the CIAT gene bank by EMBRAPA (Brazil).

The first evaluations of this material were made in Brazil in 1994 together with another 49 *Arachis pintoi* accessions. CIAT 22160 stood out for presenting high persistence during the dry season (CIAT, 1994). In Colombia, the earliest evaluation records of CIAT 22160 were documented by Moreno et al. (1999), Cárdenas et al. (1999), and Peters et al. (2000) as part of a multilocational trial with several *Arachis pintoi* accessions. The objective was to find alternatives to *Arachis pintoi* CIAT 17434 (perennial forage peanut) with higher adaptability. The evaluated accessions were acquired by CIAT between 1993 and 1994 from EMBRAPA-CENARGEN (Brazil) and the National Institute of Agricultural Technology (INTA, Argentina) (CIAT, 1994). The experiments were established between 1994 and 1995 in different locations: (i) 39 accessions in a tropical dry forest (Moreno et al., 1999), (ii) 41 accessions in a very humid premontane forest (Cárdenas et al., 1999), and (iii) 61 accessions in a very humid forest ecosystem (Peters et al., 2000). Accession CIAT 22160 was identified as promising material for very humid tropical forests, with superior characteristics to the control variety (*Arachis pintoi* CIAT 17434), such as greater rooting, faster growth, higher dry matter production and a more efficient use of Phosphorus (CIAT, 2002). The first evaluation of CIAT 22160 in the Colombian Orinoquía was conducted by Rincón (2001) with 11 *Arachis pintoi* accessions (*Arachis pintoi* CIAT 17434 as control variety). Rincón (2001) established two experiments at the CORPOICA (now AGROSAVIA) research center La Libertad in the Meta Department on poorly drained soils, leading to a preselection of the three best performing accessions according to their agronomic performance: *Arachis pintoi* 22160, 18748, and 18744. All three accessions stood out for their high dry matter production (>1 ton/ha) and level of soil cover (>70%) (Rincón, 2001).

Evaluation of *Arachis pintoi* CIAT 22160 Under Temporary Flooding Conditions

In 2013, CORPOICA (now AGROSAVIA) started the evaluation of *Arachis pintoi* under temporary flooding conditions in the Orinoquía (Rincón and Pesca, 2017). Trials were established at the research center La Libertad in the Meta Department under medium drainage conditions and included 22 *Arachis pintoi* accessions. Each accession was established in plots with an area of 6 m^2, in a complete random block design with three repetitions. From 2013 to 2014, a series of agronomic evaluations were carried out that led to the preselection of four accessions (22160, 18748, 18744, and 17434) which were then evaluated at the agronomic level during both the dry and rainy seasons. After another year of evaluation, the *Arachis pintoi* CIAT 22160 was selected for grazing trials as a result of its outstanding attributes of soil cover, persistence, competition with weeds, forage production and nutritional quality.

The grazing trials were carried out from February 2016 to April 2017 at the farm *Los Arrayanes*, and from October 2019 to February 2020 at the farm *El Recreo*, both located in the Orinoquía region and presenting temporary flooding conditions. The accession CIAT 22160 was established in August 2015 in both locations with vegetative material, in an area of 2000 m^2 and in association with the grass *Brachiaria humidicola* cv. Humidicola (grass-legume association). Productivity results were compared with data obtained from a monoculture grazing trial with the grass *Brachiaria humidicola* cv. Humidicola (grass monoculture). Animal productivity was measured in lactating cows under a dual-purpose system. The animals were supplemented, in both treatments (grass monoculture and grass-legume association) with 8% mineralized salt at an amount of 80 g AU^{-1} d^{-1} throughout the year. Cut grass silage was supplied during the daily milking time at an amount of 2 kg/animal/d for ~180 days a year. In order to maintain pasture productivity levels, maintenance fertilization and weed control were performed once a year for both treatments. Diammonium Phosphate (DAP) (100 kg ha^{-1}) and Sulgamac (100 kg ha^{-1}) were applied for the grass-legume association. For the grass monoculture urea (100 kg ha^{-1}) was added to DAP and Sulgamac. The results of these measurements (biomass production, nutritional quality and animal response) for each treatment are presented in **Table 1**, since our economic analysis is based on these technical parameters obtained in the study by Rincón et al. (2020), previously described.

TABLE 1 | Animal response data for the grass-legume association and grass monoculture.

Variable	Grass-legume association (Mean ± SD)	Grass monoculture (Mean ± SD)
Biomass production (kg DM^{-1} ha^{-1} y^{-1})	919	808
Crude protein (%)	9.2	6.6
Neutral Detergent Fiber (NDF, %)	65	75
Acid Detergent Fiber (ADF, %)	30	38
Degradability (%)	67	64
Stocking rate (AU^2 ha^{-1})	2	1.5
Milk production (l AU^{-1} d^{-1})	6.5 ± 1.34	5.7 ± 1.28
Milk production (l ha^{-1} d^{-1})	13 ± 2.68	8.5 ± 1.92

DM, Dry Matter; AU, Animal Unit.
Source: Own elaboration based on the study carried out by Rincón et al. from 2016 to 2020 (Rincón et al., 2020). The technical parameters obtained by Rincón et al. were used for the economic evaluation presented in this article.

General Characteristics of *Arachis pintoi* CIAT 22160 cv. Centauro

Arachis pintoi CIAT 22160 was identified by AGROSAVIA as a promising material to improve the quality of cattle feed in the Colombian Orinoquía, especially under poorly drained soil conditions (Rincón et al., 2020). It was released in 2020 under the common name Centauro (*Arachis pintoi* CIAT 22160 cv. Centauro), but its commercialization will only begin in 2022 in accordance with private sector seed production schedules. Centauro is a perennial herbaceous forage legume with prostrate growth. It has an average height of 20 cm and a leaf-stem ratio of 1.4 (60% leaves and 40% stems) (Rincón et al., 2020). Its flower is generally self-pollinated but can also be cross-pollinated by bees. The first flowers appear at a plant age of 14–55 days (Simpson et al., 1995). Centauro has a wide range of adaptation, from low acid soils to high fertility soils, with a soil texture ranging from sandy loam to clay loam and with good or poor drainage. In addition, it grows well in tropical regions from 0 to 1,800 m elevation and with an annual rainfall between 1,200 and 4,000 mm. It is characterized by a high biomass production and forage quality (**Table 1**), and its leaf crude protein levels vary between 15 and 18%. It adapts well in association with invasive Gramineae or in silvo-pastoral systems (good shade tolerance), has strong persistence, competes with weeds, and is tolerant to several pests and diseases. Its prostrate and invasive growth results in soil cover levels of >90%, favoring the reduction of soil compaction and erosion (Rincón et al., 2020).

Potential Ecosystem Services of *Arachis pintoi* CIAT 22160 cv. Centauro

The inclusion of Centauro in forage-based livestock systems has high potential regarding the provision of ecosystem services. In grazing systems, different studies have shown a high persistence of *Arachis pintoi* with positive effects on soil conservation and improving soil conditions (Rincón, 1999; CIAT, 2004; Castillo-Gallegos et al., 2005; Robertson, 2005; Valentim and Andrade, 2005). These positive effects are mainly associated with high production of seed below ground, a prostrate growth habit that invades bare soil, as well as tolerance to trampling and defoliation, protecting the arable soil layer and, therefore, avoiding degradation and erosion processes (Rincón, 1999; Rincón et al., 2020). Under animal grazing trials, grass-legume associations with *Arachis pintoi* have not shown signs of degradation after several years of grazing (e.g., Lascano, 1994; Rincón, 1999; Valentim and Andrade, 2005). *Arachis pintoi* has positive effects on the soil organic matter content and soil biodiversity (Rincón, 2001), improving the physical, chemical and biological soil conditions and avoiding erosion associated with overgrazing, but may reduce above ground biodiversity. *Arachis pintoi* can also improve the persistence of the associated grasses resulting from a symbiotic nitrogen fixation to the soil, which is then used by the grass (Villegas et al., 2020). For example, Dubeux et al. (2017) estimated a range of 123 to 280 kg ha^{-1} yr^{-1} of fixed nitrogen in six *Arachis pintoi* accessions, and Pereira et al. (2019) evaluated beef production in an associated system of *Brachiaria brizhanta* and *Arachis pintoi* (cv. Belomonte), estimating a minimum nitrogen fixation of 120 kg ha^{-1} yr^{-1}. The higher contribution of nitrogen not only represents a strategy for the restoration of degraded pastures, but also contributes to reducing the use of nitrogen fertilizers and, therefore, to reducing nitrous oxide emissions. Other studies focused on estimating the effect of *Arachis pintoi* accessions on carbon levels and other elements in the soil. Nutrient uptake in *Brachiaria humidicola* monoculture pastures and in grass-legume associations of *Brachiaria humidicola* and *Arachis pintoi* in acid soils with low fertility was, for example, measured in the Orinoquía, showing that the inclusion of *Arachis pintoi* increased the nitrogen, calcium, potassium and phosphorus availability in the soil by 130, 133, 19, and 13%, respectively (CIAT, 1994). In evaluations in the Atlantic coast of Costa Rica and the humid forest of the Colombian Amazon, different grass-legume associations with *Arachis pintoi* showed statistically higher levels of carbon reserves in the soil than in the native forest (Amézquita et al., 2004). In the Orinoquía, the inclusion of *Arachis pintoi* in a *Brachiaria humidicola* pasture notably increased the amount of carbon in the soil (CIAT, 1994). *Arachis pintoi* also helps in reducing greenhouse gas emissions associated with ruminal fermentation processes (higher nutritional quality of the forage) and the application of nitrogen fertilizers, while contributing to the intensification of cattle systems through productivity and increases in animal carrying capacity (Rincón et al., 2020).

MATERIALS AND METHODS

Discounted Cash Flow Model

The present study's economic analysis is based on a discounted cash flow model and the estimation of profitability indicators, such as Net Present Value (NPV) and Internal Rate of Return (IRR). These indicators are obtained assuming the most probable values of the model variables (associated with benefits and costs). The analysis is carried out by comparing the profitability indicators for the grass-legume association and the grass monoculture. The cash flow allows ordering and synthesizing the sequence of income, costs and investments associated with the evaluated technologies. The following cost categories were considered: total costs of establishment and maintenance of each treatment, opportunity costs of capital and operating costs (animal health, supplementation, permanent and occasional labor). The benefits are derived from milk production in a dual-purpose system, according to the animal response indicators obtained for each treatment (**Table 1**).

Model Assumptions

For the construction of the cash flow it is necessary to establish different economic and technical assumptions. The following sections provide detailed explanations for each of them.

Technical Assumptions

Given that productivity was measured only for daily milk production (see section Research on *Arachis pintoi* in the Neotropics), the other technical indicators are assumed to be the same for both treatments and were described by consulting average values reported for the study region: (i) 550 days calving interval; (ii) calf age of 9 months and weight of 150 kg at weaning; and (iii) lactation time of 8.5 months. AGROSAVIA researchers verified these indicators for the region.

Evaluation Horizon

The evaluation horizon is established according to the expected lifespan of a technology under evaluation. For the evaluation of the grass-legume association and the grass monoculture, a period of 10 years (2020–2029) was defined, which is in accordance with the productive lifespan for improved pastures (Riesco and Seré, 1985). It is, however, worthwhile mentioning that improved pastures can have a much longer productive lifespan if managed adequately (e.g., in terms of grazing and fertilization).

Discount Rate

The cost of financing is chosen as the discount rate according to the rural credit lines of FINAGRO (the Colombian Fund for the Financing of the Agricultural Sector). This financing cost is considered the opportunity cost of capital and is associated with a risk factor present in the activities of the rural sector. The following discount rate was therefore established: DTF (fixed-term deposit rate) + 5% effective annual interest rate. The projection of the discount rate in the corresponding periods was made following the DTF projections according to the Annual Report on Economic Projections Colombia 2020 (Bancolombia–Dirección de Investigaciones Económicas, 2020).

Permanent Labor

The required permanent labor is defined according to the weighting factors for labor established by FEDEGAN (2003). In a dual-purpose cattle system, 4.8 permanent jobs are needed for every 100 animals. The minimum salary for 2019 was used, including transportation assistance, contributions to social security, and social and parafiscal benefits, adding up to US$ 422 per month. For salary projections during the period of analysis (2020–2029), the following was assumed: Variation of the minimum salary (in %) = expected inflation (in %) + observed variation of workforce productivity (WP, in %). A WP of 1% is assumed, according to historical estimates from national statistics (DANE, 2020a).

Currency at Current Prices

Inflation is considered for estimating revenue and cost streams during the evaluation period. For revenues, the projection of the Consumer Price Index (CPI) estimated by Bancolombia–Dirección de Investigaciones Económicas (2020) for the period 2020-2023 was considered. For production costs, the Producer Price Index (PPI) estimated by DANE (2020b) was used.

Milk Price. Price information was obtained from the Milk Price Monitoring Unit (USP) for the predefined Region 2, where dual-purpose production systems predominate (MADR/USP, 2020). The prices were projected according to the CPI projections. Additionally, we included projections for the effect of extreme climatic events (El Niño and La Niña phenomena) on milk price variations. Abril et al. (2017) quantified climate impacts on food inflation in Colombia. According to their results, after the occurrence of an El Niño or La Niña phenomenon, food inflation increases significantly between four and 5 months later (increasingly when the intensity of the phenomenon is strong), and its response is asymmetric depending on the impacts and size of the shock. This directly affects the income received by producers and household purchasing power in Colombia. Regarding milk prices, variations have been >7% in the years with such climatic events, compared to variations of <1% in years without (DANE, 2020a,b). The variation margin of the CPI was assumed for the occurrence climatic phenomena as follows: (i) the spread of the CPI vs. the CPI of milk in 2018 was assumed as the spread that the price of milk would have against the national CPI for a scenario where no climatic phenomenon occurs; (ii) the spread of the CPI vs. the milk CPI of 2015–2016 was assumed as the spread that the price of milk would have against the national CPI for a scenario where a climatic phenomenon occurs; (iii) variations in the CPI of 7% with a climatic phenomenon and <1% without are considered (**Figure 1**); and (iv) both the CPI and CPI for milk were obtained from DANE (DANE, 2020b,c).

Quantitative Risk Analysis

Risk is defined as the possibility that the real return on an investment is less than the expected return (Park, 2007). Profitability is therefore associated with the variability of revenue and cost streams, and these in turn depend on the randomness of the main variables of the investment project (e.g., yields, market prices). Rural investment projects involve particular risks, and their results depend on a broad set of variables which, in many cases, cannot be controlled by the investor/producer (e.g., climatic factors). In this sense, it is necessary to incorporate risk levels associated with the profitability indicators for each of the evaluated investment alternatives. For this purpose, we apply a Monte Carlo simulation model. The accuracy of simulation models depends on the quality of the input data. In this study, for example, the milk production data under each treatment was derived from on-farm measurements carried out during representative periods (i.e., rainy and dry seasons; section Evaluation of *Arachis pintoi* CIAT 22160 Under Temporary Flooding Conditions). We consider this data reliable, reflecting the distribution and real behavior of the variable observed by the technical team. Cost data and possible variations of its values were constructed with experts from AGROSAVIA according to the real conditions of cattle producers in the region in terms of prices and quantities used.

Monte Carlo Simulation Model

Monte Carlo simulation is a method in which a random sample of results is generated for a specific probability distribution (Park, 2007). This method allows potential investors or decision makers to see all the possible results and to evaluate the impact of risk on profitability indicators in investment projects. To perform the simulation, it is necessary to determine the random input variables (those that can have more than one possible value) and the possible range values for each. These variables are assigned a probability distribution, to later calculate the determined profitability indicators. Monte Carlo simulation was performed with the software @Risk (Paladise Corporation). For the evaluated treatments, 5,000 iterations were performed with a confidence level of 95%.

Decision Criteria

As decision criteria, the mean values and the variance of the profitability indicators resulting from the simulation are used: Net Present Value (NPV) and Internal Rate of Return (IRR) [Equations (1, 2)]. The use of the mean value criterion is based on the law of large numbers, which establishes that, if many repetitions of an experiment are carried out, the average result will tend toward the expected value (Park, 2007). The variance of the indicators determines the degree of spread or dispersion on both sides of the mean value (Park, 2007). That is, the lower the variance, the lower the variability (loss potential) associated with

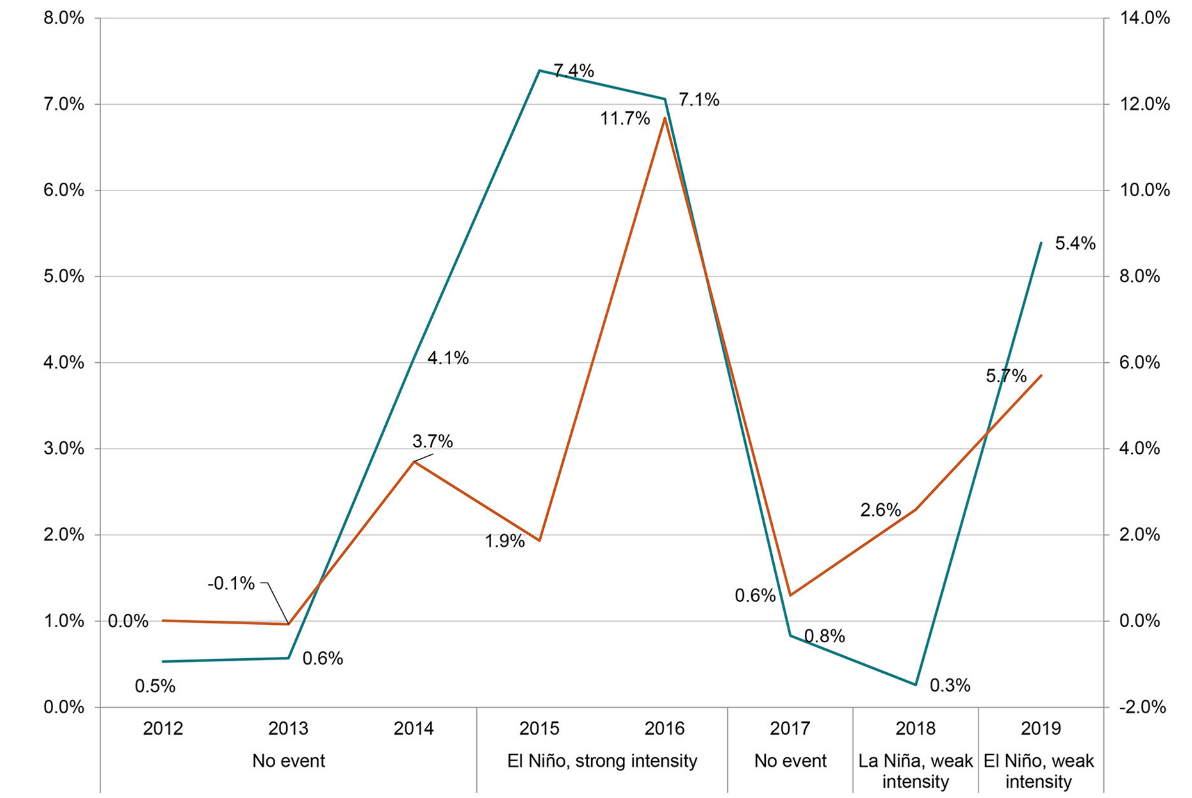

FIGURE 1 | Consumer Price Index (CPI) and Producer Price Index (PPI) behavior of milk in the face of climatic events. Source: Own elaboration based on DANE (2020b,c).

the indicators.

$$NPV_{(Mean)} = \sum_{t=0}^{n} \frac{E(FC_t)}{(1+r)^t} \quad (1)$$

$$IRR_{(Mean)} = \sum_{t=0}^{n} \frac{E(FC_t)}{(1+r*)^t} = 0 \quad (2)$$

Where,

E (FCt): Expected value of the net profit flow for period t
Var (FCt): Net profit flow variance for period t.
r: Real discount rate
r∗: Internal rate of return
t: Evaluation horizon of the project

The NPV at risk indicator (VaR) is also estimated and the probability of success of the evaluated investments is estimated (Prob NPV (mean) > 0). The VaR is defined as the maximum expected loss that the project could suffer from investment in a time interval and with a certain level of confidence (Park, 2007). The probability of success is defined as the proportion of positive results of all interactions (NPV > 0, the project is economically viable). A sensitivity analysis was performed using a tornado graph, which sensitizes each variable in order to measure its impact on the profitability indicators and to identify within the critical variables those with the greatest effects on the profitability indicators.

Simulated Variables

The study considers how sensitive the economic results are to changes in the main variables of the model. **Table 1** shows the variables identified as risk variables and the distributions and parameters used for modeling them. For modeling the milk production variable for the grass-legume association and the grass monoculture, a distribution adjustment of the data was performed with @Risk.

Economic Evaluation Under Climate Change Scenarios RCP 2.6 and 8.5

Forage Production Under Climate Change Scenarios

The effect of climate change on livestock productivity was determined by comparing forage biomass production under a baseline (current) scenario with estimated levels under climate change scenarios. We used two climate change scenarios for the region: RCP 2.6 and RCP 8.5 (Armenta et al., 2015). To identify the main environmental factors that affect the productivity of the evaluated treatments, as well as the magnitude of the effect, an analysis of variance (ANOVA) was performed. The delta identified in both climate change scenarios was applied to each of these environmental factors, to estimate the monthly biomass production per hectare. This delta refers to the change in climatic variables between one scenario and another. It is important to note that the model is only considering changes in the climatic

TABLE 2 | Variables simulated with the Monte Carlo model.

#	Variable	Distribution	Most likely value	Lower limit	Upper limit
1	Milk price (US$ l^{-1})	Triangular	0.35	0.33	0.36
2	Productivity milk GLA[a] (l d^{-1})	Pert	6.5	5.16	7.84
3	Productivity milk GM[b] (l d^{-1})	Pert	5.7	4.42	6.98
4	Establishment costs GLA (US$'$ha^{-1}$)	Triangular	642	578	706
5	Establishment costs GM (US$ ha^{-1})	Triangular	450	405	495
6	Periodicity of El Niño phenomenon (y)	Discrete uniform	n/a	2	7
7	Variation of the discount rate (%)	Triangular	0	1	2
8	Variation IPC (%)	Triangular	–0.5	0	0.5

[a]GLA, grass-legume association; [b]GM, grass monoculture.

variables of the RCP, keeping constant the assumptions of pasture and soil management, level of technology, investment in labor and animal characteristics.

In addition to possible changes at the productive level of the forage species, the change in environmental conditions under climate change scenarios can alter the potential distribution of plant species. In other words, the species would tend to modify their distribution toward latitudes and altitudes different to those where they are currently found (Walther et al., 2005). To identify this possible effect, the maximum entropy model Maxent (version 3.4.1; Phillips et al., 2021) was used. The model makes it possible to estimate the extent of future environments and to determine, in the case of the legume *Arachis*, if and where conditions similar to the current environments exist.

The Maxent model requires two input streams: (i) points of presence (distribution) of the species throughout the world and, (ii) bioclimatic variables. The current distribution points of *Arachis* were downloaded from Global Biodiversity Information Facility (GBIF.org, 2020) with more than 600 points of presence which, after cleaning outliers and anomalous data, reached just over 300 total points. The second input of the Maxent model were the bioclimatic variables for RCP 2.6 and 8.5 obtained from Navarro-Racines et al. (2020). These variables represent annual trends (e.g., mean annual temperature and precipitation), seasonality (e.g., annual ranges of temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, precipitation of humidity).

Milk Production Under Climate Change Scenarios

Based on the forage production estimates under climate change scenarios (section Potential Ecosystem Services of *Arachis pintoi* CIAT 22160 cv. Centauro), milk production estimations were performed in the LIFE-SIM model (version Dairy 15.1), developed by the International Potato Center (CIP; León-Velarde et al., 2006). In this model, milk production estimations are based on the characteristics of the animals, forages and climatic conditions (temperature, humidity and wind speed) (León-Velarde et al., 2006). **Tables 2**, **3** and **4** present the information used in the model. The analysis did not consider episodes of heat stress in the animals, which could also affect milk production.

TABLE 3 | LIFE-SIM model inputs for the animal component.

Variable	Value
(A) Existing information	
Calving interval (days)	550
Calf weaning age (months)	9
Calf weaning weight (kg)	150
Lactation time (months)	8.5
Number of lactations	9–10
Weight (kg AU^{-1})	400
(B) Estimated information	
Age of animals (years)	3
Weight after delivery (kg)	380
Duration of gestation (days)	282
Birth weight (kg)	28
Weight loss during lactation (%)	8
Expected weight at next delivery (kg)	412.2
Fat content of milk (%)	3
Protein content of (%)	3.1
Non-fat solids content of milk (%)	8.7
Animal fur thickness (mm)	2

Economic Evaluation Under Climate Change Scenarios

Based on the results of milk production under the climate change scenarios (**Table 7**) and according to the methodology presented in sections Evaluation of *Arachis pintoi* CIAT 22160 Under Temporary Flooding Conditions and General Characteristics of *Arachis pintoi* CIAT 22160 cv. Centauro, the profitability indicators were estimated for both treatments. **Figure 2** shows how the different models used in this study are interlinked (Maxent, LIFE-SIM and economic models).

RESULTS

Discounted Cash Flow Model

Table 5 shows the average costs and revenues for the grass-legume association and the grass monoculture, respectively. The models include the variable costs and revenues associated with

TABLE 4 | LIFE-SIM model inputs–climatic variables.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Average temperature (°C)												
C.S.[a]	26.1	26.6	26.7	25.7	25.4	24.8	24.6	25	25.5	25.5	25.7	25.6
RCP 2.6	28.3	28.7	28.6	27.6	27.3	26.5	26.4	27	27.3	27.6	27.7	27.5
RCP 8.5	28.6	29.2	29	27.9	27.6	26.9	26.7	27.3	27.7	27.9	28	27.9
Wind speed (km/h)												
C.S.	6	6.3	5.6	5	4.6	4.8	4.9	4.5	4.9	4.5	4.5	5.4
RCP 2.6	6.4	6.8	6.0	5.4	4.9	5.2	5.3	4.8	5.3	4.8	4.8	5.8
RCP 8.5	6.5	6.9	6.1	5.4	5.0	5.2	5.3	4.9	5.3	4.9	4.9	5.9
Humidity (%)												
C.S.	68.5	62.3	74.7	81.5	84.1	85.5	81.9	82.8	78.5	79.6	80.5	78.6
RCP 2.6	74.0	67.3	80.7	88.1	90.8	92.3	88.5	89.4	84.7	86.0	86.9	84.9
RCP 8.5	74.8	68.1	81.6	89.1	91.9	93.4	89.4	90.4	85.7	87.0	87.9	85.9

[a]*C.S., Current Scenario.*

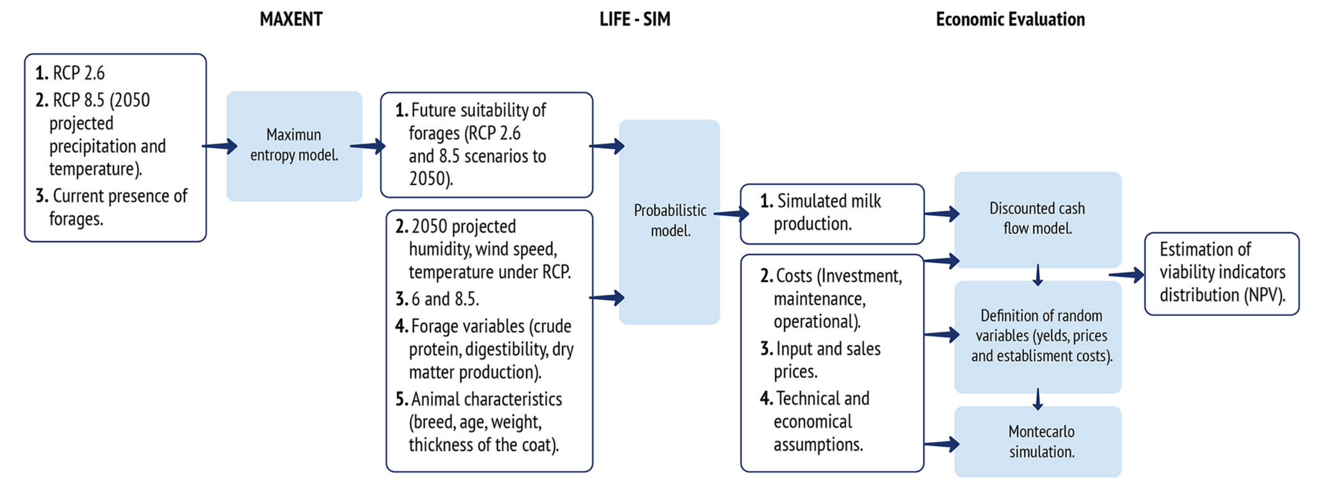

FIGURE 2 | Interdependence of the models used in this study (Maxent, Life-Sim, and economic models).

the establishment of each technology under a dual-purpose production system. The revenue results from the sale of raw milk and the sale of weaned calves (150 kg) every 550 days (calving interval). According to average daily milk production, the inclusion of Centauro in the system (grass-legume) allowed an increase in milk production per hectare by, on average, 52% when compared to grass in monoculture. Particularly during the months of minimal rainfall (dry season from January to March), grass-legume showed greater persistence and, consequently, a more stable milk production. The average production was 2,373 l ha^{-1} yr^{-1} for the grass-legume association and 1,560 l ha^{-1} yr^{-1} for the grass monoculture, respectively. This is equivalent to a gross income from raw milk sales of US$ 822 and US$ 518, respectively, representing a 58% increase for the grass-legume association.

Regarding production costs, labor (63%) makes up the largest share, followed by inputs for pastures (21%), supplements (8.5%), drugs (1.2%), and other costs (5%). The unit production cost of milk is US$ 0.23 for the grass-legume association and US$ 0.31 for the grass monoculture, respectively, representing 35% lower costs for the grass-legume association. The average net profit for the year is US$ 212 for the grass-legume association and US$ −6.95 for the grass monoculture. Under these assumptions, production under the grass monoculture is unprofitable, a consequence of the low productive indicators associated with this alternative. It is important, however, to highlight the social connotation of dual-purpose systems in the country: given the cash flow provided by the sale of raw milk, its high nutritional value and the relatively low barriers for getting involved in the business, it is still an attractive alternative for many producers, from which they derive the subsistence for their family. This exercise includes the required labor costs (permanent and occasional), valued at the minimum salary (plus benefits). These costs could, however, reflect the opportunity cost of family labor that, in many cases, is not accounted for within the cost structures, but rather represents part of the household income.

TABLE 5 | Summary of main costs and revenues for the grass-legume association and the grass monoculture.

Economic indicators	Grass-legume association	Grass monoculture
Milk production (l ha^{-1} y^{-1})	2,373	1,560
Gross income from milk sales (US$ ha^{-1} y^{-1})	834.2	548.6
Gross income from weaned calf sales (US$ ha^{-1} y^{-1})	489.5	257.0
Pasture establishment costs (US$ ha^{-1})[a]	642	450
Production costs (US$ ha^{-1} y^{-1})	787.9	699.7
Net income system (US$ ha^{-1} y^{-1})	212.0	−7.0
Unit cost of milk production (US$ l^{-1})	0.2	0.3
Milk income (US$ l^{-1})	0.3	0.3
Milk profit margin (US$ l^{-1})	0.1	0.0
Unit cost of calf production (US$ kg^{-1})	1.2	1.5

[a]*Includes the costs associated with soil analysis, machinery rental, inputs and labor required for soil preparation, fertilization, weed control, and planting of the material for both treatments. Vegetative material and labor costs for planting the legume are added to the items required for the establishment of a grass in monoculture.*

TABLE 6 | Profitability indicators of the simulation model for the grass-legume association and the grass monoculture.

Decision criterion	Indicator	Grass-legume association	Grass monoculture
NPV (US$)*	Mean[a]	121	(941)
	SD[b]	391	276
	CV	3.24	0.29
	VaR	(902)	(1,637)
	Prob < 0 (%)	60.9	100
IRR (%)	Mean	12.2	–

[a]*Mean value of the NPV obtained in the simulation (5,000 iterations).*
[b]*SD, Standard deviation of the NPV with respect to the mean value.* *Prices in US$-US$/COP XRT: Average 2019.*

TABLE 7 | Changes in milk production under climate change scenarios.

Treatment	Scenario	Milk production, dry season (average liters per AU^{-1} d^{-1})	Milk production, rainy season (average liters per AU^{-1} d^{-1})
Grass-legume association	C.S.[a]	5.49	5.59
	2050 RCP 2.6	4.2	5.4
	2050 RCP 8.5	3.66	4.53
Grass monoculture	C.S.	4.97	5.1
	2050 RCP 2.6	1.83	2.93
	2050 RCP 8.5	1.63	2.35

[a]*C.S., Current Scenario.*

Profitability Indicators and Risk Analysis

The summary of the main financial indicators obtained from the Monte Carlo simulation is presented in **Table 6** Under the assumptions used in the model and according to the indicator coefficient of variation (CV) and VaR, the inclusion of Centauro in the grass-legume association allows better economic and lower risk indicators to be obtained when compared with *Brachiaria humidicola* cv. Humidicola as a monoculture. The results indicate that the investment in the establishment of the grass-legume association is profitable, with an average NPV of US$ 121 and an IRR of 12.2%.

Regarding the probability of not obtaining financial feasibility, the results of the NPV probability distribution are presented in **Figure 3** and reflect the amplitude of the variation for the NPV indicator. For the grass-legume association, in 60.9% of the scenarios generated during the simulation, an NPV > 0 was obtained, whereas for the grass monoculture, the investment was not profitable under any of the generated scenarios.

Sensitivity Analysis

Figure 4 shows the contribution of different input variables to the NPV variance as result of the simulation. These graphs represent the correlation that each input variable simulated in the model has with the NPV profitability indicator. As can be seen, the profitability of the treatments measured by the NPV indicator is highly sensitive to changes in the milk production variable for both treatments. The correlation between the NPV indicator and the milk production variable is positive, which means that changes in daily production affect the indicator by more than 90% for both treatments. This results from the high dependence on the income generated from milk production and the productivity levels in this system.

Economic Evaluation Under Climate Change Scenarios RCP 2.6 and 8.5

Forage Production Under Climate Change Scenarios

The estimates for dry matter production for the evaluated climate change scenarios are presented in **Figure 5**. According to the results, changes in the climatic variables in the RCP scenarios have notable impacts on the productivity of both treatments. For the grass-legume association, biomass production was reduced, on average, by 7.74 and 16.62% under RCP 2.5 and 8.5, respectively. For the grass monoculture, the reductions were 14.95 and 35.27% under RCP 2.5 and 8.5, respectively. The most

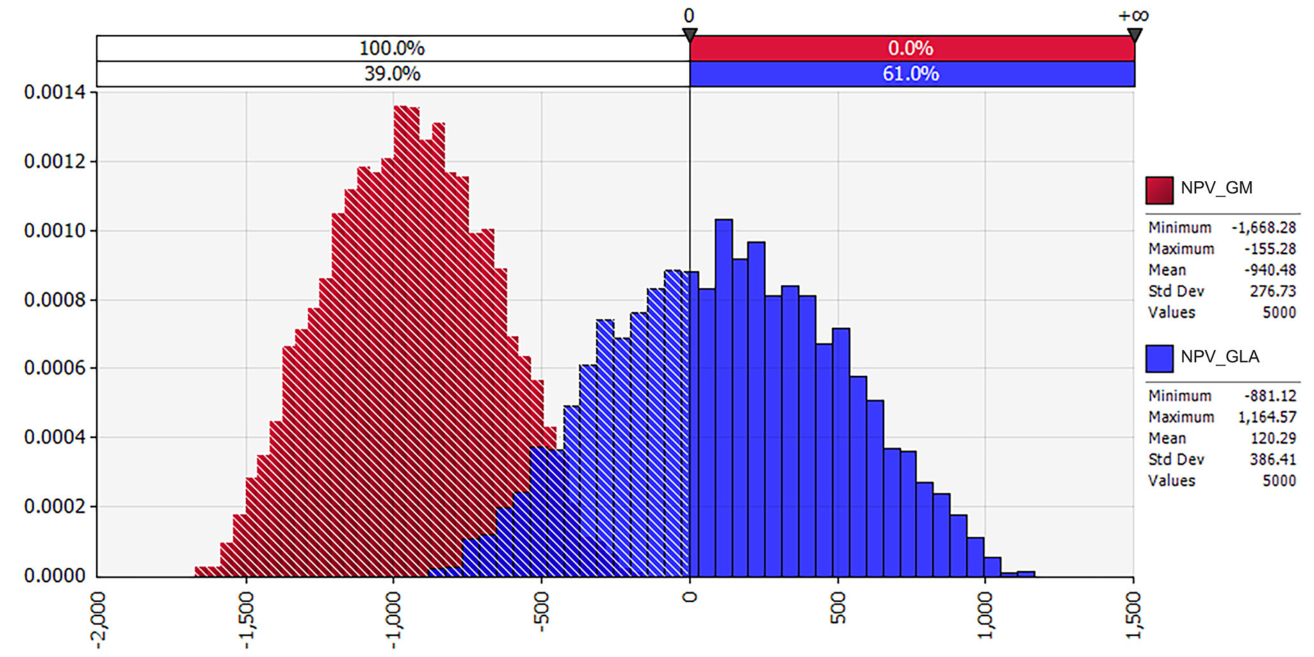

FIGURE 3 | Probability density distributions for NPV for the grass-legume association and the grass monoculture. GLA, Grass-legume association; GM, Grass monoculture.

influential environmental factors on productivity were average temperature and bio3[2] for the grass-legume association and precipitation7[3] and bio3 for the grass monoculture.

In addition to the possible changes at the productive level of *Arachis*, projected changes in environmental conditions under climate change scenarios can alter its potential distribution. According to the results of the Maxent model, under RCP 2.6 (until 2050) a shift of suitable areas for *Arachis* toward higher altitudes would occur (**Figure 6**), meaning a decrease in their suitability for lower altitudes like the foothills or savannas. Under RCP 8.5, in addition to the described shift of suitable areas, a total reduction of the potential area for *Arachis* would occur (**Figure 6**).

Milk Production Under Climate Change Scenarios

The effects of the climate change scenarios on forage biomass production translate into a strongly marked decrease in milk production for both treatments (**Table 6**). For the grass-legume association, during the first 3 months of the year (dry season) and compared to the current scenario, a decrease of close to 23% would occur under RCP 2.6 and 33% under RCP 8.5, respectively. These reductions are less marked during the rainy season but are still relevant for the low production volumes under this system.

In the case of the grass monoculture, the change in climatic variables would cause a reduction of milk production during dry season of 63 and 67% for RCP 2.6 and RCP 8.5, respectively. During rainy season, the effect on productivity would be above 40% for both RCP scenarios.

Profitability Indicators Under Climate Change Scenarios

The reduced milk production under both climate change scenarios leads to a leftward shift of the distribution curves for the NPV indicator in both treatments, when compared to the current scenario (**Figure 7**). The net income is reduced by 60 and 90% for the grass-legume association, and 113 and 131% for the grass monoculture under the scenarios RCP 2.6 and RCP 8.5, respectively. Although including Centauro in the system has highly positive effects at the productive level and therefore on the economic performance, both climate change scenarios would affect the system in such a strong way that the investment in any of the treatments would not be profitable.

DISCUSSION

The inclusion of the legume *Arachis pintoi* CIAT 22160 cv. Centauro in a monoculture of *Brachiaria humidicola* cv. Humidicola allows an improvement in the technical parameters of the production system and results in better economic indicators. At the productive level, this association increased daily milk production by 14% on average and the animal stocking rate by 33% compared to the monoculture. This results from the higher crude protein content of the diet (39%), the higher

[2] bio3 is a percentage indicator that shows the variability of the diurnal temperature range with respect to the annual temperature range. Bio3 = (Bio2/Bio7)*100; Where: Bio2 = Mean Diurnal Range [Mean of monthly (max temp – min temp)] and Bio7 = Temperature Annual Range (Max Temperature of Warmest Month – Min Temperature of Coldest Month).

[3] precipitation 7 is a continuous variable representing the amount of rainfall per m^2 at a geographic point during the seventh month of the year (July).

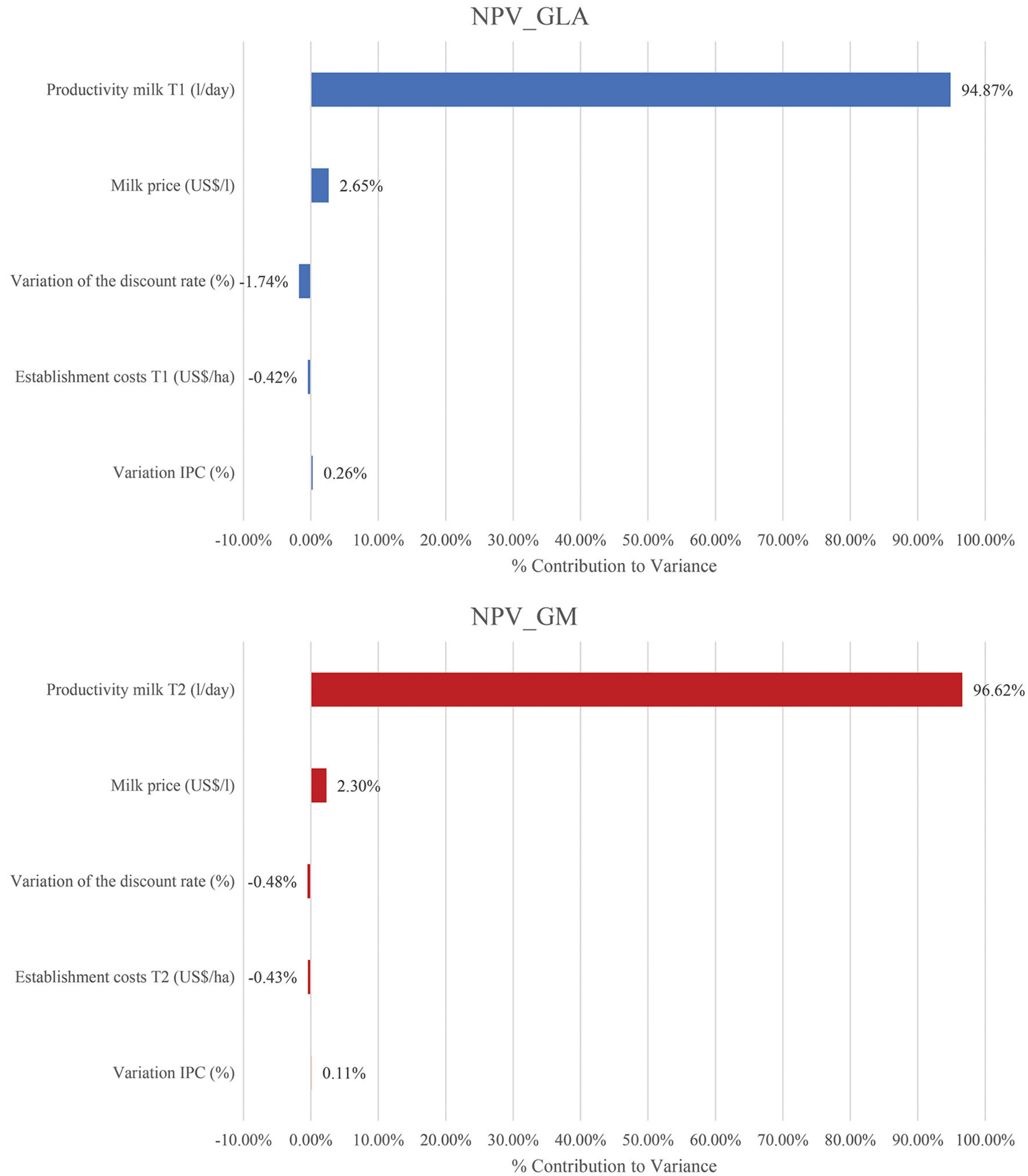

FIGURE 4 | Contribution to the NPV variance for the grass-legume association and the grass monoculture. GLA, Grass-legume association; GM, Grass monoculture.

dry matter production (14%) and the lower proportion of Acid Detergent Fiber that favors digestibility and, therefore, a better use of the available forage (Rincón et al., 2020). The higher milk production level in turn helps to improve the financial indicators of the association compared to the monoculture base scenario.

These results (i.e., the increased milk productivity by 52% and the related increased income from milk sales by 58%) are consistent with (and even surpass the results of) different studies that have evaluated the potential of *Arachis pintoi* accessions (mainly CIAT 17434) in integrated grass-legume systems for livestock production in the tropics. These studies highlight, in comparison with monoculture pastures, improvements in both forage quantity and quality, a strong compatibility with aggressive *Brachiaria* species, as well as higher meat and milk production levels and stocking rate (Peters et al., 2011; Crestani et al., 2013; Pereira et al., 2019; Boddey et al., 2020; Villegas et al., 2020). Other studies show average increases in milk production of 31% in Colombia (Rivas and Holmann, 2000), 7 and 11.4%

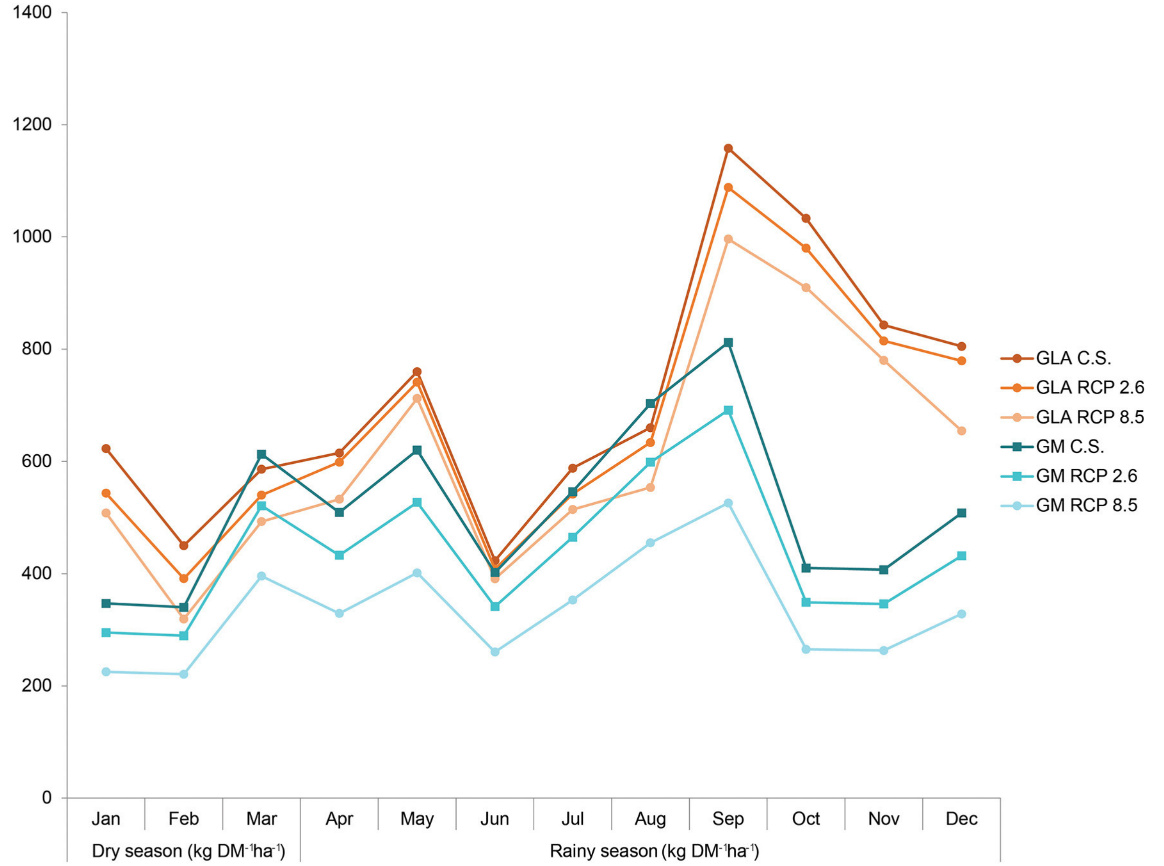

FIGURE 5 | Dry Matter (DM) production under climate change scenarios (RCP 2.6 y 8.5) and Current Scenario (C.S). GLA, Grass-legume association; GM, Grass monoculture.

in Costa Rica (Peters et al., 2001; Romero and González, 2004), and 20% in Peru (Lara and Reategui, 2004). In addition to milk yield increases, Romero and González (2004) found differences regarding the milk composition: both the milk protein (3.66 vs. 3.54%) and total solid contents (13.89 vs. 13.73%) were higher in a grass-legume association with *Arachis pintoi* than in a grass monoculture with *Brachiaria*. Regarding the animal stocking rate, the reported increases are between 33 and 50% in Colombia (1.5-2 AU ha^{-1} vs. 1 AU ha^{-1}; Holmann, 2004), 29% in Brazil (2.26 vs. 1.6; Vasques et al., 2019), 50% in the Peruvian Amazon (4.13 vs. 2.07; Lara and Reategui, 2004), and 25% in Costa Rica (4.6 vs. 3.7; Romero and González, 2004). Other studies highlight successful cases of early adoption of *Arachis* accessions in livestock systems, e.g., in western Brazil (Valentim and Andrade, 2005), in the Colombian Caquetá Department (Lascano et al., 2005) and in northern Costa Rica (Wunscher et al., 2004), suggesting the relevance of, and the potential for dissemination, across different regions, for the technology evaluated in our study.

The improvements in the economic indicators resulting from the inclusion of Centauro are also associated with improvements in the risk indicators. The probability of obtaining economic loss was reduced from 100 to 39.1%, for example. The sensitivity analysis shows that the daily milk production variable has the highest impact on the economic performance indicators. The monoculture is more sensitive to small reductions in milk production as when associated with Centauro. Changes of just 1% in milk production lead, however, to changes in profitability indicators of more than 90% in both systems. Since different empirical studies have shown that risk factors (perception of risk about future returns from implementing a new technology, and level of risk aversion of the producer) are determining factors in technology adoption (e.g., Marra et al., 2003; van Winsen et al., 2014; Trujillo-Barrera et al., 2016), there is reason to believe that the lower risk levels resulting from the inclusion of Centauro in the cattle production system will enhance technology adoption.

The inclusion of different climate change scenarios (RCP 2.6 and RCP 8.5) in our models revealed the substantial impact that climatic variables have on forage production, both in terms of geographic distribution and available forage biomass. Until 2050, the available forage biomass would reduce in both systems, the grass monoculture and the grass-legume association. For the latter, however, reductions would be of a lower magnitude (maximum reduction of 16.6 vs. 35.3% for the monoculture). The highest losses are to be expected during dry season from January to March. These effects on forage productivity are the

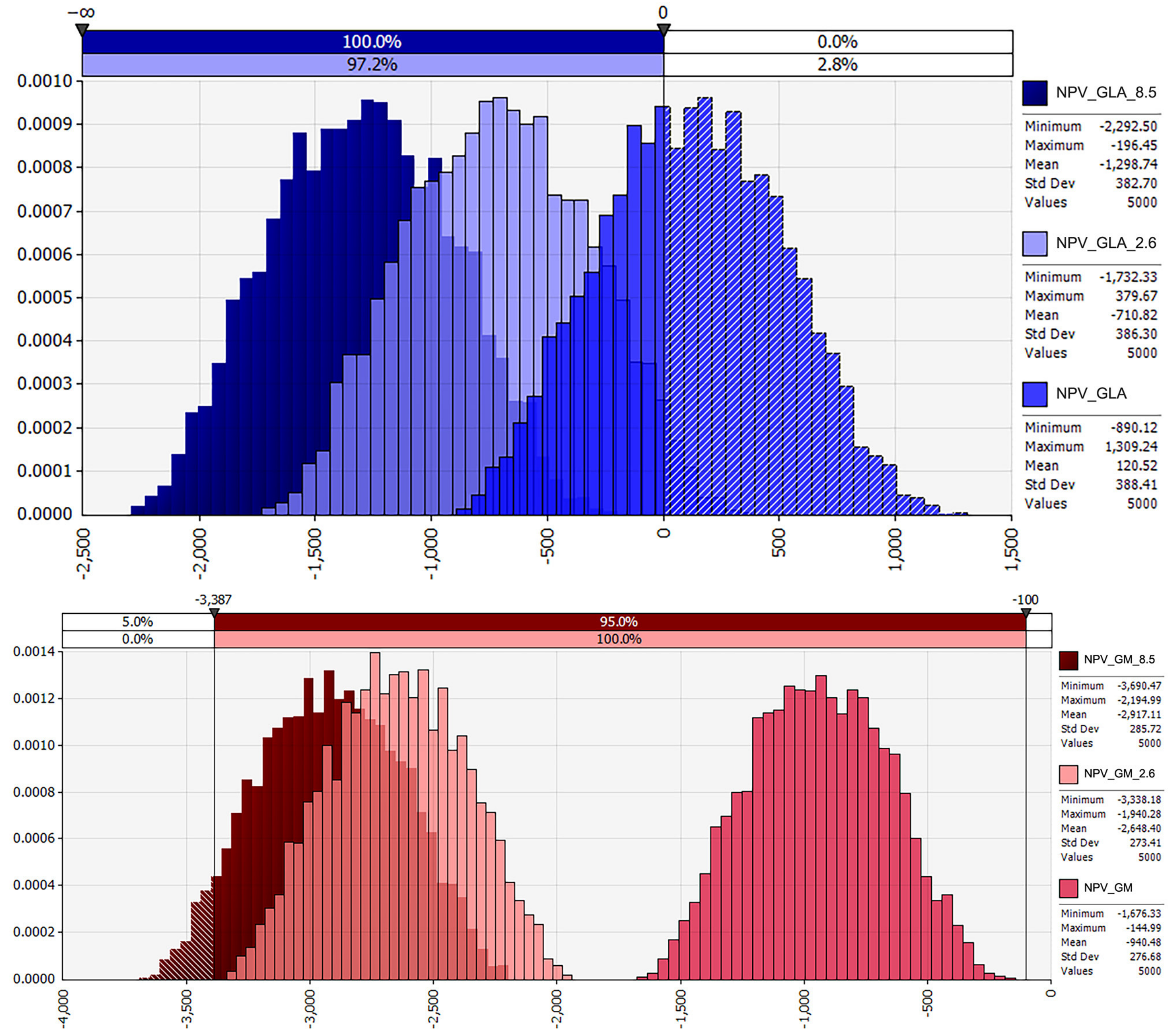

FIGURE 6 | Estimated change in suitable areas for Centauro by 2050 under RCP 2.6 **(left)** and RCP 8.5 **(right)**.

result of a combination of increased temperatures, variations in precipitation levels and atmospheric CO_2 concentrations caused by climate change (Thornton et al., 2009; Rojas-Downing et al., 2017). Not only would forage productivity be affected if the favorable environmental conditions changed but also the potential distribution of Centauro and other *Arachis* varieties. Centauro would migrate to higher altitudes more favorable for its development and the overall potential area for distribution would decrease. This could pave the way for the arrival of new (invasive) species or native grasses with a better adaptation capacity to the conditions projected for 2050. Although in our model we only made projections for Centauro, the impacts of climate change in the Orinoquía would also affect the distribution, quantity and quality of other forage species by up to 60% according to estimates provided by CIAT CORMACARENA (2018), putting livestock production in a difficult position.

Although the modeled impacts from the climate change scenarios were relevant for both alternatives, the grass monoculture and the grass-legume association, the latter shows a better adaptation capacity. This can be attributed to the symbiotic effect between the legume and the grass associated with the contribution of nitrogen-fixing (Dubeux et al., 2017; Pereira et al., 2019; Villegas et al., 2020). The higher availability of N improves both the yields and persistence of the grass, and comes with the co-benefit of mitigating GHG emissions through reducing (i) methane emissions (as a result of an improved diet), and (ii) synthetic fertilizer use (resulting in lower N_2O emissions). Simultaneously, *Arachis* has positive

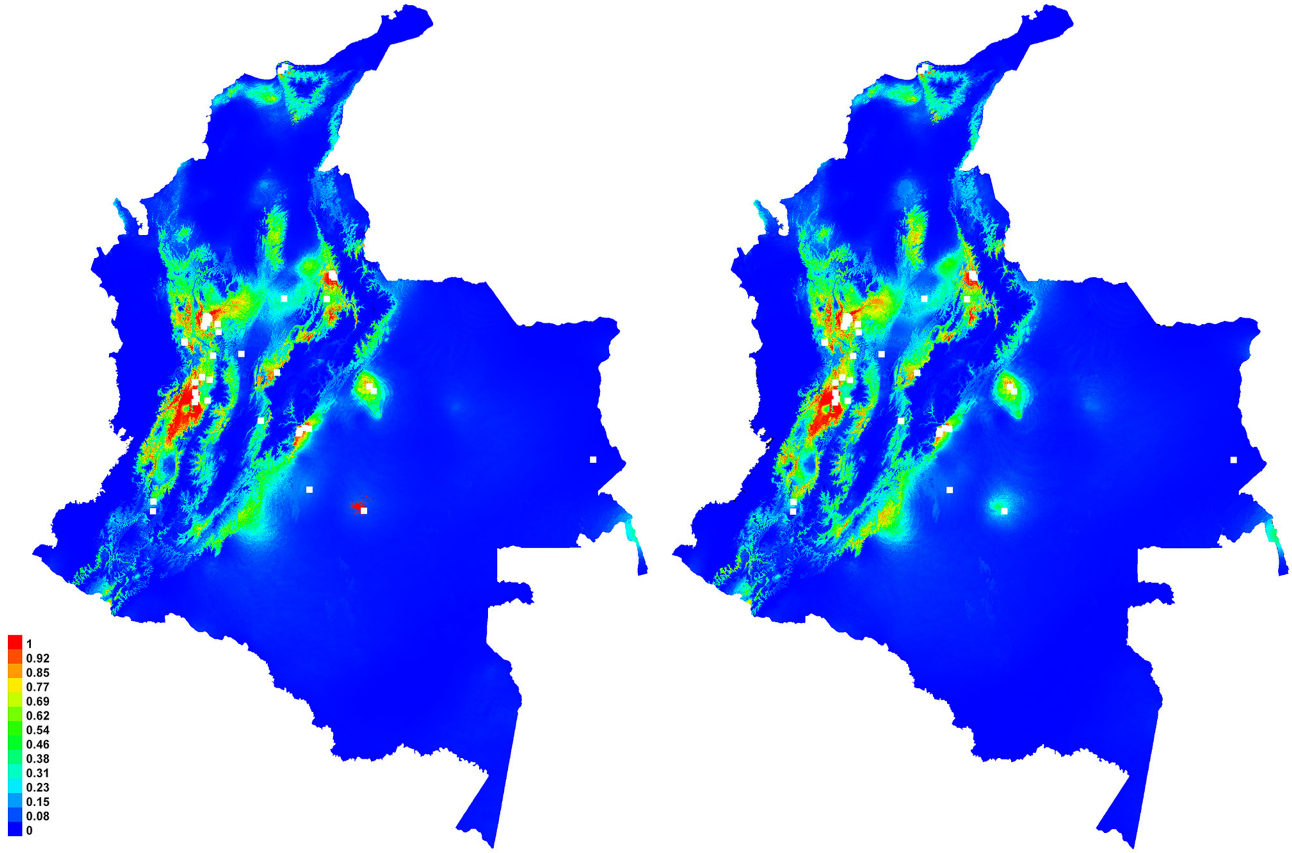

FIGURE 7 | NPV probability distribution for the grass-legume association and the grass monoculture under climate change scenarios. GLA, Grass-legume association; GM, Grass monoculture.

impacts on the physical and chemical properties of the soil, and contributes to increasing both soil microfauna and organic matter (Schultze-Kraft et al., 2018). *Arachis* accessions in particular, offer dense soil cover and, therefore, prevent soil erosion problems (Schultze-Kraft et al., 2018).

The effects of climate change on forage biomass production would lead to a strong decrease in milk production in both systems. The grass-legume would, however, be less affected (-19%) than the grass monoculture (−56%). This in turn would lead to significant economic losses in the dual-purpose production systems in the region. Given the low productivity levels and values of technical indicators in dual-purpose livestock systems, profitability margins are inherently sensitive to small changes in production levels. The effects of climatic variations on livestock production have been identified in the literature as one of the main impacts (e.g., Garnett, 2009; Thornton et al., 2009; Nardone et al., 2010; Henry et al., 2012). The severity or level of the impact varies significantly between regions, however (Rojas-Downing et al., 2017). For the Orinoquía region, CIAT CORMACARENA, 2018 predict that the impact of climate change would lead to significant losses in cattle live weight gains and dairy production, and lower birth and increased mortality rates. As mentioned in the methodology, our study only considers changes in dairy production as a result of the impacts of climate change on pasture productivity. Production could also be affected, however, by other possible effects not considered in our study, such as effects on health, growth and reproduction, water availability, and the distribution of pests and diseases (Garnett, 2009; Thornton et al., 2009; Rojas-Downing et al., 2017). In particular, periods of heat stress could become the main source of loss at the productive level in the livestock sector (Garnett, 2009; Nardone et al., 2010). This scenario does not, however, consider potential technological changes nor the inclusion of other (new) species better adapted to the predicted regional climate change scenarios. Likewise, our study does not include any potential benefits that might be derived from culled cows on the beef market.

Despite the benefits of including *Arachis pintoi* and other legumes in cattle systems, adoption levels remain low. Several studies have identified some of the factors that limit the adoption of *Arachis* accessions in countries such as Colombia and Costa Rica, including a lack of commercial seed availability, high establishment costs of planting material, limited technical information on the establishment and management of the material in pastures, a lack of promotion and little knowledge about its benefits (CIAT, 2004; Wunscher et al., 2004; Lascano et al., 2005). A particularly important issue is seed supply, which also continues to be a restriction in the dissemination and adoption processes for the new Centauro accession. In this sense, it is necessary to develop focused strategies, for example, artisanal seed production by cattle producers, as this could not only contribute to generating higher technology adoption levels. Focused strategies could also play an important role in providing additional income, assuring income diversification, and opening new business alternatives for young people and women. In the long run, this would strongly contribute to supporting both the rural economy and sustainable intensification processes in the region. In addition, the increased demand and adoption of the legume could generate interest from the private sector for seed production.

In addition to the above-mentioned limiting factors, there are structural conditions that could slow down or discourage sustainable intensification. The prevailing tradition of extensive production systems and low land prices, for example, make it more efficient to acquire more (new) land than to intensify existing land (White et al., 2001). In particular, in regions such as the Orinoquía, where land is relatively abundant and are prices low, producers continue to favor more extensive systems at the cost of deforestation processes. Even if the costs of implementing new technologies are below land prices, cattle producers may not reduce the area, since one of the main reasons for land expansion is to secure land ownership rights (Kaimowitz and Angelsen, 2008). This may be favored by speculation processes in land prices, where a high price generates additional incentives for extension, given the increase in the value of capital gains (Smith et al., 1997). These speculation processes could also, however, promote intensification if the amount of land that can be acquired is reduced, for example by regulations (Smith et al., 1997). Unfortunately, in most cases producers may not be willing to intensify until land is scarce and most forests are gone (Kaimowitz and Angelsen, 2008). Similarly, if producers have few alternatives to invest their savings other than cattle production, this can contribute to the expansion of pasture areas. This situation is further aggravated by the precarious controls on land tenure and the lack of monitoring and control regarding the expansion of the agricultural frontier. Positive advances have been documented in Costa Rica, where the agricultural frontier cannot be expanded any further as a result of the little remaining forest area and high land prices, forcing cattle producers to use their land more efficiently, e.g., through incorporating *Arachis pintoi* in their pastures and adopting *Cratylia* protein banks (White et al., 2001). The opposite was documented in Peru, where land is still abundant and cheap, and market access is limited. Producers failed to adopt legumes such as *Arachis* in Peru, given the higher level of investment required (White et al., 2001).

Intensification strategies in the Orinoquía have been a subject of debate, mainly in environmental terms, since the introduction improved forages (*Brachiaira* species) in the 1970s. On the one hand, different studies have reported positive impacts associated with intensification processes with improved forages, such as (i) lower incidence of degradation of native savannas in intensified areas with improved forages, since they reduce the pressure to produce animal feed in the native savannas (Smith et al., 1997); (ii) reduction of greenhouse gas emissions associated with burning native savannas (Smith et al., 1997), carried out to increase grassland and savanna productivity in the short term at the expense of eliminating vegetation cover and nutrient availability in the long-term (Peñuela et al., 2014); and (iii) reduction of nitrous oxide emissions, associated with Biological Nitrification Inhibition (BNI) in *Brachiaria humidicola* pastures (Subbarao et al., 2009, 2017; Moreta et al., 2014). On the other hand, however, negative effects have also been reported and include (i) the loss and degradation of native savannas and threats to biodiversity (decrease in bird, animal and fish species), with gallery forests being the ecosystems under the greatest threat in the most intensified areas (Smith et al., 1997); (ii) increases in deforestation levels to expand grazing areas with introduced forages and, therefore, compromising ecosystem stability and functions (e.g., altering microclimates and shifting the rates of consumption and supply of light, water and mineral nutrients), and increasing greenhouse gas emissions due to land-use changes (Williams and Baruch, 2000; Reid et al., 2010; Peñuela et al., 2014; CIAT CORMACARENA, 2018); (iii) a displacement of native species given the aggressive growth characteristics, invasive behavior and fire resistance of *Brachiaria* species, particularly *Brachiaria humidicola* in savannas and highlands (Peñuela et al., 2014); (iv) increased soil erosion processes (Peñuela et al., 2011); and (v) increased frequency and intensity of fires due to establishment and management processes and the large standing necro mass left by grasses of African origin (such as *Brachiaria*) at the end of the dry season that facilitate the combustion (Williams and Baruch, 2000). In the case of the new Centauro variety, although its introduction into livestock systems provides a strategy to restore degraded areas, improve productivity and provide ecosystem services, it could also be a technology that promotes deforestation processes and has negative impacts on protected ecosystems. The higher profitability associated with new technologies, such as Centauro, could, for example, lead producers to increase their herd size and hence their pasture area. Likewise, profitable technologies can also provide farmers with the additional capital they need to finance livestock expansion (Kaimowitz and Angelsen, 2008).

In this sense, diffusion and adoption processes of new technologies like Centauro must be accompanied by land use governance and management policies. These policies require a multidimensional approach that includes the development of coordinated land tenure security policies, specific economic incentives aimed at promoting sustainable intensification (e.g., special credit lines, conservation requirements to access benefits or credits), integrated planning and zoning of land use, protection of forests and ecosystems, and tracking and monitoring of land use change, particularly at the agricultural frontier. This also implies greater institutional coordination and coordination between national policies (e.g., related to land use, agriculture, rural development), partnerships between the public and private sectors, and local communities that increase the effectiveness of policies and other instruments. Brazil for example, has reported a notable reduction in deforestation rates in the Amazon region, which has been the result of a combination of multiple public and private mechanisms for the protection of forests (FAO, 2016). For example, the new Brazilian Forest Code (Federal Law No. 12,651/2012; Presidência da República, 2012) obliges rural land owners to submit data with geographic coordinates for the registration of private rural properties, certify their intention to comply environmental regulations, and in cases where this does not occur, land owners are subject to administrative, civil or criminal processes and charges. Commercial banks are required (in accordance with the Forest Code) to request rural land owners and holders to provide a registration certificate from the Rural Environmental Registry before granting loans for agricultural purposes. Zero deforestation agreements for livestock signed by major beef companies have helped in reducing deforestation in certain parts of Brazil (Gibbs et al., 2015) and the Brazil Green Bag Initiative (Presidência da República, 2012) is a conditional cash transfer program with a commitment to responsibly manage resources and conserve ecosystems (FAO, 2016).

CONCLUSIONS

The results of this study suggest that integrating the legume *Arachis pintoi* CIAT 22160 cv. Centauro in a *Brachiaria humidicola* cv. Humidicola monoculture has great potential to improve both productive and economic indicators in the dual-purpose cattle production system of the Orinoquía region. Not only that, Centauro also helps in generating important ecosystem services with positive effects on, for example, the quality and persistence of the associated grass (restoration of degraded pastures), the soil system and biodiversity. Centauro improves the system's resilience to climatic variations, which is especially important considering the rather pessimistic climate projections for the region. These attributes make the inclusion of Centauro in the production system a key alternative for sustainable intensification in the region, and thus also contributes to achieving other environmental objectives such as the liberation of areas for reforestation purposes or the protection of local ecosystems.

It is important to mention, however, that, despite their numerous environmental benefits (see section Potential Ecosystem Services of *Arachis pintoi* CIAT 22160 cv. Centauro) and because of their economic and social benefits, forage technologies that are selected for intensification purposes (even if sustainable such as Centauro), bear a risk of misuse in regions and contexts where neither grasses nor legumes should be planted. This could lead to results contrary to the objectives of sustainable intensification and could therefore negatively affect local landscapes with significant ecological consequences. In the case of the Orinoquía region, this includes for example the promotion of deforestation or the penetration of important local ecosystems (such as native flooded savannas). The Orinoquía has a high degree of vulnerability to changes generated by human actions, which include transformations of productive models that are ignorant of the natural cycles threatening the ecosystem balance. The continuous search for productivity increases has led to significant changes in the productive models of the region, including, for example, the introduction of improved pastures in floodable savannas (Peñuela et al., 2011). This situation is likely to worsen considering the imminent effects of climate change, and threatens the savanna ecosystem as it could become subject to desertification processes as a consequence of inadequate natural resource management (Peñuela et al., 2011). To avoid such unwanted consequences, effective technology diffusion approaches need to be applied (which include extension and training programs), involving institutions relevant to the region within a context that helps to close information and monitoring gaps. In the case of Centauro, the focus of such information efforts should be on the correct establishment and management of the legume, highlighting both potential economic benefits and environmental threats. This needs to go hand in hand with strong inter- and intra-institutional coordination, and the development of public policies and comprehensive monitoring and control mechanisms. National and regional multi-stakeholder platforms, such as the Colombian Roundtable for Sustainable Beef and Dairy (MGS) and its regional sub-roundtables, can fill some of the gaps—at least in short- to medium-term, e.g., through providing targeted information campaigns and trainings or developing indicators and frameworks for sustainable intensification of the sector. In the long term, however, and based on the abovementioned efforts of multi-stakeholder platforms, comprehensive public policies need to be developed, applied and monitored.

Accelerating climate change will also affect the Orinoquía region. Our study suggests that variations in the local climatic conditions would have significant impacts on the economic viability of the dual-purpose cattle systems of the region. It is necessary, therefore, to implement regional climate change adaptation and mitigation measures that include specific strategies for the local context. Among others, animal breeding strategies that improve cattle by crossing with rustic breeds, silvo-pastoral systems or scattered trees in pastures for heat stress reduction, and water harvesting for animal consumption, can significantly increase the adaptation potential of dual-purpose systems, particularly during dry seasons.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data was retrieved from another research project and is not publicly available. Requests to access these datasets should be directed to s.burkart@cgiar.org.

AUTHOR CONTRIBUTIONS

SB, KE, and DR: conceptualization and methodology. KE, DR, and ÁR: formal analysis. KE, DR, ÁR, and SB: writing the original draft, review, editing, and resources. SB: supervision, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

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Classification of Megathyrsus Maximus Accessions Grown in the Colombian Dry Tropical Forest by Nutritional Assessment During Contrasting Seasons

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The diversity and use of tropical forages for cattle feeding are the protagonists in livestock systems. The production and nutritional quality of forages represent a strategy of continuous research in animal feeding to help mitigate the environmental impact generated by tropical livestock. The objective of this study was to classify the nutritional behavior in contrasting seasons and the relationship with agronomic traits of a collection of 129 CIAT (Centro Internacional de Agricultura Tropical) accessions of *Megathyrsus Maximus* established in the Colombian dry tropics. By means of the near-infrared reflectance spectroscopy (NIRS) technique, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and *in vitro* dry matter digestibility (IVDMD) were determined under rainy and dry seasons as fixed effects. We measured plant height, dry matter biomass (DMB) and flowering in field. Aspects such as plant height and DMB did not show correlation with nutritional aspects, whereas flowering was correlated with the content of structural carbohydrates. Despite genotype and precipitation affecting nutritional value, there is relative nutritional steadiness in NDF, ADF, and IVDMD between seasons for some accessions. According to the cluster analysis carried out for each season, it was evidenced that from the total collection, 51.2% of the accessions during the dry season and 19.4% of the accessions during the rainy season were classified with a better nutritional profile, thus, showing a higher number of materials with better nutritional behavior in the dry season. Both the genotypic characteristics of *M. maximus* and environmental conditions during contrasting seasons are factors that might influence the variability of the nutritional content, productive parameters, and flowering. Additionally, fodder material classification under Hotelling's T-squared test and Nutritional Classification Index suggests accessions that might be promising for resilient nutritional quality and adequate DMB, which proves that *M. maximus* could become an alternative for animal feeding and sustainable livestock production during critical dry periods in tropical agroecosystems.

Keywords: forages, grassland, Guinea grass, livestock, *Panicum*

INTRODUCTION

The expansion of the agricultural frontier with crops and pastures in tropical regions of developing countries for food production requires implementing production strategies with an eco-efficient focus to sustainably meet the increasing demand for food (Rao, 2013).

The major part of livestock activity in intertropical regions is carried out under grazing systems and mixed model systems (concentrated pastures), (Gerber et al., 2015). Food for these livestock systems based on pastures is developed through the production of forages, which depends on the rainfall pattern (Castañeda et al., 2015; Gándara et al., 2017; Marcillo et al., 2021), which is influenced by the consequences of climate change. The instability in forage production brings along with it an increase in production costs because of the use of supplements (concentrates), (Morales-Vallecilla and Ortiz-Grisales, 2018) and nutritional variables that influence productivity (Cooke et al., 2020), thus, compromising both cattle feeding efficiency and the sustainable management of herds (Paul et al., 2020).

The diversity and use of tropical forages for livestock feeding are protagonists in tropical livestock systems. Characteristics such as biomass yield and nutritional quality depend on genetics, environment, and some other factors (Paul et al., 2020). Investigating and evaluating these characteristics will contribute to the development of forages adapted to the specific edaphoclimatic conditions of the tropics and identifying genotypes capable of producing "more with less," which, according to Rao (2013), is important for advancing toward an eco-efficient livestock system.

Megathyrsus maximus–Panicum maximum (Cook and Schultze-Kraft, 2015) is an African species that has been widely distributed in the warm areas of Colombia. Under edaphoclimatic conditions of the Colombian dry tropical forest, the response in terms of production is adequate during low-precipitation periods. Also, this grass has short recovery periods, tolerance of shade and moderate drought periods, tolerance of short flooding periods (Morales-Velasco et al., 2016; Matínez-Mamian et al., 2020), and an adequate response in association with forage legumes (Matínez-Mamian et al., 2020) and with silvopastoral systems (Barragán-Hernández and Cajas-Girón, 2019). This grass is promising for environmental management of cattle because of its potential for biological nitrification inhibition (IBN), (Carvajal-Tapia et al., 2021) and is outstanding for its nutritive value, perenniality, and adaptive potential, and for showing diversity among cultivars in terms of yield, forage quality, and response to nutrient fertilization (Benabderrahim and Elfalleh, 2021).

The nutritional quality of *M. maximus* in terms of protein and fiber content, and digestibility, has a wide range of values generated by different edaphoclimatic, genotypic, and management conditions. The attributes of adaptation to edaphoclimatic limitations, forage quality, and seed production facilitate the development of superior cultivars in current grass breeding activities (Rao, 2013). However, identifying the nutritional behavior of the species in a potential livestock area can help to find a versatile feeding alternative for the establishment and development of eco-efficient livestock production or to select material with improved fodder quality (Ramakrishnan et al., 2014).

The nutritional quality and association with the productive parameters of a broad range of accessions of *M. maximus* in Colombian tropical regions have not been described in detail or correlated with climatic factors. This is a relevant aspect in the identification of resilient forage species, particularly for the agricultural sector that faces the consequences of climate change. Therefore, we propose the hypothesis that the rainfall pattern that determines two contrasting seasons (rainy and dry) in tropical regions influences not only the agronomic behavior of the collection of *M. maximus* but also the nutritional composition and at the same time can be related to the productive variables of forages.

NIRS (near-infrared reflectance spectroscopy) is a fast and accurate technique with an eco-friendly technology to diagnose the nutritional quality of tropical forages (International Organization for Standardization ISO 12099:2017., 2017; Parrini et al., 2018; Mazabel et al., 2020). Since 2015, the CIAT forages and animal nutrition quality laboratory has worked on the development of NIRS predictive models, in particular, for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and *in vitro* dry matter digestibility (IVDMD) for tropical forages.

With the purpose of helping to identify promising forage crops for tropical areas and to classify potential germplasm for smallholder farmers or plant breeding programs, the object of this study was to classify the vegetative material of *M. maximus* established in the Colombian dry tropics according to nutritional behavior using NIRS methodology during contrasting seasons and the relationship with plant height, forage production, and flowering with nutritional quality.

MATERIALS AND METHODS

Location

The experiment was conducted in a tropical dry forest agroecosystem in the Patía Valley, which is located in the department of Cauca in southwestern Colombia, with an average temperature of 27.9°C and bimodal cycle with average annual precipitation of 1,414 mm (**Figure 1**). To guarantee the process of establishing experimental plots, we used water irrigation and mechanical weed control.

The local soil is a medium-fertility Mollisol. Chemical analysis in the 0-to 20-cm layer showed pH of 6.26, organic matter content of 4.50%, phosphorus content of 6.3 ppm, and calcium, magnesium, and potassium content of 14.58, 6.91, and 0.59 cmol/kg, respectively. 1 year after establishment of the experimental plots, we applied fertilizer only once at a rate of 150 kg N/ha and 95 kg P/ha.

Experimental Design in Fields

For the agronomic and nutritional evaluation in December of 2015, 129 accessions of *M. maximus*, including commercial varieties provided by the germplasm bank of the International Center for Tropical Agriculture (CIAT) and two improved

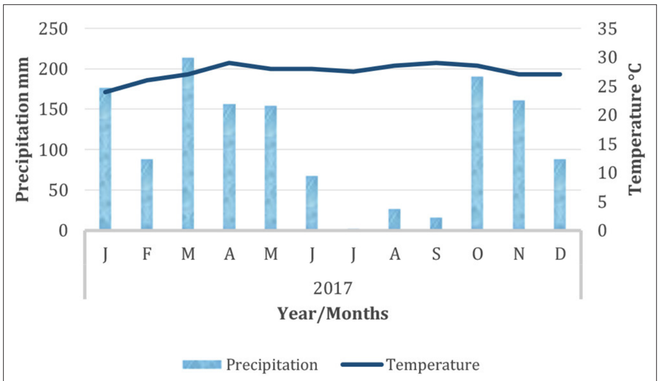


FIGURE 1 | Average temperature values and rainfall accumulation during experiments in field trials. Coordinates: N: 1°59′13″; W: 77°5′57′″, Patía Valley. Source: NUTRIFACA Weather Station, 2016–2018.

TABLE 1 | Centro internacional de agricultura tropical (CIAT) accession numbers and origin of evaluated *Megathyrsus maximus* and commercial cultivars.

Origin	CIAT accessions
Kenya	622, 688, 691, 692, 693, 6,526, 6,536, 6,571, 6,890, 6,891, 6,893, 6,897, 6,898, 6,900, 6,901, 6,903, 6,906, 6,912, 6,915, 6,918, 6,923, 6,981, 6,982, 6,983, 6,984, 6,986, 6,990, 6,996, 16,003, 16,004 y 16,005
Tanzania	6,927, 6,928, 6,929, 6,944, 6,945, 6,948, 6,949, 6,951, 6,954, 6,955, 6,960, 6,963, 6,967, 6,968, 6,969, 6,975, 16,011, 16,017, 16,018, 16,019, 16,021, 16,023, 16,025, 16,027, 16,028, 16,034, 16,035, 16,036, 16,038, 16,039, 16,041, 16,044, 16,046, 16,048, 16,049, 16,051, 16,054, 16,055, 16,057, 16,058, 16,059, 16,060, 16,061, 16,062, 16,064, 16,065, 16,068, 16,069 y 16,071
Unknown	673, 685, 6,094, 6,095, 6,171, 6,175, 6,461, 6,497, 6,500, 6,501, 6,525, 6,658, 6,784, 6,787, 6,796, 6,799, 6,805, 6,831, 6,836, 6,837, 6,839, 6,840, 6,842, 6,843, 6,855, 6,857, 6,864, 6,866, 6,868, 26,723, 26,906, 26,911, 26,917, 26,923, 26,924, 26,925, 26,936, 26,937, 26,939, 26,942, 26,944 y 26,947
Ivory Coast	6,872
Rwanda	26,360
Commercial	6,962 Mombasa, 6,826 Coloniao, 16,031 Tanzania, 6,299 Tobiatá, 26,900 Vencedor y Massai

Urochloa species (*U. brizantha* cv. Toledo and hybrid cv. Cayman) as controls (**Table 1**), were established in plots using a randomized complete block design with three replications. The experimental units (plots) measured 4 m^2, and the plants had 10–12 tillers. The distance between plots was 1 m, and the distance between blocks was 2 m (**Figure 2**).

To determine the number of regrowing days and provide homogeneous conditions for all accessions, a standardization cut was applied. It was a mechanical cutting of plots at a residual height of 30 cm above the soil. Seasonal conditions in the field area and harvesting age are shown in **Table 2**.

We measured (a) plant height according to the methodology of Toledo and Schultze-Kraft (1982) and (b) flowering (FW). We used observations and calculated the percentage of flowering present in the experimental plot in a range of 0–100% at the time of evaluation. For dry matter biomass (DMB), we estimated the availability of green forage (GF) after cutting at the height of 30 cm from the ground and measuring the weight per plot in the field. Out of all the GF, we weighed subsamples of ∼200 g. These were dried in an oven with controlled ventilation at a temperature of 60°C (140°F) until reaching constant weight (48 to 72 h). With the final weight of the subsamples, we estimated dry matter.

Near-Infrared Reflectance Spectroscopy Testing in the Laboratory

The subsamples obtained in the field to determine DMB were analyzed in the CIAT forages and animal nutrition quality laboratory, where they were pulverized using a Retsch SM 100 (Retsch GmbH, Haan, Germany) with a 1-mm bottom screen. For NIRS processing, we used a Foss 6,500 model and ISIS software (IS-2,250) version 2.71 (FOSS and Infrasoft International, USA, 2005). For each sample, duplicates of the spectra were taken in separate quartz cells of 3.5-cm internal diameter and 1-cm thick. The wavelength range was from 400 to 2,500 nm.

The values obtained through wet chemistry were used to build chemo metric models (Mazabel et al., 2020) and generate predictive equations in NIRS. Chemical analyses were performed in duplicate for each accession in both seasons (rainy and dry) under the guidelines of the 21st edition of the Official Methods of Analysis of (AOAC International, 2002). Crude protein content was determined using the FOSS Kjeltec™ 8,100 (Foss Company, HillerØed, Denmark). An ANKOM 2,000 fiber analyzer (ANKOM Technology Corporation, Macedon, NY, USA) was used for NDF and ADF (Van Soest et al., 1991) and for IVDMD (Tilley and Terry, 1963).

The results of the reference chemical analysis and the spectral signals of each sample were processed using Win ISI software version 4.0. Then, the results were incorporated in equations generated at the CIAT forages and animal nutrition quality laboratory, as follows: R^2 of 0.93, 0.98, 0.85, and 0.98 and standard error for cross validation (SECV) of 2.11, 1.22, 2.78, and 0.61 for NDF, ADF, IVDMD, and CP, respectively (Molano et al., 2016). This increases the action range and accuracy of the model.

Data Analysis

Descriptive statistics and Pearson correlation coefficient for every season were obtained with SAS Statistical Software (Statistical Analysis System) version 9.4 (2018) (SAS, 2016). Figure of correlation was obtained with package corrplot in R (Wei and Simko, 2017). Cluster analysis was used, and principal components were calculated using the library "FactoMineR" and package "Factoextra" (Kassambara and Mundt, 2020) with the variables NDF, ADF, CP, and IVDMD for every season. Figures were created in R using the package "ggplot2" (Wickham, 2016). Wilcoxon sum rank test was used to compare differences between means in terms of the season for each of the variables in R version 4.0.3 (R Core Team, 2020).

To find a classification index for the fodder material according to nutritional content, multicriteria weighted indices were adapted (Contreras et al., 2004). To obtain a level

FIGURE 2 | Aerial view of the field experimental design. R, replications.

TABLE 2 | Seasonal conditions and plant harvesting parameters for agronomy and nutritional evaluation in the Patía Valley, Cauca, Colombia.

Season	Plant harvesting parameter		Period of evaluation from	Temperature (°C)			Humidity average %	Total precipitation (mm)
	Regrowing	Average height (cm)		Minimum	Maximum	Average	Average	
Rainy	6 weeks or 41 days	130.7	March 24 to May 4, 2017	21.5	31.8	26.7	77	172.1
Dry	8 weeks or 55 days	55.2	June 30 to August 24, 2017	19.6	36.1	27.8	61.7	22.8

of classification, a value was assigned to each variable considering the relative importance with regard to nutritional assessment of CP, NDF, ADF, and IVDMD in consumption, use, and rumen degradability-diet composition (Van Soest, 1982; Barahona-Rosales and Sánchez-Pinzón, 2005). The Nutritional Classification Index was calculated as follows:

NCI = (IVDMD R *8 + IVDMD D *7 + CP R *6 + CP D *5 + NDF R*4 + NDF D *3 + ADF R*2 + ADF D *1)/8,

where NCI is the Nutritional Classification Index, IVDMD R is the *in vitro* dry matter digestibility rainy season, IVDMD D is the IVDMD dry season, CP R, is the crude protein rainy season, CP D is the CP dry season, NDF R is the neutral detergent fiber rainy season, NDF D is the NDF dry season, ADF R is the acid detergent fiber rainy season, and ADF D is the ADF dry season.

To select accessions without significant changes in nutritional composition in the evaluation from one season to the next, the Hotelling T-squared test was performed using the Hotelling library and package corpcor in R (Schafer et al., 2017).

RESULTS

The contrasting seasons present in the Colombian dry tropics might explain the differences found in this research regarding the agronomic and nutritional behavior of *M. maximus*. Flowering, plant height, BDM, and CP decreased during the dry season compared with the rainy season at 64.8, 57.8, 43.1, and 27.7%, respectively (**Table 3**). Low precipitation, the lowest relative humidity, and the highest temperature (**Table 2**) were determining factors for the changes observed mainly in the agronomic variables. The average NDF, ADF, and IVDMD contents of the *M. maximus* collection differ from 1 to 2% from one season to the other. The Wilcoxon test for comparison of means indicates statistical differences when the accessions are under different rainfall conditions (**Table 3**).

Commercial cultivars of *M. maximus* show a similar nutritional behavior as the rest of the studied collection. During the dry season, NDF content increased slightly except in

TABLE 3 | Descriptive statistics and significance between seasons of the nutritional composition and agronomic traits of a collection of *Megathyrsus maximus* in Colombian dry tropical.

Variable	Season	$\bar{x}$	Median	SD	Minimum	Maximum	*p*-value
NDF (%)	Rainy	66.5	66.5	1.47	63.2	70.7	0.00118
	Dry	67.2	67.1	2.17	62.1	74.0	
ADF (%)	Rainy	39.2	39.0	1.48	35.3	42.6	0.00000
	Dry	38.3	38.2	1.54	34.7	44.0	
CP (%)	Rainy	10.1	10.1	0.95	7.6	13.9	0.00000
	Dry	7.3	7.3	0.98	4.9	10.5	
IVDMD (%)	Rainy	57.9	58.0	2.13	52.3	62.6	0.00126
	Dry	59	59.2	2.78	50.0	65.3	
Height (cm)	Rainy	130.7	132.7	19.27	74	163.3	0.00000
	Dry	55.2	55.0	7.87	35	76.7	
Biomass (t/ha)	Rainy	5.8	5.6	1.42	2.5	9.5	0.00000
	Dry	3.3	3.2	0.79	1.6	5.3	
Flowering (%)	Rainy	76.2	100	34.19	34.1	100	0.00000
	Dry	26.8	20	25.78	0	100	

NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; IVDDM, in vitro digestibility of dry matter.

Mombasa, Massai, and Coloniao. In contrast, ADF content decreased, except in Tanzania. Tanzania shows higher CP content and the lowest NDF y ADF content during the rainy season. Mombasa and Coloniao stand out for featuring the lowest NDF and ADF content during the dry season. Vencedor and Coloniao showed high IVDMD during the rainy season and Mombasa in the dry season (**Figure 3**).

Analysis using Pearson's correlation coefficient shows that different degrees of associativity exist, highlighting values highly significant and superior ($r \geq 0.3$). Among the agronomic measurements, plant height is directly related to DMB in a positive manner ($r = 0.41$ and 0.48, rainy and dry season, respectively), whereas with flowering, it is related in a negative manner in the rainy season ($r = 0.39$). This could be interpreted as a high forage yield being estimated for the tall accessions in the rainy season during 42 days, and not presenting flowering or having low flowering upon finalizing the cutting period.

The positive relationship existing between flowering and structural carbohydrate content is evidenced in the two seasons. This suggests that physiological traits such as flowering could have a stronger relationship with the nutritional parameters in the *M. maximus* collection under the edaphoclimatic conditions of the Colombian dry tropical forest. Likewise, in **Figure 4**, a higher degree of associativity is noted among the traits estimated in the nutritional evaluation.

In both seasons, the structural carbohydrate content of *M. maximus* influenced CP content in a negative manner. The correlation is higher for ADF content.

In the rainy season, ADF ($r = 0.65$) shows a moderate and negative correlation with IVDMD, higher than when we refer to NDF ($r = 0.49$). NDF and ADF have an evident positive correlation, resulting from the use of NDF content in the ADF calculation (**Figure 4**).

For the cluster analysis, three clusters (Cl) were defined (**Table 4** and **Figure 5**) considering the degree of resemblance in specific characteristics of the accessions for each cluster. For both seasons, the best nutritional composition corresponds to accessions of Cl 1; some accessions and material of genus *Urochloa* have lower NDF and ADF and higher CP and IVDMD, contrary to what Cl3 shows, with accessions having lower nutritional *content* with higher NDF and ADF and lower CP. Cl2 materials are characterized by having an intermediate composition between Cl1 and Cl3 (**Tables 4**, **5**). In dry and rainy seasons, 51.2 and 19.4% of the collection, respectively, stands out for its nutritional profile. Therefore, a higher number of accessions have a great nutritional profile during the dry season in the tropics and are available for further study.

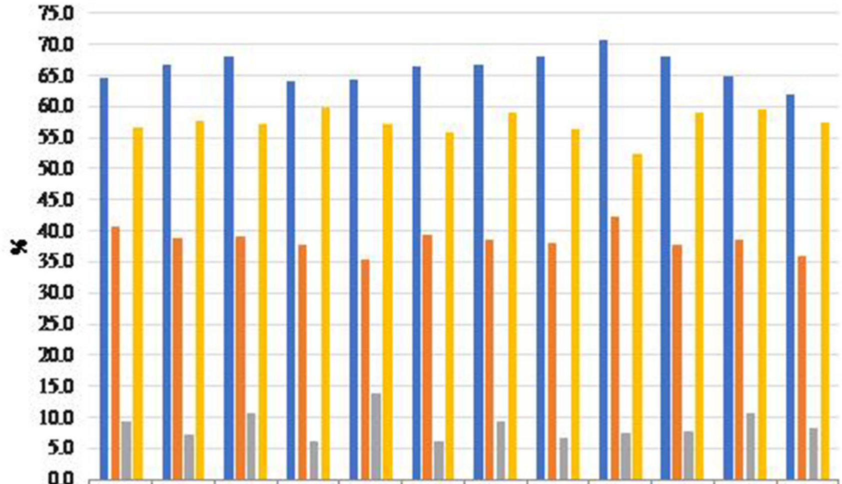
The distribution of the clusters (**Figure 5**) shows the description of the correlations and the different nutritional behavior from *Megathyrsus* and *Urochloa* species, during both seasons. Also, during the rainy season, the response of Tanzania stands out.

In each season, the following accessions stand out for being part of the 41.9% of the collection with DMB above average at 5.9 and 3.4 t/ha in the rainy and dry season, respectively, and being classified in the cluster with the best nutritional profile (Cl).

In the rainy season, accessions CIAT 6,501, 6,842, 6,868, 16,004, 16,023, 16,048, 16,062, 16,071, and 26,723 stand out; in the dry season, accessions CIAT 693, 6,171, 6,497, 6,658, 6,836, 6,891, 6,898, 6,903, 16,005, 16,011, 16,025, 16,027, 16,034, 16,035, 16,036, 16,038, 16,039, 16,044, 16,049, 16,058, 16,059, 26,936, 26,937 and Massai stand out.

For the NCI, the highest indices correspond to accessions 685 (199.05) and 6,864 (197.30), belonging to Cl1 in both seasons. Accession CIAT 26,911 had one of the highest values for NDF, also standing out for its value in NCI (198.91).

On the other hand, Hotelling's multivariate T-squared test showed that accessions 6,968, 26,360, and 26,947 did not feature



	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
	Tobiata		Mombasa		Tanzania		Vencedor		Massai		Coloniao	
NDF (%)	64.6	66.7	68.2	64.0	64.4	66.5	66.8	68.0	70.7	68.2	64.8	62.1
ADF (%)	40.7	38.8	39.1	37.8	35.3	39.4	38.5	38.1	42.3	37.8	38.6	35.9
CP (%)	9.3	7.1	10.8	6.2	13.9	6.1	9.4	6.8	7.6	7.8	10.6	8.3
IVDDM (%)	56.5	57.6	57.1	59.7	57.2	55.8	58.9	56.5	52.3	58.9	59.6	57.3

NDF (%) ADF (%) CP (%) IVDDM (%)

FIGURE 3 | Comparative analysis between commercial cultivars of *Megathyrsus maximus* in terms of CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVDMD, *in vitro* dry matter digestibility.

significant changes from the rainy to dry season in NDF, ADF, CP, and IVDMD, and their NCI surpassed 189.94.

Discussion

Edaphoclimatic stress factors are abiotic indicators that become important in the search for forage material adapted for intensive production in a sustainable manner (Rao, 2013). In the Patía Valley region, a representative dry tropical agroecosystem, the evaluations set up in this research during contrasting seasons allowed us to compare the agronomic and nutritional behavior of a collection of *M. maximus*, helping to identify physiological mechanisms and the association of flowering with nutritional traits, which contributes to the selection of interesting traits. This provides tools so that breeding programs can broaden their research when seeking forage material resilient to climate change.

Plant height, flowering, DMB and crude protein of the collection were higher during the rainy season, contrasting with stress, growth, and production limitations during the dry season (Hare et al., 2015), which indicates that the water supply favors agronomic characteristics and protein content (Larsen et al., 2021). Weather characteristics have an effect on agronomic and nutritional parameters for *M. maximus* (Machado, 2013; Lemos et al., 2017; Maranhão et al., 2021; Marcillo et al., 2021).

Productive Measurements and Flowering

The mean values for plant height and DMB reached by the *M. maximus* germplasm were similar and superior to those registered in other tropical regions (Machado, 2013; Benabderrahim and Elfalleh, 2021), with fertilization (Braz et al., 2017) or higher rainfall (Macedo et al., 2017).

Studies with commercial varieties suggest that, at 70-to 90-cm height, a higher quantity of biomass is generated with adequate grassland recovery for the next grazing (Soares Filho et al., 2015; Carvalho et al., 2017). In the rainy season, the entire collection reached the mínimum value of the range; whereas, in the dry season, this was obtained only by accessions 16,035, 691, 6,982, 6,960, and 6,915 (**Supplementary Material**). For DMB, an important variable for adoption processes by farmers in tropical countries (Mwendia et al., 2019), the mean and maximum values (5.8 and 9.5 t/ha, respectively) of the collection during the rainy season were similar to those reported in previous studies in the same zone with commercial cultivars (6.3 and 9.8 t/ha, every 45 days) (Vivas-Quila et al., 2015). In spite of the dry season, the average and maximum values of DMB declined notably (3.3 and 5.3 t/ha, respectively). The values obtained were also higher than those obtained with naturalized species in the Patía Valley region, and in different tropical regions such as Brazil (Macedo et al., 2017) and Cuba (Machado, 2013). These values were improved only in Thailand with nitrogen fertilization (Hare et al., 2015). In addition, the positive correlation between plant height and DMB (**Figure 4**) might indicate that the evaluated collection presents adequate DMB yield under the edaphoclimatic conditions of the Patía Valley.

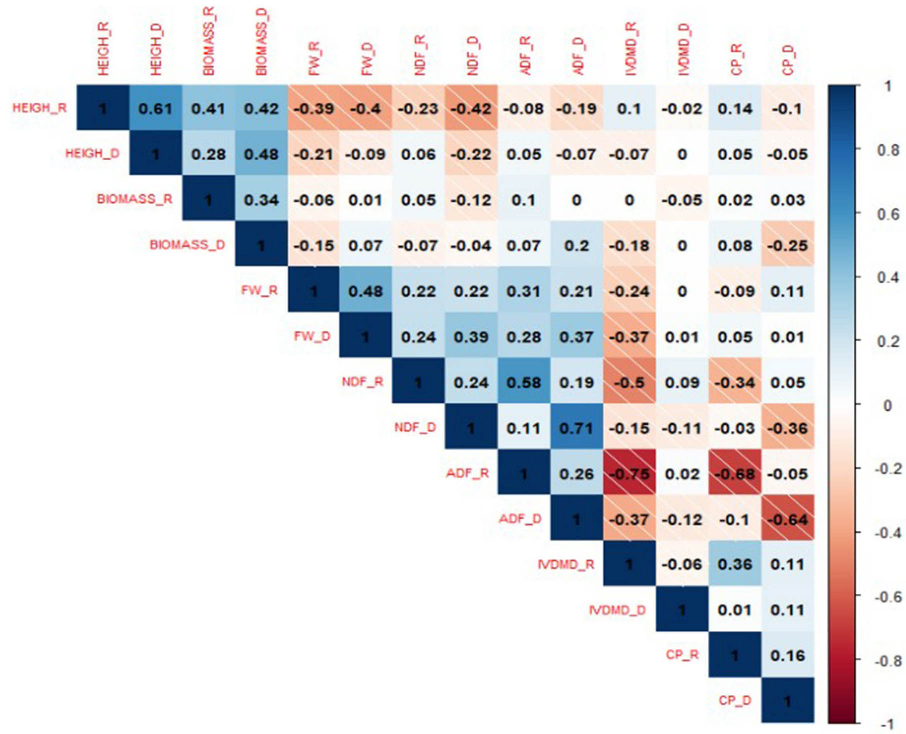


FIGURE 4 | Correlograms with Pearson coefficient to visualize correlation among agronomic and nutritional variables of the *Megathyrsus maximus* collection in the Patía Valley of Colombia. BIOMASS_R, biomassa dry matter in rainy season; BIOMASS_D, biomassa dry matter in dry season; Heigh_R, in rainy season; Heigh_D, in dry season; FW_R, flowering in rainy season; FW_D, flowering in dry season; NDF_R, neutral detergent fiber in rainy season; NDF_D, neutral detergent fiber in dry season; ADF_R, acid detergent fiber in rainy season; ADF_D, acid detergent fiber in dry season; CP_R, crude protein in rainy season; CP_R, in dry season; IVDMD_R, *in vitro* dry matter digestibility in rainy season; IVDMD_D, *in vitro* dry matter digestibility in dry season.

TABLE 4 | Nutritional behavior per cluster in a *Megathyrsus maximus* collection during rainy and dry seasons in Colombian dry tropical forests.

Cluster	Number of accessions		NDF (%)		ADF (%)		CP (%)		IVDMD (%)	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
1	25	66	64.7 ± 1.6c	65.9 ± 1.7c	37.0 ± 1.4c	37.2 ± 1.1c	11.4 ± 0.7a	8.0 ± 0.8a	59.9 ± 1.7a	60.2 ± 2.0a
2	55	30	66.6 ± 1.3b	67.4 ± 1.8b	38.6 ± 0.7b	38.6 ± 1.0b	10.0 ± 0.7b	7.1 ± 0.6b	59.0 ± 1.0b	55.9 ± 1.9c
3	51	35	67.4 ± 1.1a	69.5 ± 1.9a	40.6 ± 0.8a	40.2 ± 1.3a	9.5 ± 06c	6.4 ± 0.6c	55.8 ± 1.3c	60.0 ± 1.9b

Note. NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; IVDDM, in vitro digestibility of dry matter. Different letters denote statistical differences according to analysis of variance and Tukey HSD test (α = 0.05).

Megathyrsus maximus is usually described as drought resistant (Rodríguez et al., 2017) with adaptation to varied edaphoclimatic conditions because of its clumps and strong root system (Kissmann and Groth, 1995; Benabderrahim and Elfalleh, 2021). However, it expresses its productive potential during the rainy season. Under the edaphoclimatic conditions of the Patía Valley and during the rainy period, it is possible to consider a recovery period of about 35 days, and it is advised to consider irrigation during the dry season to reach the potential of the species.

Flowering is a determining variable for plant breeding technology adoption processes. It is related to forage yield (Casler et al., 2018; Casler, 2019). Flowering determines nutritional composition (Gusha et al., 2019), specifically in this research with NDF and ADF content and persistency in the field. Light intensity might also affect flowering (Tavares de Castro and Carvalho, 2000). During the dry season, no flowering occurred, or it was lower than 10% for accessions: 622, 688, 693, 6,094, 6,175, 6,299 Tobiatá, 6,497, 6,500, 6,525, 6,658, 6,796, 6,837, 6,857, 6,868, 6,897, 6,901, 6,906, 6,918, 6,923, 6,927, 6,928, 6,948, 6,962, 6,963, 6,968, 16,003, 16,017, 16,023, 16,027, 16,028, 16,034, 16,035, 16,036, 16,038, 16,039, 16,048, 16,049, 16,051, 16,055, 16,061, 16,062, 16,069, 16,071, 26,360, 26,900 vencedor, 26,906, 26,923, 26,924, 26,925, 26,937, and 26,939 (39.5% of the collection), and during the rainy season for accessions 6,299 Tobiatá, 6,962 Mambasa, 6,963, 16,027, 16,028, 16,035, 16,044, 16,051, 16,061, 16,069, 16,071, 26,723, and 26,925.

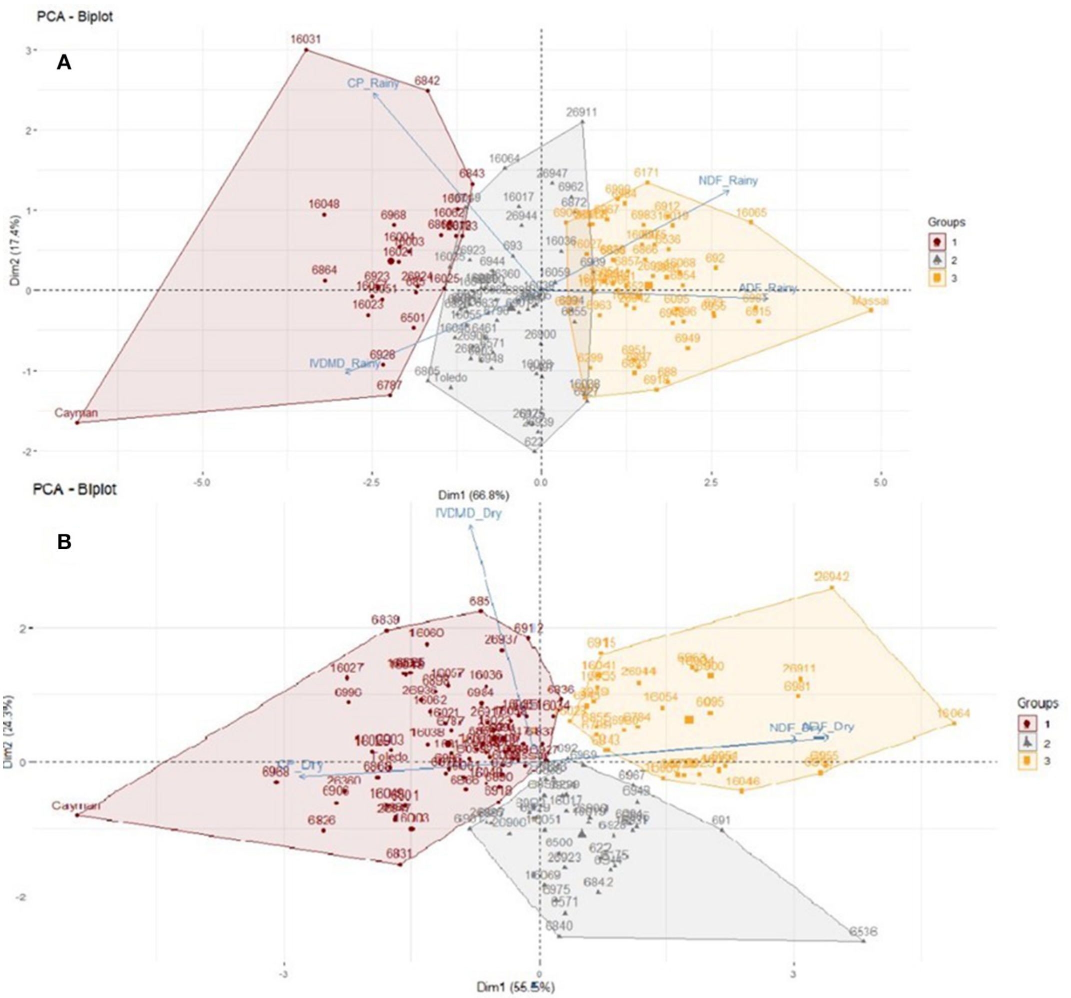


FIGURE 5 | Cluster analysis based on principal components of the germplasm collection of *Megathyrsus maximus*. Cumulative variance accounts for 86 and 80% for the rainy and dry season, respectively. ADF, acid detergent fiber; CP, crude protein; IVDMD, *in vitro* dry matter digestibility; NDF, neutral detergent fiber. **(A)** Rainy season. **(B)** Dry season.

Flowering was the variable that declined the most when it was evaluated in the dry season vis-à-vis the rainy season. Lower flowering in germplasm during the dry season despite better light conditions in the tropics could be associated with hydric stress (Wilson and Ng, 1975) and high evaporation, with the possibility that this could generate a negative hydric balance for forage production and the production process of grasses (Rao, 2013). According to (Atencio Solano et al., 2018) , there is an evident effect of the dry season on vegetative development, which influences flowering of the species. This matches the negative correlation between flowering and plant height in the rainy season ($r = 0.39$).

Nutritional Composition

Factors such as management, regrowth age, fertilization, cut height, phonological aspects, growth under shade, and season might have a significant effect on the nutritional value of forages (Van Soest, 1982; Velásquez et al., 2010; Santiago-Hernández et al., 2016; de Vasconcelos et al., 2019; Schnellmann et al., 2020; Tesk et al., 2020), which affects digestibility in animals (Valente et al., 2010). Variability in structural carbohydrates (NDF, ADF) in the *M. maximus* collection might be influenced by characteristics related to the accessions' own physiological and metabolic aspects such as the conversion efficiency of nitrogen and flowering rate (dos Costa et al., 2017), which might generate a wide range of available accessions and could be used in plant breeding programs (Deo et al., 2020) to produce or select materials with the best IVDMD (Barahona-Rosales and Sánchez-Pinzón, 2005).

The protein content decline during low precipitation periods, similar to that found by Larsen et al. (2021), might be caused by the lack of production of new leaves and tillers. Also, the senescent material decreases cellular content, in particular, protein (Vargas Junior et al., 2013). *M. maximus* shows a higher protein content during the rainy season and under shady

TABLE 5 | Grouping of the *M. maximus* collection by nutritional behavior in rainy and dry seasons of the Patía Valley, Cauca, Colombia.

Season	Cluster 1
Rainy	685, 6,501, 6,787, 6842, 6,843, 6,864, 6,868, 6923, 6,928, 6,968, 16,003, 16,004, 16,018, 16,021, 16,023, 16,025, 16,031, 16,048, 16,051, 16,057, 16,062, 16,071, 26,723, 26,924 and Urochloa hibrido cv Cayman
Dry	673, 685, 688, 693, 6,171, 6,461, 6,497, 6,501, 6525, 6,658, 6,787, 6,826, 6,831, 6,836, 6,837, 6,839, 6,864, 6,866, 6,868, 6,872, 6,890, 6,891, 6,898, 6903, 6,906, 6,912, 6,918, 6,927, 6,962, 6,968, 6,983, 6,984, 6,986, 6996, 16,003, 16,005, 16,011, 16,018, 16,021, 16,023, 16,025, 16,027, 16,034, 16,035, 16,036, 16,038, 16,039, 16,044, 16,048, 16,049, 16,057, 16,058, 16,059, 16,060, 16,061, 16,062, 16,071, 26,360, 26,917, 26,924, 26,936, 26,937, 26,947, Massai, Urochloa brizantha cv toledo and Urochloa hibrido cv Cayman
	Cluster 2
Rainy	622, 693, 6,094, 6,175, 6461, 6,497, 6,500, 6,571, 6,784, 6,796, 6,799, 6,805, 6,826, 6,831, 6,837, 6,839, 6,855, 6,872, 6,890, 6,901, 6,903, 6,927, 6,929, 6,944, 6,948, 6,960, 6,962, 6,969, 6,982, 16,005, 16,017, 16,028, 16,034, 16,035, 16,036, 16,038, 16,039, 16,044, 16,046, 16,049, 16,055, 16,059, 16,061, 16,064, 26,360, 26,900, 26,906, 26,911, 26,923, 26,925, 26,937, 26,939, 26,944, 26,947 and *Urochloa brizantha* cv toledo
Dry	622, 691, 692, 6,094, 6,175, 6,299, 6,500, 6,536, 6,571, 6,805, 6,840, 6,842, 6,857, 6,893, 6,897, 6,901, 6,928, 6,929, 6,944, 6,948, 6,954, 6,967, 6,969, 6,975, 6,982, 16,017, 16,019, 16,031, 16,051, 16,069, 26,900, 26,906, 26,923, 26,925 and 26,939
	Cluster 3
Rainy	673, 688, 691, 692, 6,095, 6,171, 6,299, 6,525, 6,536, 6,658, 6,836, 6,840, 6,857, 6,866, 6,891, 6,893, 6,897, 6,898, 6,900, 6,906, 6,912, 6,915, 6,918, 6,945, 6,949, 6,951, 6,954, 6,955, 6,963, 6,967, 6,975, 6,981, 6,983, 6,984, 6,986, 6,990, 6,996, 16,011, 16,019, 16,027, 16,041, 16,054, 16,058, 16,060, 16,065, 16,068, 16,069, 26,917, 26,936, 26,942 and Massai
Dry	6,095, 6,784, 6,796, 6,799, 6,843, 6,855, 6,900, 6,915, 6,923, 6,945, 6,949, 6,951, 6,955, 6,960, 6,963, 6,981, 6,990, 16,004, 16,028, 16,041, 16,046, 16,054, 16,055, 16,064, 16,065, 16,068, 26,723, 26,911, 26,942 and 26,944

conditions (Dele et al., 2017; Barragán-Hernández and Cajas-Girón, 2019). In contrast, other authors argue that higher values for protein can be found during the dry season (Rodríguez et al., 2017).

The preservation of beef cattle is an important goal in the Patía Valley region, where animals lose weight and mortality increases because of the lack of water and good-quality feed. Considering the challenging hydric conditions of the tropical zone during the dry season, the average protein content of 7.3% and the maximum of 10.5% in *M. maximus* stand out. These nutritional values contribute to preserving rumen functionality. A relevant consideration to keep a functional rumen in bovines is the minimum required nitrogen amount equivalent to 8% of CP (Gaviria et al., 2015). Also, considering that in this region most of the plants for a complementary diet are grasses, fodder legumes, and other plants rich in protein, the contribution of *M. maximus* could be ideal to avoid a loss of rumen functionality and to support livestock production during the dry season.

A high negative correlation exists between structural carbohydrate content and digestibility (Jung et al., 1997) in the *M. maximus* collection in the rainy season. This might have incremented IVDMD by 1.86% during the dry season. Therefore, the results of this parameter highlight the potential of this species as an alternative during low-precipitation periods, for both biomass production (Morales-Velasco et al., 2016) and steady relative quality.

During the dry season, Tobiatá, Mombasa, Tanzania, Vencedor, Massai, and Coloniao had protein content of 7.09, 6.24, 6.13, 6.72, 7.82, and 8.30%, respectively. These values were higher than those found in commercial cultivars in important tropical livestock areas (dos Costa et al., 2017; Silva et al., 2017; da Silva et al., 2018). However, in the same research location where this experiment took place, and with a similar number of regrowing days and average height in Massai, Ruiz et al. (2015) showed 14.20% CP. This could possibly be due to fertilization at establishment and evaluation during the rainy season.

In tropical regions of Colombia, productive differences exist between commercial cultivars and genotypes of the evaluated collection in this research, which could be associated with aspects inherent to morphology (Patiño-Pardo et al., 2018) and nutritional profile. These are advantageous characteristics in terms of adaptation to different livestock systems.

Some studies suggest that *in vitro* and *in vivo* digestibility of organic matter increases with the rainy season (Vargas Junior et al., 2013; Silva et al., 2017), and others show that water stress did not significantly affect organic matter digestibility (OMD), (Fariaszewska et al., 2020). The findings in this research suggested that ADF decreased similar to that reported by Larsen et al. (2021) and IVDMD increased slightly during the dry season vis-à-vis the rainy season. This condition might be related to the average height of germplasm of 130.7 vs. 55.2 cm during the rainy and dry seasons, respectively. Therefore, growth in height could result from a decrease in leaf material and the respective digestibility (Kalmbacher et al., 1980), and drought stress might delay maturity, which can improve the OMD of forages (Fariaszewska et al., 2020). The correlations found in the *M. maximus* collection were similar to those reported by Stabile et al. (2010) with commercial cultivars.

The classification of the accessions under multivariate tests (by cluster analysis and Hotelling's T-squared test) and NCI shows that the genotypic and physical characteristics specific to each accession (not included in this study) as well as morphological aspects (Santos et al., 2010), leaf-to-stem ratio (Homen et al., 2010), and maturity or metabolism rate (dos Costa et al., 2017) may have influenced the classification of materials with a low or high nutritional profile.

This classification shows that some accessions respond to prolonged tropical dry periods and possibly show promise for resilient nutritional quality with adequate DMB. In addition, *M. maximus* outperforms other forage species used for grazing under semiarid or dry tropical conditions (Coêlho et al., 2018). For a diversity of agronomic parameters and nutritional composition related to genetic aspects, *M. maximus* shows promise for breeding programs.

Agronomic and nutritional analysis, in general terms, allows us to learn about a large group of *Megathyrsus maximus* accessions as potential options for the establishment and management of productive and efficient cattle raising under the agro ecological conditions of the Patía Valley, thus, contributing to the agricultural development of the region and the quality of life of its producers.

The *M. maximus* collection contains several materials that stand out for their nutritional value (CP, NDF, ADF, and IVDMD), which, although they did not show a relationship with DMB, have sufficient productive yield. They also have adaptation potential for drought or low-rainfall conditions in tropical regions. Therefore, they represent a suitable option for sustainable livestock systems. Furthermore, they help subsequent plant breeding programs to contribute to finding alternative materials to maintain adequate feeding efficiency for cattle and mitigate the effects of climate change.

Both the genotypic characteristics of *M. maximus* and environmental conditions during contrasting seasons are factors that might influence the variability of nutritional content, productive parameters, and flowering of the evaluated germplasm. This allows a classification of forage material according to specific or preferential criteria of farmers and plant breeders.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JC-T carried out the experimental work, statistical analyses, wrote the manuscript, the original draft, and the methodology. JM performed the experiment based on NIRS Technology. NV-Q handled the supervision, the project administration, the acquisition of funds, helped on the conceptualization, validation, and the writing of the original draft. All authors contributed to the analysis and interpretation of data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.684747/full#supplementary-material

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Avena sativa AV25-T (Altoandina) Supplementation as Alternative for Colombia's High-Altitude Dairy Systems: An Economic Analysis

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In the Colombian high-altitude tropics (2,200–3,000 m.a.s.l.), Kikuyu grass (*Cenchrus clandestinus*) is the main feed source for the dairy system. This grass species has good characteristics regarding adaptability and productivity, but is affected by frost, grass bugs (*Collaria spp*.) and precipitation-related production seasonality. Forage deficits might thus be a problem at several times in a year. As a strategy to maintain production stable, dairy farmers use commercial feed concentrates increasing their production costs. Agrosavia, as a response to this, started in 2005 with the evaluation and selection of new forage species for the Colombian high-altitude tropics. The oat *Avena sativa* AV25-T was identified as promising alternative to supply the requirements of dry matter in times of deficit and released as cultivar in 2018 under the name Altoandina. The objective of this study was to evaluate the economic viability of Altoandina in Colombia's high-altitude dairy systems. Altoandina (Aa) was provided as silage in two different diets: 35%Aa−65% Kikuyu (Yellow Diet) and 65%Aa-35% Kikuyu (Red Diet). The diet for comparison was traditional grazing with 100% Kikuyu grass (Blue Diet). All diets were supplemented with 6kg commercial feed concentrate, 0.5 kg cotton seeds and 0.5 kg Alfalfa meal per cow/day, respectively. To estimate economic indicators, we used a cashflow model and risk assessment under a Monte Carlo simulation model. Including Altoandina incremented productivity per hectare by 82.3 and 220% in the Yellow and Red Diets, respectively. According to the results of our economic model, the Yellow Diet is the best alternative. Its average Net Present Value (NPV) was superior in >80% and showed a lower variability. The indicators Value at Risk (VaR) and probability (NPV < 0) show the Yellow Diet to have the lowest risk for economic loss under different yield/market scenarios. The Yellow Diet also has the lowest unit production costs and uncertainty of productive parameters. According to our findings, supplementation with Altoandina at 35%, i.e., during critical times, has high potential to improve efficiency and profitability. This information is key for the decision-making process of dairy farmers on whether to adopt this technology.

Keywords: sustainability, Monte Carlo simulation, silage, oat, dairy system

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INTRODUCTION

The livestock sector and, particularly the cattle subsector, is a critical component of food systems since it provides food with high quality protein (i.e., 14% of the calorie and 33% of the protein intake of the global human diet comes from livestock) that is in most cases produced on marginal lands not suitable for crop production. Additionally, livestock provides people with incomes, assets, alternative energy, animal draft power, and livelihoods (FAO, 2018). Especially, dairy production is crucial for income generation and food security, mainly in (the rural areas of) developing countries where the dairy sector is dominated by smallholder production systems (World Bank, 2005; Reisinger and Clark, 2018). Globally, there are around 300 million poor people whose livelihoods depend on the daily income and nutrition provided through milk production (World Bank, 2005). The dairy sector is of great economic and social importance in Colombia. It contributes with 36.7% to the national livestock and 12% to the agricultural Gross Domestic Product (GDP), respectively, and generates 20% of the jobs in the agricultural sector (MADR, 2020). According to the Colombian Cattle Federation (FEDEGAN, 2018), there are about 319,000 milk-producing families in Colombia, and the dairy sector is predominated by small-scale or subsistence producers (with less than 10 animals). Milk production in the country happens under two differentiated systems linked to specific environmental conditions. First, the specialized dairy systems, located in the higher tropics (>2,000 m.a.s.l.), mainly in the departments of Antioquia, Boyacá, Cundinamarca, and Nariño, which provide 45% of the total national milk supply and use only 6% of the total cattle inventory (1.72 million heads) (Carulla and Ortega, 2016; FEDEGAN, 2020b). Second, the dual-purpose production systems, located in the lower tropics (<1,200 m.a.s.l.), which contribute with 55% of the national milk supply using 39% of the total cattle inventory (10.08 million heads) (FEDEGAN, 2018, 2020a).

The dairy sector has had high growth rates in the last two decades, with an increase in total milk supply of 35% between 2000 and 2019, which is equivalent to a production of 5,295 and 7,257 ml, respectively (FEDEGAN, 2020b). Production and productivity, however, are strongly linked to the local climatic conditions present in the production areas (FEDEGAN, 2018), making the dairy sector dependent on rainfall regimes and periods of drought that affect the availability and quality of the forages used as animal feed (FEDEGAN, 2018). Because of climate change, this situation has been aggravating in recent years, given the progressive increase in global and local average temperatures and variations in rainfall patterns. This is directly affecting cattle production through impacts on pasture availability, animal comfort (heat stress), water availability and biodiversity (Rojas-Downing et al., 2017). In addition to the above, the increasingly frequent occurrence of extreme climatic phenomena in the country, such as *La Niña* and *El Niño*, causing heavy rainfall, flooding, and extreme droughts, makes the situation even more critical, particularly when it comes to milk production, since dairy cows are more susceptible to heat stress (SIPSA/DANE, 2016). This is evidenced by milk production decreases of on average 4.9% in years with presence of the *El Niño* phenomenon [UNGRD (Unidad Nacional para la Gestión del Riesgo de Desastres-Colombia), 2016].

In the specialized dairy systems of Colombia, the predominant feed base is grazing of Kikuyu grass (*Cenchrus clandestinus*) and the use of supplementation with commercial concentrates, the latter representing a significant percentage of the total production costs (∼37%) (Cárdenas, 2003; Campuzano et al., 2018; Castillo et al., 2019). Kikuyu grass, although with good characteristics in terms of adaptability and productivity (biomass production), is affected by frost and grass bugs (*Collaria scenica*) (Campuzano et al., 2018). It also has nutritional limitations that can affect the production and compositional quality of milk, such as high levels of soluble nitrogen and low levels of non-structural carbohydrates (Correa et al., 2008). In addition, the production systems based on Kikuyu are associated with deficient pasture management, mainly in terms of fertilization (Campuzano et al., 2018), and residual grass management, restricting both levels of production and productivity. This leads to impacts at the environmental level, since soil and water are being contaminated with nitrogen (N) that is not usable by the animal and released with the urine (given the levels of soluble N in Kikuyu, the inadequate management of grazing and low levels of supplementation) (J. Castillo, Agrosavia, personal communication).

Consequently, there are important bottlenecks related to the deficit of forage at different times of the year, high production costs of animal feed and negative effects at the environmental level. Considering the climate change scenarios for the region, this situation is likely to worsen: The Colombian Institute of Hydrology, Meteorology and Environmental Studies (IDEAM) forecasts for the Departments of Cundinamarca, Boyacá, and Antioquia (which make up 40% of the national dairy production mainly under specialized dairy systems) increases in precipitation levels of more than 4% and in temperature of at least 2°C until the year 2100 (IDEAM, 2015). This would lead to a lower water use efficiency and possibly greater water stress for the Kikuyu grass (Vargas-Martínez et al., 2018) and largely affect dairy production in those regions.

In this sense, the Colombian Agricultural Research Corporation (ICA and CORPOICA before, now Agrosavia) has conducted forage research to improve the efficiency and reduce the seasonality of milk production in the higher tropics of Colombia. These studies have focused on seeking strategies for soil recovery and renovation of pastures, establishment and management of forage grazing systems, and production of forage crops for ruminant feeding systems (Castillo et al., 2019). Although there is no germplasm improvement and evaluation program specifically for the higher tropics, the research processes carried out by Agrosavia have led to the release of six oat cultivars in the country since the 1960s: ICA Bacatá (*Avena fauta*) (1963), ICA Soracá (*Avena byzantina*) (1965), ICA Gualcalá (*Avena byzantina*) (1968), ICA Cajicá (*Avena sativa*) (1976), Avena Obonuco Avenar (*Avena sativa*) (2003) and Avena Altoandina (AV25; High-Andean Oat) (2018) (Bustamante, 1965; Arias et al., 1972; Bolaños-Alomía et al., 2003; Campuzano et al., 2018). Despite its release over 45 years ago and the release of other cultivars thereafter, ICA Cajicá still predominates on the market and is one of the most used oats for animal feeding (through silage). It is, however, susceptible to rust (*Puccinia spp.*) which is predominant in many parts of the Colombian higher tropics. The cultivar Altoandina, released in 2018, is the most recent oat made available to dairy producers, and is the result of an evaluation process which began in 2005. Compared to the previously released materials and commercial oats used in the region, Altoandina stands out for its higher biomass production, better nutritional quality, and greater resistance to rust and overturning (Campuzano et al., 2018, 2020), making it a promising alternative for supplying the forage deficit of the prairies in times of scarcity (drought) and improving the productivity of the specialized dairy systems in the Colombian higher tropics. In general terms, oats stand out as a forage crop widely used as a source of animal nutrition throughout the world, especially in European countries and the United States (Fraser and McCartney, 2004; Suttie and Reynolds, 2004; Harper et al., 2017). *Avena sativa* is predominant there and used either in grazing systems or as supplement in the form of hay and silage. In South America, a harvested area of 806,000 hectares was registered for 2019, with an average annual growth rate of 8% between 2010 and 2019 (FAOSTAT, 2021), indicating the interest of dairy producers in this material. In Colombia, oats are mainly used as basis for silage production in the higher tropics, but, to a limited extent also for grazing in the lower to medium tropics. Using oats has been gaining importance in cattle production, especially in the technified dairy systems in the higher tropics, but adoption rates remain low on farms with less technical level (FEDEGAN, 2012).

The technical evaluation of oats in Colombia is being led by Agrosavia, which has focused on evaluating the effects of using it as a supplementation strategy in critical times (through silage) on the production and composition of milk in the higher tropics (Barahona et al., 2003; León et al., 2008; Mojica et al., 2009; Campuzano et al., 2018, 2020). Although variable effects on production have been reported, most of these studies have shown how the use of oats allows maintaining milk production stable when compared to feeding strategies solely based on Kikuyu grass (León et al., 2008; Mojica et al., 2009; Campuzano et al., 2018, 2020). Studies on the economic viability of including oat varieties in cattle systems were, however, not conducted yet for Colombia. Even though oats (due to their beneficial characteristics such as higher biomass availability, maintenance of production levels in critical times, and reduction in the use of commercial concentrates) have positive impacts on economic viability and economic indicators, it is also evident that the implementation of feeding strategies based on oats imply higher costs at the productive level compared to grazing systems, making it necessary to provide information on the profitability of these technologies in order to facilitate dissemination and adoption processes.

In this sense, the present study aims to evaluate, from an economic perspective, the viability of the oat Avena AV25-T (Altoandina) as a feeding strategy for dairy systems in the Colombian higher tropics. Altoandina (Aa) was provided as silage in two different diets: 35%Aa−65% Kikuyu (Yellow Diet) and 65%Aa−35% Kikuyu (Red Diet). The diet for comparison was traditional grazing with 100% Kikuyu grass (Blue Diet). Through a discounted cash flow model and a quantitative risk analysis using a Monte Carlo simulation, we provide economic indicators, such as Net Present Value (NPV), Internal Rate of Return (IRR), and Benefit-Cost Ratio (B/C), that help in identifying the best diet for the system under evaluation. This document is structured as follows: after this introduction, the main characteristics of the evaluated variety are presented [Section Description of the technology: Avena AV25-T (Altoandina)]. The methodology, assumptions, and data sources used are explained in Section Materials and Methods, the results are provided in Section Results and discussed in Section Discussion, and conclusions and recommendations for various stakeholders are presented in Section Conclusion.

DESCRIPTION OF THE TECHNOLOGY: AVENA AV25-T (ALTOANDINA)

In 1992, the oat accession with the experimental name AV25 was introduced to the National Germplasm Bank System for Food and Agriculture of Colombia (SBGNAA) managed by Corpoica (now Agrosavia). The accession was delivered by the International Maize and Wheat Improvement Center (CIMMYT) from Mexico. The evaluation process of this accession began in 2005 with the aim of offering forage alternatives for the cattle systems in the Colombian higher tropics. In total, 18 oat genotypes from New Zealand, CIMMYT, SBGNAA and commercial national varieties were evaluated. The AV25 genotypes were selected for presenting high Dry Matter yields, tolerance to overturn and resistance to leaf and stem rust (Campuzano et al., 2020). From 2016 to 2017, agronomic evaluations were carried out in eight locations in the Colombian Andean region, selecting the cultivar AV25-T (Altoandina) as most promising material for covering the feed requirements of the high-altitude dairy systems during critical times (Campuzano et al., 2018), particularly for milk production in the subregions of the savannas of Bogotá, upper Chicamocha, the Ubaté and Chiquinquirá valleys, and the highlands of the Nariño Department (Campuzano et al., 2018).

Altoandina is a forage oat with a semi-erect growth habit with an average height of 108–143 cm and an average density of 27 leaves per plant. It adapts well to altitudes between 2,600 and 3,000 m.a.s.l. and to soils with a moderately acidic to neutral PH value. Compared to other commercial oats (e.g., Cayuse), Altoandina has a shorter flowering time (92–107 days compared to 110-150), being considered an intermediate cycle oat. The average harvest period until a state of milky to pasty grains [7.9 points on the Zadoks growth scale (Zadoks et al., 1974)] is reached, varies between 130 to 140 days. It is characterized by high biomass production (up to 64.9 t ha^{-1} of green forage and up to 25 t ha^{-1} of Dry Matter, depending on the management and environmental conditions), resistance to overturning (5.2% compared to 30% for commercial varieties), low incidence of leaf and stem rust (*Puccinia spp.*) (<20% compared to 60% for commercial varieties), and higher crude protein values in the

TABLE 1 | Forage production and nutritional quality of Altoandina and commercial oat varieties.

Variable	Altoandina (Mean ± SD)*	Commercial varieties (Mean ± SD)*
Biomass production (t DM^{-1**} ha^{-1})	10.6-24.8	3.6-19.3
Crude protein (%)	7.5 ± 1.4	4.7 ± 1.27
Neutral Detergent Fiber (NDF, %)	57 ± 3.15	58 ± 3.16
Total digestible nutrients (TDN, %)	51 ± 3.15	50 ± 3.24

*Mean values and standard deviations reported for a total of 6 evaluations: two each in the Nariño, Boyacá, and Cundinamarca Departments; **DM, Dry Matter.*
Source: Own elaboration based on the study carried out by AGROSAVIA "Evaluación y selección de nuevas especies forrajeras, and estrategias para mejorar la competitividad y sostenibilidad de los sistemas de producción de leche y/o carne en la región andina" (Campuzano et al., 2018, 2020; LF. Campuzano, Agrosavia, personal communication). The technical parameters obtained by Campuzano et al., were used for the economic evaluation presented in this article.

TABLE 2 | Composition of the evaluated diets.

Category	Composition	Evaluated diets		
		Blue	Yellow	Red
Forage composition (%)	Kikuyu grass	100%	65%	35%
	Altoandina silage	0%	35%	65%
Supplements (kg $AU^{-1*}d^{-1}$)	Feed concentrate Standard 70 (kg/DM)	6.0	6.0	6.0
	Cotton seeds (kg/DM)	0.5	0.5	0.5
	Alfalfa flour (kg/DM)	0.5	0.5	0.5
Consumption (kg $AU^{-1}d^{-1}$)	Kikuyu grass	57.5	33.4	16.7
	Altoandina silage	0.0	12.0	25.9
	Supplements	7.0	7.0	7.0

AU, Animal Unit. One Animal Unit is equivalent to an adult cow of 450 kg live weight.

milky to pasty grain state, where starch levels are at their highest point and improve the nutritional quality of the forage (59% higher than for the commercial varieties Cayuse and Cajicá) (Campuzano et al., 2018). A summary of the characteristics of Altoandina is provided in **Table 1**. Altoandina was released by Agrosavia in 2018 and is commercially available to cattle producers since then.

In the present study, Altoandina was evaluated as silage for supplementation in times of feed scarcity in the higher tropics of Colombia. The evaluation considered two different silage supplementation percentages of the total diet: 35% (Yellow Diet) and 65% (Red Diet) of Altoandina silage. This was compared with a traditional grazing scenario with 100% Kikuyu grass (Blue Diet) (see **Table 2**). Prior to the entry of the animals to the systems, the chemical composition of the Kikuyu grass and the Altoandina silage were measured. In the case of the Altoandina silage, the levels of Crude Protein were 8.7%, Neutral Detergent Fiber 51.5%, and Total Digestible Nutrients 52.6%, respectively. For the Kikuyu grass, the levels of Crude Protein were 17.8%, Neutral Detergent Fiber 58.1%, and Total Digestible Nutrients 24.7%, respectively (J. Castillo, Agrosavia, personal communication). The composition of the diet presented in **Table 2** refers to the percentages available and supplied to the animals. The actual consumption of the animals, might differ since animals were offered voluntary feed intake. To ensure that each cow ate the planned amount, the silage was supplied individually, and the silage surplus was weighed daily. The residual silage did not reach higher levels than 3.9 and 3.6% for the two evaluated diets (65 and 35% of Altoandina silage) (A. Albarracín, Agrosavia, personal communication).

In the three diets, additional supplementation was carried out with Standard 70 feed concentrate, cotton seed and Alfalfa flour, at an amount of 6, 0.5, and 0.5 kg AU^{-1} d^{-1}, respectively. These amounts are assumed as constant throughout the year and are identical for the three evaluated diets. The productivity data for Altoandina were obtained from field evaluations carried out by Agrosavia in 2008 in the municipality of Tibasosa in the Boyacá Department in Colombia (5°44′53″ north latitude and 72°59′56″ west longitude, at an altitude of 2,528 m.a.s.l.). The experiment was carried out between July and August 2007, during the dry season of the second semester of the year. The average temperature there is 13°C with fluctuations between 0 and 20°C and a relative humidity of 80 to 85%. Frosts occur in the area in the months of January, February and early August, the average annual rainfall is 528.9 mm. Altoandina was sown in an area of 5,500 m^2, on soils with moderate to strong acidity (PH 5.9), medium percentages of organic matter, medium levels of Phosphorus (P) and Sulfur (S), and a low level of Boron (B). The oat harvest for silage production was carried out 119 days after sowing when 70% of the crop was in the state of milky to pasty grains, with an approximate Dry Matter production of 20 t ha^{-1}. Animal productivity was evaluated in 15 Holstein cows in a specialized dairy system under conditions of the higher tropics (2,200–3,000 m.a.s.l.). The animal productivity evaluations were performed in a crossover design with three treatments, where the experimental unit consisted of five Holstein cows in the first third of the lactation period. Each treatment involved three groups each of five cows who had between three and five calvings in the past. The silage supply was offered individually in the pasture with portable feeders, dividing the daily amount of silage into two fractions supplied after each milking process. The total evaluation period was 21 days, with daily milk yield measurements in seven-day blocks. To determine grazing area in each diet, the total available forage was calculated, and to determine the dry matter intake, the weight of the cows was measured. The measurements of forage availability were made before and after grazing to determine the consumption of Kikuyu grass. For the Blue Diet (100% Kikuyu grass), forage was provided to the animals through grazing on a daily plot size of 241 m^2 and the total area used was 4824 m^2. For the Yellow (35% Altoandina silage) and Red (65% Altoandina silage) Diets, Kikuyu forage was provided to the animals through grazing on a daily plot size of 140.1 and 69.9 m^2, and the total area used was 2802 and 1398 m^2, respectively.

MATERIALS AND METHODS

Discounted Cash Flow Model

A cost-benefit analysis (CBA) was carried out to determine the viability of the different interventions with Altoandina as a supplementation strategy in critical times. The CBA is based on a discounted free cash flow model and a quantitative risk analysis. The analysis was carried out by comparing the profitability indicators of the technology in different diets (Red Diet and Yellow Diet) and the traditional scenario (Blue Diet) for the study region. For each case, the economic costs and benefits were determined. Regarding the cost categories, the following have been considered (per hectare): total costs of establishment and maintenance, opportunity costs of capital, and operating costs (e.g., for animal health, supplementation, permanent and occasional labor). The benefits are derived from the production of milk in a specialized dairy system, according to the animal response indicators obtained for each diet. The estimated profitability indicators include the total production costs, the gross income, the net profit, the profit margin per liter of milk, and financial indicators such as the Net Present Value (NPV) and the Internal Rate of Return (IRR).

Model Assumptions

For the construction of the cash flow, it was necessary to establish different economic and technical assumptions, which are in detail described below.

Technical Parameters of Dairy Production

Since animal productivity was only measured in terms of milk production per day, the other technical parameters are the same for the three diets according to the average indicators for the study area: (i) a milk production period of 305 days; (ii) a calving interval of 401–450 days; and (iii) a productive lifespan of dairy cattle of 6 years. The purchase price of dairy cattle (US$ 812 AU^{-1}) was amortized for the period of analysis and the price for culled cows was adjusted for inflation at 6 years and added in the last year (US$ 406 AU^{-1}).

Sowing Frequency of Altoandina

Altoandina is sown twice a year—in March/April and October/November. Oat silage is prepared and offered to the animals in periods of frost or drought to cover the supply of forage required in the diet—usually from December to February and July to September. In other words, oat supplementation is assumed for a total of 180 days per year for the Red and Yellow Diets. It is necessary to emphasize that the planting of Altoandina must be linked to a farm development plan to fulfill this assumption. If the supply of forage is low, two sowings are planned, otherwise the producers sow oats, especially between March and April.

Pasture Renewal of Kikuyu Grass

The renewal is assumed once every 2 years, according to the trend in the region (J. Castillo, Agrosavia, personal communication). This is done to improve the physical and chemical quality of the soil, as well as to recover the productive capacity and quality of the Kikuyu grass.

Evaluation Horizon

The evaluation horizon is established according to the lifespan of the main assets for each diet. In the case of Altoandina, an evaluation period of 6 years was considered (from 2020 to 2025), according to the productive lifespan of the Holstein cows used in the specialized dairy system in the Colombian higher tropics (M. Sotelo, Alliance of Bioversity International and CIAT, personal communication).

Discount Rate

The financing cost is chosen as the discount rate in accordance with the rural credit lines of the Colombian Fund for the Financing of the Agricultural Sector (FINAGRO). This financing cost is considered the opportunity cost of capital and is associated with a risk factor present in the activities of the rural sector. Therefore, the following discount rate was established: Fixed-term deposit rate (DTF) + 5% effective annual interest rate. The projection of the discount rate in the corresponding periods was carried out following the DTF projections, according to the Annual Report of Economic Projections Colombia 2020 (Bancolombia, 2020).

Permanent Labor

The need for permanent labor was established according to the labor weights of FEDEGAN (2003), referring to a need of 7.8 permanent workers for every 100 animals in specialized dairy systems. The 2019 basic salary, transportation assistance, social security contributions, social and parafiscal benefits were considered for establishing the cost of one permanent farm worker, which is US$ 422 per month. For the projection of wages during the period of analysis, the universal rule was assumed: Variation of the minimum salary (in %) = expected inflation (in %) + observed variation of workforce productivity (WP, in %). A WP of 1% is assumed, according to historical estimates derived from the National Administrative Department of Statistics of Colombia (DANE, 2020a).

Taxes

Income tax was considered as dictated by law 2010 of 2019 (Congress of the Republic, 2020). Here a rate of 32% was established for 2020, 31% for 2021 and 30% for 2022, remaining fixed at the latter value for the subsequent years.

Currency at Current Prices

Inflation was considered to estimate income flows and costs in the evaluation period. In the case of income, the projection of the Consumer Price Index (CPI) estimated by Bancolombia (2020) for the period 2020–2023 was considered. For production costs, the Producer Price Index (PPI) provided by the National Administrative Department of Statistics of Colombia (DANE, 2020b) was considered.

Milk Price

Price information was obtained from the Milk Price Monitoring Unit for Region 1, where specialized dairy production systems predominant (MADR/USP, 2020). The prices were projected according to the CPI projections. Additionally, this projection included variations in milk prices, associated with the presence of extreme weather events such as *El Niño* and *La Niña*. According to Abril et al. (2017), the occurrence of these phenomena caused a significant increase in food inflation, particularly when the phenomenon is of a strong category. In Colombia, milk prices have had variations of more than 7% in the years with the presence of these events, compared to variations of less than 1% in the years without phenomenon (DANE, 2020b,c).

Quantitative Risk Analysis

Risk is defined as the possibility that the real return on an investment is less than the expected return (Park, 2007). Therefore, profitability is associated with the variability of the flows of benefits and costs, and these in turn of the randomness of the main variables of the investment project (e.g., yields, market prices). Investment projects at the rural level pose a high risk, resulting from a dependence on a wide set of variables, in many cases, not controlled by the producer (e.g., climatic factors). In this sense, it is necessary to incorporate risk levels associated with the profitability indicators of each of the diets evaluated. For this, a Monte Carlo simulation model was carried out. The simulation was performed for a total of 5,000 simulations or iterations, with a 95% confidence level, with the software package @Risk (Paladise Corporation). The objective of this analysis is to determine the standard deviation mean values of the profitability indicators through the variable parameters: price per liter of milk, milk production per day in each of the diets, fertilization costs, variation in the discount rate and in the CPI indicator. These variables are assigned a probability distribution according to their empirical behavior, literature or based on expert interviews. The yields were modeled according to expert knowledge and the best fit in @Risk following a Pert distribution, where the predominance of values in the most probable range was assumed. In the case of costs and price variations, a triangular distribution was assumed according to the reported minimum and maximum values and assigning a greater probability to the extremes. **Table 3** shows the simulated variables, the range values, and the probability distributions used. In the simulation, values of the variables identified as critical are randomly assigned, according to their probability distribution functions, to later calculate the determined profitability indicators.

Decision Criteria

As decision criteria, the mean values and the variance of the profitability indicators resulting from the simulation are used. The use of the mean value criterion is based on the law of large numbers, which states that if many repetitions of an experiment are carried out, the average result will tend toward the expected value (Park, 2007). The variance of the indicators determines the degree of spread or dispersion on both sides of the mean value (Park, 2007). In other words, the lower the variance, the lower the variability (loss potential) associated with the indicators.

$$NPV_{(Mean)} = \sum_{t=0}^{n} \frac{E(FC_t)}{(1+r)^t} \quad (1)$$

$$IRR_{(Mean)} = \sum_{t=0}^{n} \frac{E(FC_t)}{(1+r^*)^t} = 0 \quad (2)$$

Where,

E (FCt) = Expected value of the net profit flow for period t
Var (FCt) = Net profit flow variance for period t
r = Real discount rate

TABLE 3 | Variables simulated in the Monte Carlo model.

Variable	Distribution	Most likely value	Lower limit	Upper limit
Milk price (US$ l^{-1})	Triangular	0.31	0.28	0.34
Milk productivity Blue Diet* (l AU^{-1} d^{-1})	Pert	20.48	17.63	23.32
Milk productivity Yellow Diet** (l AU^{-1} d^{-1})	Pert	21.67	19.09	24.24
Milk productivity Red Diet*** (l AU^{-1} d^{-1})	Pert	19.01	16.85	21.17
Fertilizer/corrective costs for Kikuyu renewal (US$ ha^{-1})	Triangular	80	54	303
Periodicity of the *El Niño* phenomenon	Discreet uniform	n.a.	2	7
Variation of the discount rate (%)	Triangular	0%	1%	2%
Variation of the CPI (%)	Triangular	−0.50	0	0.50

**100% Kikuyu grass, **35% Altoandina silage and 65% Kikuyu grass, ***65% Altoandina silage and 35% Kikuyu grass.*

r* = Internal Rate of Return
t = Evaluation horizon of the project

The NPV at risk indicator (VaR) and the probability of success of the evaluated diets [Prob (NPV (Medium)>0] were also estimated. The VaR is defined as the maximum expected loss of the investment project in a time interval and with a certain level of confidence (Manotas and Toro, 2009). Additionally, a sensitivity analysis was performed using a tornado graph, which sensitizes each variable in order to measure its impact on the profitability indicators and to identify within the critical variables those with the greatest effects on the profitability indicators.

RESULTS

Figure 1 shows the information corresponding to the technical indicators of animal productivity for each of the evaluated diets. These indicators show that the inclusion of Altoandina silage in a percentage of 35% (Yellow Diet) allowed to increase the daily milk production per cow by 5.8% and per hectare by 82.3% compared to the Kikuyu grazing system (Blue Diet). When the percentage of silage in the diet increased by 65% (Red Diet), daily milk production per cow was reduced by 7.7% and per hectare increased by 220% compared to the Blue Diet. The higher per hectare milk production is associated with the higher availability of forage and, therefore, an increase in the animal stocking rate of 42% and 71% for the Yellow and Red Diets, respectively. In addition, the inclusion of Altoandina silage makes it possible to reduce the rate of milk production decline in critical times and, in the end, to increase milk production per unit area. It should be noted that, of the evaluated diets, the highest variability in animal production is observed for the Red Diet, measured by the standard deviation indicators and coefficient of variation. It is important to highlight that, as mentioned in the methodology, the data were collected during the dry season of the second

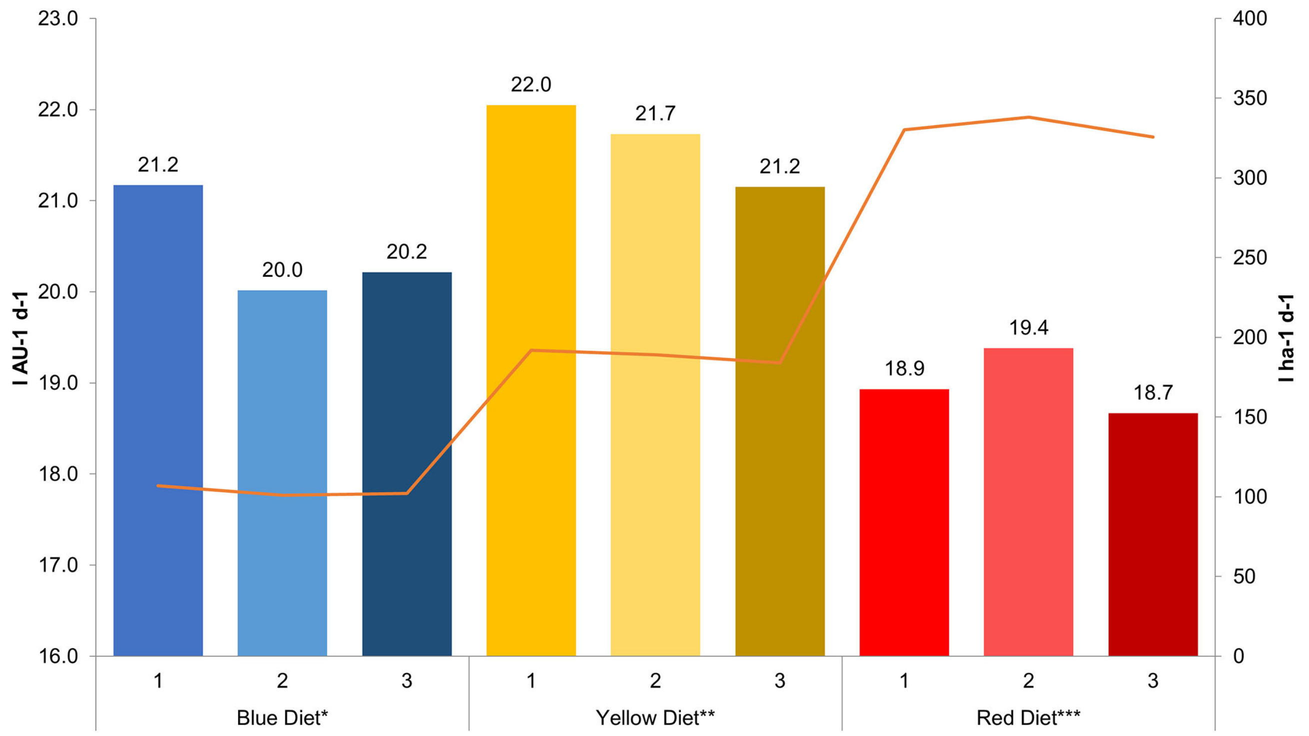

FIGURE 1 | Milk production per three cycles for the diets with Altoandina and the Kikuyu diet (each cycle was a 7-day evaluation, the total evaluation period was 21 days). *100% Kikuyu grass, **35% Altoandina silage and 65% Kikuyu grass, ***65% Altoandina silage and 35% Kikuyu grass.

semester and were used to estimate the total annual production under each diet. However, given that production levels tend to be higher in rainy seasons, which is associated with the better forage availability, the data estimations used in this study could be underestimating production levels for the whole year. In this sense, better annual milk yields could be expected.

Table 4 presents the summary of the average costs and income for each of the evaluated diets. The cash flow models include the variable costs and revenues associated with the establishment of each technology (Altoandina, Kikuyu). The income results from the sale of raw milk under a specialized dairy production system, according to the technical parameters presented in **Figure 1**. The average annual milk yields are 31,522, 57,316 and 101,543 L/ha for the Blue, Yellow and Red Diets, respectively. This results in a gross income for the sale of raw milk of US$ 10,091 for the Blue, US$ 18,335 for the Yellow, and US$ 32,483 for the Red Diet, respectively. Regarding production costs, animal feed and labor costs are the most significant items in this production system, making up 52 ± 3% and 23 ± 1% of the total cost of each diet. The costs corresponding to inputs for pastures, animal health, and others add up to the remaining 25%, which results in a production cost per liter of milk of US $0.31 for the Blue, US$ 0.29 for the Yellow, and US$ 0.34 for the Red Diets, respectively. The feed cost includes those costs related to supplementation with Standard 70 concentrate, cotton seed and Alfalfa flour, at an amount of 6 kg, 0.5 kg, and 0.5 kg AU^{-1} d^{-1}, respectively, adding to a total cost of US$ 2.34 AU d^{-1} and US$ 836 AU y^{-1}. This amount is assumed constant throughout the year and the same for the three evaluated diets. The net profit per hectare and year was US$ 1,226, US$ 2,620, and US$ 2,351 for the Blue, Yellow and Red Diets, respectively.

TABLE 4 | Overview of principal economic indicators per diet.

Economic indicator	Blue Diet*	Yellow Diet**	Red Diet***
Milk production (l ha^{-1} y^{-1})	31,544	57,316	101,544
Gross income from milk sales (US$ ha^{-1} y^{-1})	11,355	20,631	36,552
Production Costs (US$ ha^{-1} y^{-1})	9,695	16,815	34,383
Net utility (US$ ha^{-1} y^{-1})	1,381	2,949	2,646
Unit Production Cost (US$ l^{-1})	0.31	0.29	0.34
Milk price (US$ l^{-1})	0.36	0.36	0.36
Unit Profit Margin (US$ l^{-1})	0.05	0.07	0.02
Financial Viability indicators[a]			
NPV_mean	5,194	11,842	7,853
IRR	40.8%	49.9%	23.5%

[a]*NPV and IRR; NPV mean value obtained through Monte Carlo simulation (5,000 repetitions with a 95% confidence level).*

**100% Kikuyu grass, **35% Altoandina silage and 65% Kikuyu grass, ***65% Altoandina silage and 35% Kikuyu grass.*

From a purely technical point of view, the Red Diet presents the highest values for the indicator milk production per hectare. When estimating the costs and economic viability indicators,

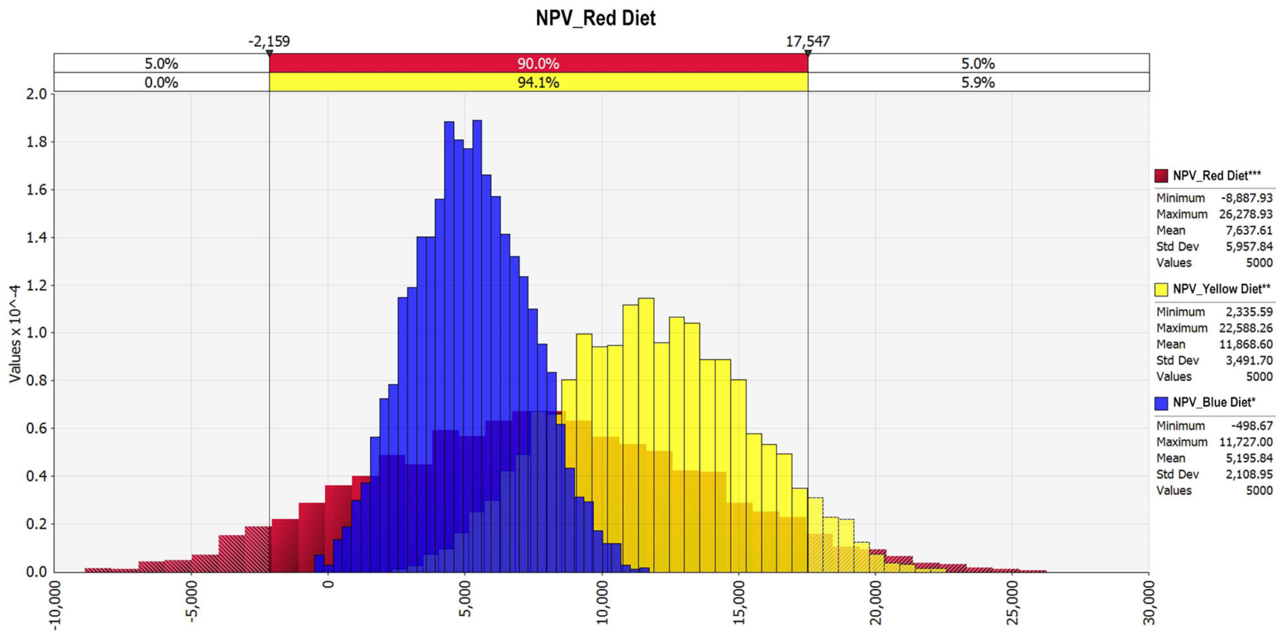

FIGURE 2 | Probability density of the NPV per evaluated diet. *100% Kikuyu grass, **35% Altoandina silage and 65% Kikuyu grass, ***65% Altoandina silage and 35% Kikuyu grass.

however, the Yellow Diet turns out to be the more efficient one with lower unit production costs and higher daily milk productivity per cow. Therefore, a higher profit margin can be obtained per liter of milk produced. The cost of establishing one hectare of Altoandina is estimated at US$ 886, which includes the costs required in its establishment and for ensilaging. The green forage yield is 46,545, the amount silage obtained from that is 41,891, and the DM production is 14,155 kg ha^{-1}, respectively. The cost per kg of DM produced is estimated at US$ 0.06.

The summary of the main financial indicators obtained from the Monte Carlo simulation is presented in **Table 4**. Under the assumptions used for the modeling, all diets result in economically viable alternatives (NPV>0). The best indicators are, however, associated the Yellow Diet. Its mean NPV is 128% and 55% higher than the ones of the Blue and Red Diets, respectively, and a lower dispersion of the indicators is observed according to the Coefficient of Variation (29%, compared to 41% and 76% for the Blue and Red Diets, respectively). Regarding the probability of not obtaining financial feasibility of the three diets, the results of the probability distribution of the NPV are presented in **Figure 2**. Here, the amplitude of the variation for the NPV indicator can be observed with a confidence level of 95%. For the Blue Diet, the indicator can take negative values close to US$ 990 and positive values close to US$ 11,554, with a probability of economic loss of less than 1%. For the Yellow Diet, the distribution curve shifts to the right, with a range that varied between US $2,075 and US$ 23,050. The curve for the indicator for the Red Diet presents a more dispersed behavior around the mean value, reaching minimum values close to -US$ 9,862 and maximum values of US$ 27,278, where 10% of the simulated scenarios presented an NPV<0.

For all three evaluated diets, the economic viability indicators are highly sensitive to changes in the daily milk production variable, meaning that 70, 62.9, and 60% of the variations in the NPV indicator of the Blue, Yellow and Red Diets can be explained by variations in daily milk production. The second most impactful variable is milk price, which explains on average 30% of the variations in the NPV. The Red Diet is the most sensitive to changes regarding milk price (38.7%), which suggests that it would pose a greater risk in the face of market conditions that cause price reductions (**Figure 3**).

DISCUSSION

The use of Altoandina as a supplementation strategy in times of food scarcity proved to be a viable alternative at both the technical and economic levels in specialized milk production systems in the Colombian higher tropics. The higher availability of feed in the evaluated diets based on Altoandina silage allow to increase milk production per hectare substantially (82 and 220% for the Yellow and Red Diets). The daily milk production is, however, 7.7% lower for the Red Diet (which has the highest share of Altoandina silage with 65%) than for the Blue Diet (control scenario, 100% Kikuyu), which is associated with the lower nutritional quality of the silage compared to the higher quality of Kikuyu grass. According to literature, although the effects on milk production can be highly variable, most studies have reported how the use of oats has allowed to maintain and even improve production in critical times. For example, some studies report that the

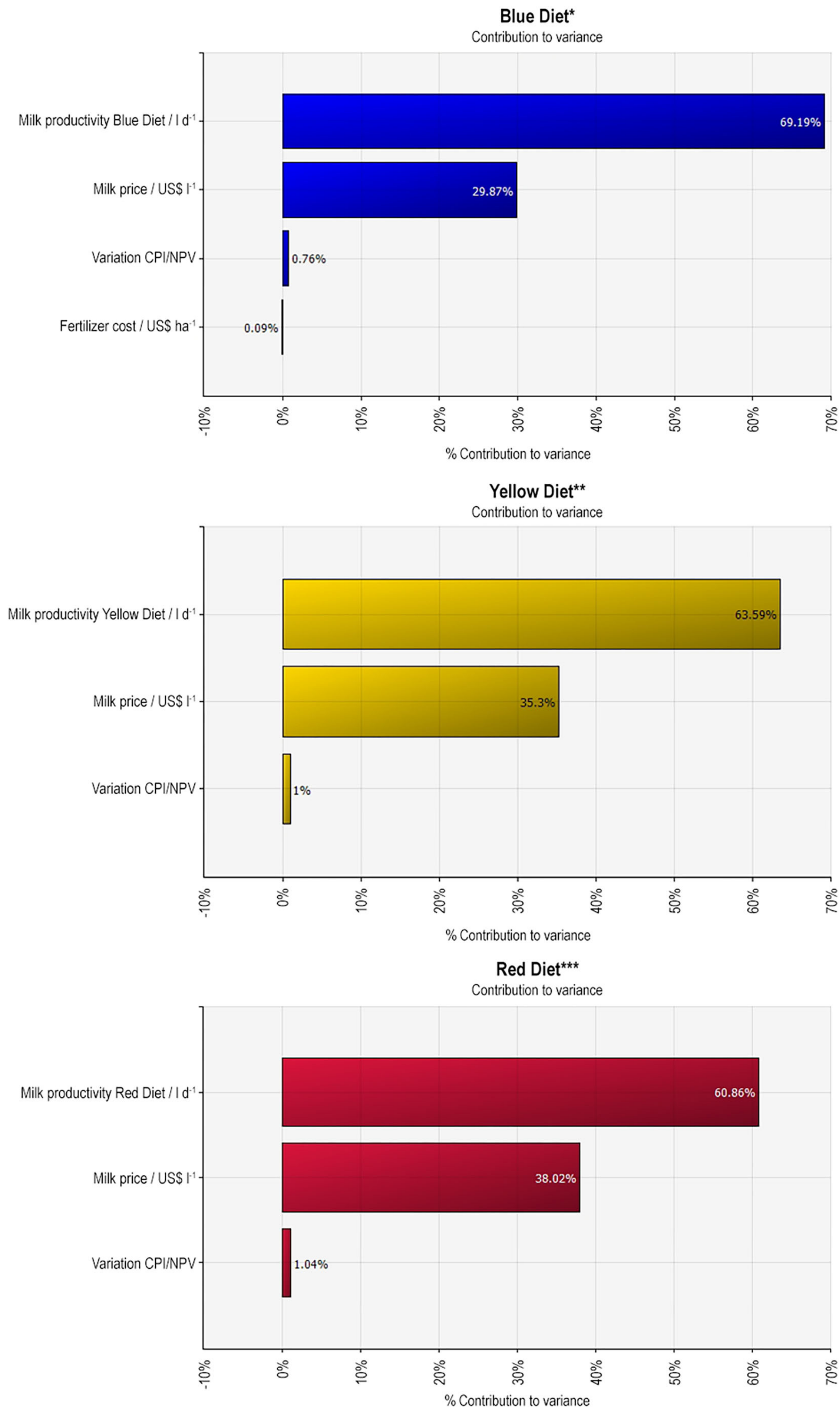

FIGURE 3 | Contribution of variables to the NPV variance for the evaluated diets. *100% Kikuyu grass, **35% Altoandina silage and 65% Kikuyu grass, ***65% Altoandina silage and 35% Kikuyu grass.

supplementation with oat silage has allowed increases in the production and percentage of milk fat, without detriments to protein and total solids (Campuzano et al., 2018). This increase is associated with the greater supply of forage available in diets that include silage, which balances a diet rich in protein and energy (J. Castillo, Agrosavia, personal communication). Mojica et al. (2009) found a higher milk production in cows fed with Kikuyu grass with a supply of oat silage (*Avena sativa*) of 0.7 kg DM per 100 kg of live weight (equivalent to a supplementation of 17.5% of the total diet), although this increase in production was statistically similar to the diets where only Kikuyu grass was fed. Similarly, León et al. (2008), Harper et al. (2017), Burbano-Muñoz et al. (2018), and Castro-Rincón et al. (2020) report no significant differences in the DM consumption, milk production and composition for supplementation diets with 10–35% oat silage (*Avena sativa*). These results show that the inclusion of oat silage in low percentages of the diet does not affect the nutritional value of forage and, therefore, production is maintained. On the contrary, León et al. (2008) and Mojica et al. (2009) reported reductions in milk production when up to 1.4 kg DM of oat silage per 100 kg liveweight were incorporated into the diet (33–36% of the total diet). This effect was associated with a possible negative effect on the nutrient balance since DM consumption was similar with respect to the diet based only on Kikuyu grass. Barahona et al. (2003), however, reported an optimal level of silage utilization for supplementation of up to 75% of the total diet, with acceptable and profitable levels of milk production. In general, these variable results regarding the effects of oat silage on milk production can be associated with multiple factors, such as nutritional quality and cutting age of the oat (variation in the amount of nutrients), the type of silage and its interaction with the grass feed base, lactation (differences in nutritional requirements), availability and level of DM consumption, and level of energy consumption (Bhandari et al., 2008; León et al., 2008; Mojica et al., 2009; Harper et al., 2017).

At an economic level, the results indicate the Yellow Diet as the best alternative, yielding an average NPV higher than for the other alternatives and a lower variability for said indicator. Similarly, the risk indicators VaR (Value at Risk at 95% confidence) and Prob (NPV < 0) are more favorable for this diet. These results are associated with greater efficiency in terms of production costs, which allows for increasing the profit margin per liter of milk produced. The Yellow Diet with 35% Altoandina silage can therefore be considered the best alternative from an economic point of view under different performance scenarios and market conditions. Sections 1 and 2 of this article evidenced the lack of economic studies regarding the implementing of oat supplementation strategies in the Colombia. In fact, the only study we found was conducted in the highlands of Mexico (Burbano-Muñoz et al., 2018). According to the results, production costs per kilogram of milk increased by 25 and 50% for inclusion levels of *Avena sativa* cv. Chihuahua oat silage of 3 and 6 kg DM per cow and day, respectively. Since there were no significant differences in yields or milk composition, the diet with only Kikuyu grass had the highest profit margin. This study, however, highlights the importance of this feeding strategy to maintain production levels in places where grazing conditions are limited. Likewise, the use of oat silage makes it possible to reduce the use of feed concentrates or expensive by-products for feeding animals—which are mainly imported at high prices and are subject to often strong price fluctuations. Both are also important attributes observed in our study. In addition, Altoandina has tolerance to rust (*Puccinia spp.*), higher drought tolerance and resistance to frost, which make it an option less likely to be affected by specific climatic conditions and pests present in the Colombian higher tropics. Altoandina can also be conserved for up to 3e years when proper oat conservation processes are guaranteed (silo, silage), which helps in reducing production seasonality and improving productive parameters.

Given the presence of periods of drought or frost that reduce the biomass supply in grazing systems in the Colombian high tropics, alternatives, such as supplementation with oat silage, that allow to maintain milk production levels stable throughout the year, are of great importance for the dairy sector. Achieving stable milk production would improve the income level of producers, contributing to their livelihoods, but also to food security and a better nutrition in the region. Although there is a visible trend toward using feed supplementation strategies in dairy farms in the high tropics (e.g., hay and silage in critical times), this rather applies to the more technified farms. Farms with low to medium technification are more reluctant resulting in low levels of adoption of such supplementation strategies, which is evidenced by less than 5% and 20% of the producers using hay and silage supplementation, respectively (FEDEGAN, 2012). Among the main barriers that limit the adoption of supplementation strategies are the lack of equipment to chop the silage (Reiber et al., 2010, 2013; Bernardes and do Rêgo, 2014), and the lack of labor (Bernardes and do Rêgo, 2014). On the other hand, factors that favor the adoption of supplementation strategies are financial and agricultural resources, continuity and intensity in rural extension, access to demonstration farms and the participation of key innovators, the lack of alternative feeds for the dry season, the perceived benefits of silage feeding, and the presence of a favorable milk market (Reiber et al., 2010, 2013). This highlights the importance of providing support in the diffusion processes of these technologies in terms of training and education on the use of supplementation strategies as well as their technical and economic benefits. Likewise, facilities for producers to access the required equipment (e.g., machine rings) can help in technology adoption and diffusion processes.

The inclusion of oat silage in animal diets can also have positive effects at the environmental level, given the reduction of greenhouse gas emissions in the specialized dairy systems of the higher tropics. In Colombia, those systems present a high level of emissions of both Nitrogen (N) and Phosphorus (P) (León et al., 2008), which is associated with the levels of conventional fertilization with N used for the maintenance of (Kikuyu) pastures (around 400 kg N ha^{-1} y^{-1} are used), the high levels of protein consumption (e.g., 17–21% of protein levels in Kikuyu), and the consumption of P (through mineralized salts) not fully used at the ruminal level (León et al., 2008).

Different studies have proposed the use of cereal silages rich in starches as a strategy to reduce the consumption of N and P, increasing the efficiency in the use of these minerals and, therefore, reducing greenhouse gas emission levels. For example, León et al. (2008) evaluated the balance of N and P in 18 cows under grazing of Kikuyu grass and compared the results with a diet based on the inclusion of oat silage (*Avena sativa*). According to their results, the decrease in nutrient consumption through supplementation with oat silage decreased the excretion of N in the urine and reduced the P balance. On the other hand, it increased the excretion of N in feces which is associated with the lower degradability of the silage compared to Kikuyu grass. The above-described changes were not affecting milk production levels and composition. The authors state that the reduction of N in the urine significantly contributes to the reduction of greenhouse gas emissions, since it degrades faster than fecal N. Dhiman and Satter (1997) observed that the total excretion of N to the environment was reduced from 6 to 15% with diets that contained corn silage. Ramin et al. (2021) described that a higher inclusion of oats linearly reduced CH_4 emissions from 467 to 445 g d^{-1}, and the intensity of CH_4 from 14.7 to 14.0 g per kg of milk, without having adverse effects on productivity or energy balances. Other studies have confirmed that reducing the level of protein in the diet (i.e., from 18% to 15%) does not affect production, but reduces the excretion of N into the environment (Wattiaux and Karg, 2004; León et al., 2008). In summary, including grain silage, such as Altoandina, into the cattle diet may help to reduce greenhouse gas emissions without affecting productivity levels and thus, has positive effects on the environment when compared with traditional diets based on grazing (of Kikuyu) and feed concentrates. To achieve the maximum benefits in this regard, it is, however, important to ensure that the oats are being harvested at the optimum time (milky-pasty grains) and that the grains are being mixed with the forage.

CONCLUSIONS

The results of this study suggest that supplementation with Altoandina oat silage is an efficient alternative to meet feed requirements in critical times of milk production in the Colombian higher tropics. The inclusion of Altoandina silage as supplement into the Kikuyu dairy cattle diet in a 35% :65% proportion (Yellow Diet) results in the best per animal milk productivity indicators, whereas in a proportion of 75:25% (Red Diet), daily milk production declines. This is associated with the loss of nutritional quality of the forage at a level of 75% oat silage supplementation, affecting the nutrient balance and, therefore, the daily per animal milk productivity. This is consistent with other studies, which suggest oat silage supplementation as a promising alternative to maintain milk production levels in times of forage scarcity. Prior to the planting forage crops such as oats, it is, however, important to conduct technical and economic evaluations focused on the use of supplements to lower the excess protein levels that Kikuyu grass could present, according to the productive potential of the animals and the goals proposed in farm development plans. In addition, we recommend including the supply of supplements, such as Altoandina oat silage, into forage budget calculations (feed budget) to estimate the actual supply and demand of feed of the dairy herd, and to assess production costs for grass and supplements. Finally, it is important to carry out or publish results of the protein-energy balance in the Colombian higher tropics, focusing on the efficiency and importance of balancing diets based on forage crops such as oats.

According to the economic evaluation, the Yellow Diet turned out to be the best alternative to improve efficiency and profitability at the farm level when facing problems of seasonality in dairy production and increasing the income of producers. The evaluation also shows that implementing this diet is less risky than implementing the traditional diet based on Kikuyu (Blue Diet) and, considering the risk aversive behavior of many dairy farmers, this is a key aspect to promote diffusion and adoption. Altoandina also shows tolerance to stem rust (*Puccinia ssp.*) and drought, as well as resistance to frost, which makes it a valuable option for specific climatic conditions and pests in the Colombian higher tropics that can contribute both to reducing the seasonality of production and improving production parameters. Likewise, when there is an excess of protein in the pasture (as in the case of the 100% Kikuyu grass diet), supplying oat silage with high starch levels helps balancing the protein:energy ratio and thus, improves the efficiency of the system.

The use of supplementation alternatives such as oats contributes to achieving more sustainable food systems, through improving the efficiency of animal feeding. This leads to an increase in the availability of milk for consumption, which is key to nutrition and food security, and to improvements in the livelihoods of the producers. Commercial seed for growing oats is easily accessible and the establishment of the materials is relatively easy for the producers, making supplementation an attractive alternative to them. The use of Altoandina as supplementation thus helps improving the feeding efficiency by either maintaining the same production levels but reducing the use of more expensive feeds (e.g., concentrates) or producing more milk at lower per unit costs. This stabilizes the income flow of the dairy producers and, therefore, improves their livelihoods. The increased availability of milk for consumption also contributes to improving food security and the nutrition of, above all, the rural population. In addition, oats can also be a nutrient-rich food source for human consumption and contribute to the nutrition of the producer households. Likewise, the use of oats as a supplementation strategy also contributes to the reduction of N and P emissions to the environment, since oats, in their milky to pasty grain state, increase starch levels and balance the protein:energy ratio, and thus, contribute to reducing greenhouse gas emissions while improving economic efficiency. This makes oat supplementation a triple win alternative: more efficient production, increased livelihoods, reduced emissions. Although the experiments used as a basis for this study were carried out in the Boyacá Department of Colombia, it is important to note that they served as an important input for technology scaling processes and further evaluations in other high-altitude regions of the country with similar specialized dairy systems, such as in the Nariño (Castro-Rincón et al., 2020), Cundinamarca, and Antioquia Departments as well as in other areas of the Boyacá Department (J. Castillo, Agrosavia, personal communication). Likewise, the economic results obtained in this study have been key to identifying the percentage of the diet with the best economic viability at the producer level and helped to define a pathway for scaling this technology package in larger areas of the high-altitude tropics of Colombia. In this sense, Agrosavia in 2021 has been working on a plan for promoting Altoandina at the regional level, by providing dairy producers with technical recommendations and supporting them in increasing the planted areas. It is recommended, however, to conduct further trials and analyses in other countries with similar conditions (e.g., Ecuador, Peru, Bolivia) to support technology release and adoption processes there, too.

We also recommend including measurements at the environmental level in future studies on Altoandina, so that the technology's potential for reducing greenhouse gas emissions can be quantified and other potential ecosystem services identified. Such measurements should be included in the agronomic evaluations, which would then allow for accounting greenhouse gas emission reductions in the economic valuation exercise and to project them as additional benefits derived from the dairy system. Likewise, we recommend evaluating the use of Altoandina as dual-purpose crop, meaning in a mixed grazing-cutting system, where the animals graze the oat in the stuffing state, and after that fertilizer is being applied and the oat is being harvested for silage production once the grains reach the milky-pasty state. This approach could increase system efficiency and land use optimization. In addition, Altoandina is frost resistant, and intercropping with Kikuyu grass could help mitigating the effects of frost on the production system through improving the total on-farm DM availability. We thus recommend evaluations for determining the intercropping potential of Altoandina and its effects when it comes to the adaptation to climate change.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SB, KE, and JC: conceptualization, methodology, and formal analysis. KE, LA, LC, and SB: writing the original draft and review and editing. JC, KE, LA, LC, MS, and SB: resources. SB: supervision and funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

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Forage-Fed Insects as Food and Feed Source: Opportunities and Constraints of Edible Insects in the Tropics

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Farmed insects can provide an alternative protein source for humans, livestock, and fish, while supporting adaptation to climate change, generating income for smallholder farmers, and reducing the negative impacts of conventional food production, especially in the tropics. However, the quantity, nutritional quality and safety of insects greatly relies on their feed intake. Tropical forages (grasses and legumes) can provide a valuable and yet untapped source of feed for several farmed insect species. In this perspective paper, we provide a viewpoint of how tropical forages can support edible insect production. We also highlight the potential of tropical forage-based diets over those using organic agricultural or urban by-product substrates, due to their versatility, low cost, and lower risk of microbial and chemical hazards. The main bottlenecks relate to dependence on the small number of farmed insect species, and in public policy and market frameworks regarding the use of edible insects as food, feed and in industrial processes. This perspective will serve interested stakeholders in identifying urgent issues at the research, ethical, marketing and policy levels that can prevent the emergence of new, insect-based value chains and business models, and the nutritional, economic and environmental benefits they promise.

Keywords: edible insects, food security, sustainable development, business models, entomophagy policies

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INTRODUCTION

Rapid population growth, climate change, and environmental degradation have put food security and nutrition at risk, especially in the global tropics. The need to feeding a growing population has resulted in the exploration of new food sources for humans, livestock, and fisheries. In recent years, insects have been proposed as an alternative food source for humans and livestock. Food derived from insects is considered more resource efficient (needing less land and water) than traditional livestock production systems (Payne et al., 2016). Several studies highlight the benefits of edible insects for human and animal health. Crickets (*Orthoptera*), flies (*Diptera*), and beetles (*Coleoptera*) do not differ significantly in their nutritional composition from traditional protein sources such as beef, chicken, and pork (van Huis et al., 2013; Payne et al., 2016; Frigerio et al., 2020; Stull, 2021). The use of insects as food for humans or feed for livestock is, however, not a new concept. Humans have used insects in their diets throughout history (van Huis et al., 2013).

More recently, insects have been seen as viable and sustainable protein sources for livestock (Chia et al., 2019). The increased relevance of insects as feed is reflected by a rapid increase in the number of patent applications related to insect food processing methods; a growing number of companies offering insects for human and animal consumption; and increased research on edible insects and greater social acceptance of such (Müller et al., 2016; Kim et al., 2019). The boom in interest in insects as food and feed is tracking attention across the globe as evidenced by the development of legislative frameworks for insect-based products (European Food Safety Authority, 2021); and projected increases in the global market volume from US$ 400 million to between US$700 million and US$1.2 billion by 2024 (Dunkel and Payne, 2016).

In this perspective article, we provide a viewpoint of how different tropical forage crops available from international gene banks and grown on farms can support the current insect farming industry, and how their incorporation in insect diets has potential for addressing food safety concerns while maintaining the high nutritional quality of insects for human and animal nutrition. The article is structured as follows: section Insect Farming as a Food Source in the Tropics provides an overview of insect farming as a feed and food source in the tropics; section Tropical Forages as a Feed Alternative for Farmed Insects focuses on feeding insects with tropical forages; section Examples of Successful Projects provides insights into some successful pilot projects; and section Toward Responsible Insect Farming in the Tropics sheds light on how to move toward responsible insect farming in the tropics. Section Concluding Remarks and Forward Look provides concluding remarks that help interested stakeholders in developing forage-based insect value chains in the tropics.

INSECT FARMING AS A FOOD SOURCE IN THE TROPICS

Leakey (2020) projects increasing food insecurity and environmental degradation in the tropics if the business-as-usual scenario continues. As a result, there is an urgent need for a paradigm shift where environmental sustainability, dietary diversity and productivity have equal value. Insect farming to produce food is a promising intervention. Compared to traditional livestock production systems, insect farming uses 50–90% less land per kg of protein produced and 40–80% less feed per kg of edible weight; produces 1.2–2.7 kg less greenhouse gas emissions per kg of live weight gain; and uses 1,000 L less water per kg of live weight gain (Payne et al., 2016). The tropics, where most insect species occur (Chapman, 2005), are very favorable for insect production since the edaphoclimatic conditions assure a steady production throughout the year under constant environmental conditions, and the natural occurrence of a broad variety of insect species eliminates the need to introduce non-native species that represent a risk of biological invasion (Jansson et al., 2019; Bang and Courchamp, 2020). Currently, most farmed insects at the industrial scale, however, belong to few species (Jansson et al., 2019), 12 in total, despite the existence of around 2,100 edible species (Jongema, 2017). This can exacerbate problems that exist in other food chains (e.g., crops, livestock) (Tisdell, 2001; Fanzo and Mattei, 2010; Bruford et al., 2015), such as diversity loss from overexploitation (Ramos-Elorduy, 2006; Malinga et al., 2020) and the risk of biological invasion in non-native regions, as well as create genetic erosion if no preventive measures are taken.

Insects also constitute a feasible alternative for animal feed, such as soybean and fishmeal, which is generally the largest expense in livestock production, representing 60–70% of the total production costs (Alqaisi et al., 2011; van Huis et al., 2013). As a result, small- and medium-scale farmers need alternatives that are both effective and affordable (Chia et al., 2019). Several cost factors are involved in insect farming, including facilities (i.e., laboratories and other infrastructure and resources), labor requirements (e.g., natural oviposition vs. artificial larvae infestation in the substrate), lifecycles and diets of insects (Chia et al., 2019).

TROPICAL FORAGES AS A FEED ALTERNATIVE FOR FARMED INSECTS

For insects to be considered viable as a food for humans or livestock, they must be provided with an adequate diet. Most often, small-scale farmed insects are herbivores that rely on crop residues (Chia et al., 2018; Jansson et al., 2019). Larger-scale insect farming is sometimes based on feeds that are in direct competition with human diets (e.g., maize, soybean, oats, wheat; see **Table 1**), and may contain ingredients with associated environmental impacts (Miglietta et al., 2015). For instance, some commercial diets for crickets include grains and fish meal to supply protein requirements, decreasing the sustainability of the entire chain (Lundy and Parrella, 2015; Bawa et al., 2020). Based on that, we propose that tropical forages can be used as an additional feed source in insect production.

TABLE 1 | Commonly farmed insects for food and feed.

Common name	Species
Industry-scale farmed insects for food and feed[a]	
Crickets	*Acheta domesticus*
	Gryllodes sigillatus
	Gryllus bimaculatus
Mealworms	*Tenebrio molitor*
	Zophobas morio
	Alphitobius diaperinus
Black soldier flies	*Hermetia illucens*
House flies	*Musca domestica*
Wax moths	*Galleria mellonella*
Locusts	*Locusta migratoria*
Sun beetles	*Pachnoda marginata peregrina*
Cockroaches	*Blaptica dubia*

Source: own elaboration based on [a]Jansson et al. (2019).

Tropical forages refer to planted grasses and legumes that are used to feed livestock in the tropics and include species such as *Megathyrsus maximus* (syn. *Panicum maximum*), *Urochloa* spp. (syn. *Brachiaria* spp.) or *Arachis pintoi* (see **Table 1**). Most often, tropical forages are used in places where other crops cannot be produced (e.g., on low-fertility and marginal soils). Among the common features of this group of plants are their relatively high biomass production and adaptation to continuous clipping, browsing, or grazing from animals, followed by vegetative re-growth (Capstaff and Miller, 2018). Tropical forages can supply enough biomass and serve as a steady supply of vegetative material to feed herbivore and omnivorous insects over one to several seasons. Tropical forages can also be conserved when there is a production surplus, e.g., as hay or silage with potential for insect feeding.

It is possible to enhance the nutritional content of insects by using tropical forages (Oonincx et al., 2020). Recent studies report that the protein content of crickets increases according to the protein supplementation of feed. Feeding for example dry pumpkin pulp or enriched flaxseed oil increases the vitamin B and omega 3 and 6 contents, respectively (Bawa et al., 2020; Oonincx et al., 2020). Tropical forages have better nutritional values than e.g., crop residues, and herbivore insects prefer most often soft (e.g., green leaves from forage crops) over hard plant material (e.g., stubble from crop residues) (Caldwell et al., 2016). Additionally, insects fed with tropical forages would not compete with food production for human consumption as is the case with grain-based insect feeds. In Uganda, the edible cricket *Ruspolia differens* (Orthoptera: Tettigoniidae) was found feeding on 19 grasses, including *Megathyrsus maximus, Urochloa ruziziensis, Chloris gayana, Cynodon dactylon, Setaria sphacelate,* and *Pennisetum purpureum*, preferring inflorescences or seeds over stems or leaves and showing a variability in host plant preference through the different life stages (Opoke et al., 2019). Also, diets based on grass inflorescences from different species influence maximal weight, survival, shorter development time and content of fatty acids of *R. differens,* being *U. ruziziensis, P. purpureum, S. sphacelata*, and *C. gayana* efficient for rearing insects for food and feed in sub-Saharan Africa (Rutaro et al., 2018; Malinga et al., 2020).

However, there is significant uncertainty about what constitutes optimal diets for farmed insects. Insects can compensate for the detrimental effects of an unbalanced diet through different physiological and behavioral mechanisms. Adequate food ingestion with the proper protein and carbohydrate ratios, however, results in better insect performance (Barragán-Fonseca, 2018). The nutritional requirements vary for each insect species and diets determine their nutritional content. For omnivorous farmed insects, these are complex and difficult to determine because of the broad variety of feed sources and substrates, but this characteristic also allows for more versatile diets to ensure their growth and development (Cortes Ortiz et al., 2016; Barragán-Fonseca et al., 2017; Hanboonsong and Durst, 2020).

There exists a large diversity of tropical forages, with great variation in terms of forage yield, agricultural suitability, nutrient content, and production constraints (Martens et al., 2012; Lee, 2018). An important collection of tropical forage diversity is safeguarded in the CGIAR gene banks of the Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) and the International Livestock Research Institute (ILRI), with over 22,000 accessions of tropical forage grasses and legumes from over 75 countries (The Alliance of Bioversity International CIAT, 2021). This diversity is a forage resource yet to be explored and used in insect farming. **Table 1** provides an overview of commonly farmed insects and **Table 2** on the forages that could potentially be used as diet, based on the comparison of the nutritional contents of commonly used diets and tropical forages. For crickets, *Andropogon* spp. could potentially replace whole yellow corn flour, mealworms could be fed with *Megathyrsus maximus* instead of white wheat, and sun beetle diets could be changed from brewer's yeast to *Arachis pintoi*, among others. Creative approaches are needed to identify the best-suited forages and to mix them in adequate ratios to supply insects with the required nutrients, increasing their productivity, and thereby, contributing to the sustainable intensification of animal-source food production systems.

Tropical forages available in the international gene banks, but also on farms, have the potential to become a part of the diets of farmed herbivorous insects. Forage-based insect diets would also contribute to the transition to circular economies for the agricultural sector. Insects produced with such diets can be used for both human consumption and as feed for poultry, swine, or fish. This would lead to numerous benefits and opportunities, such as the creation of new industries, small-scale businesses and jobs, income diversification, more balanced human diets, the protection of endangered species and ecosystems (e.g., marine ecosystems or forests), the reduction of greenhouse gas emissions, increases in above- and below-ground biodiversity and the protection of water resources, and thus contribute to achieving some of the Sustainable Development Goals (UN, 2021), i.e., those related to ending poverty, zero hunger, climate action, clean water and sanitation, decent work and economic growth, industry innovation and infrastructure, responsible consumption and production, life below water and life on land (Chia et al., 2019).

EXAMPLES OF SUCCESSFUL PROJECTS

Two projects in Kenya and Colombia show the impact of insect production as feed in small and medium-sized farms. In Kenya, the International Centre for Insect Physiology and Ecology (ICIPE) and Wageningen University trained more than 1,000 farmers on the production of black soldier fly larvae in organic waste substrates for feeding their animals and selling larvae to feed mills, resulting in 37 new insect-based enterprises and the establishment of cost-effective modular insect production systems (Dicke, 2019; Barragán-Fonseca et al., 2020). In Colombia, the National University of Colombia implemented different projects related to insect production for replacing 15% of traditional fish feed by black soldier fly larvae, with ex-combatants of the FARC-EP guerrilla in the Tolima

TABLE 2 | Content of common diets use in large-scale insect industry and potential forage species as alternatives for insect feed.

Common diets - nutritional content[a]			Potential forages as alternatives for insect feed[c]		
Source	**Protein**	**Crude fiber**	**Protein[c]**	**Crude fiber[b]**	**Species**
Whole yellow corn flour	6.9	7.3	8.3	31.6	*Andropogon* spp.
Carrot, dehydrated	8.1	23.6	9.7	36.1	*Pennisetum purpureum*
Dry potato flour	8.3	6.6	9.8	31.3	*Cynodon dactylon (cultivars and hybrids)*
White wheat	11.3	12.2	11.2	37.3	*Megathyrsus maximus*
Crude wheat bran	15.5	42.8	14	34.2	*Stylosanthes* spp.
Alfalfa pellets	16	27	14.2	31.5	*Paspalum notatum*
Dry egg yolk	32.2	0	14.6	29.9	*Urochloa* spp. (cultivars and hybrids)
Whole soy flour	34.5	9.6	18.9	30.7	*Centrosema molle*
Dry milk, skim	36.2	0	20.6	26.1	*Cratylia argentea*
Baker's yeast	38.3	21	20.6	26.1	*Desmodium heterophyllum*
Brewer's yeast	53.3	20	21.4	27.3	*Arachis pintoi*
Dry beef liver	68	0	23.3	19.9	*Leucaena leucocephala*
			9.0	36.9	*Chloris gayana*
			7.7	38	*Setaria sphacelata*

Source: own elaboration based on [a]Cortes Ortiz et al. (2016); [b]Of fresh aerial part; INRAE et al. (2020); [c]Rao et al. (2015); Schultze-Kraft et al. (2018).

Department, also addressing SDG 16 on peace, justice and strong institutions (Barragán-Fonseca et al., 2020). Currently there are research initiatives led by the International Centre of Insect Physiology and Ecology (ICIPE), academic institutions (e.g., University of Copenhagen, Wageningen University) and governmental institutions (e.g., The Netherlands Organization for Scientific Research), such as GREEiNSECT and ILIPA, which aim at producing scientific evidence for insect production in small-, medium- and large-scale industries and developing the commercial potential for food and feed, contributing enormously to the growth of this sector in the tropics.

Apart from their use as food and livestock feed, insects can also be sold (alive or processed) on other niche markets with price premiums, such as to zoos or pet owners, generating additional income for producers. Processing methods range from more artisanal (e.g., sun and oven drying, smoking, curing, grounding) to more refined industrial techniques (Melgar-Lalanne et al., 2019). New products are being developed constantly to satisfy the increasing demands of different niche markets. For human diets, a broad range of insect-based ingredients and products are already available on the market, which include cricket powder and food coloring or oils, as well as dishes in restaurants and snacks. For instance, in Thailand, where most of the sector is on a small-scale in rural areas, new market opportunities in gourmet restaurants and gastronomy tourism allowed the development of edible crickets and silkworm products and their industrialization in the main cities of the country (Halloran et al., 2016). Forage-based insect diets help to reduce the microbiological and chemical hazard (i.e., microorganisms, viruses, prions, pesticide residues) associated with substrates like animal or agriculture by-products or kitchen waste (EFSA Scientific Committee, 2015; Dobermann et al., 2017; Gałecki and Sokół, 2019), resulting in higher food safety of the derived products for both human and animal consumption.

TOWARD RESPONSIBLE INSECT FARMING IN THE TROPICS

The European Union (EU) followed by the United States and Canada leads the global edible insect market and industry (Bermúdez-Serrano, 2020). Consequently, the most complete and strict legislation related to the use of edible insects is found in the EU, where the insects (whole or parts of) are considered a novelty food that can be marketed throughout the region. Policies that regulate the type and quality of insect feed, insect commercialization, and more recently, the safety of specific species for human consumption are decreed by the European Food Safety Authority (EFSA), EU member countries, and Switzerland (Der Schweizerische Bundesrat, 2021). In January 2021, dried larvae of the species *Tenebrio molitor* (mealworms) were declared safe for human consumption by the EFSA, highlighting that the levels of contaminants will depend on those present in the substrates used as insect feed. A review by Lähteenmäki-Uutela et al. (2017) showed that, despite the increasing number of companies involved in the development of insect-based products and the growing insect market, the United States, Canada, China and Mexico lack regulations regarding the safety of insect food and feed products. Australia and New Zealand have regulations in the Food Standard Code for the species *Zophobas morio*, *Acheta domesticus*, and *Tenebrio molitor*, without clear definitions regarding food and feed safety (Lähteenmäki-Uutela et al., 2021). A high quantity of biological, chemical and allergenic risks are associated with this industry, as with any other kind of food (EFSA Scientific Committee, 2015), highlighting the urgent need for research on this matter. In addition, the participation of non-governmental institutions like the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) is necessary to guarantee the safety of insect products and to establish an international market, yet no such standards are included in the Codex Alimentarius Commission (Lähteenmäki-Uutela et al., 2021).

In tropical countries in Asia and America, legislative frameworks for insect production, commercialization and consumption are either insufficient or non-existent. In several countries, insects are not even considered food, undermining their potential role in the diets of humans and animals (Bermúdez-Serrano, 2020). In Thailand, the use of edible insects is an ancestral practice and although there are no food safety policies, licenses are needed to establish large-scale cricket farms, which are issued by the Food and Drug Administration of Thailand. Also, governmental institutions have released guidelines for cricket farming (Halloran et al., 2015, 2017; FAO, 2021). The situation is similar in Mexico, where insect production is regulated by the organic products law, which focuses on the promotion, conservation and avoidance of overexploitation of only four species: *Aegiale hesperiaris*, *Liometopum apiculatum*, *Cerambycidae* larvae and ant eggs (Lähteenmäki-Uutela et al., 2021). Other Latin American countries, such as Colombia, Brazil, or Argentina, do not have explicit regulations in this regard and tend to follow the Codex Alimentarius Commission standards. In contrast, there is legislation in place regarding edible insects in most tropical African countries (Grabowski et al., 2020). Kenya and Uganda are the two counties currently leading the setting up of standards for the use of insects as food and feed on the African continent (Egonyu et al., 2021). However, such standards still need to fully facilitate the potential of edible insects as an industrial endeavor (Musundire et al., 2021).

CONCLUDING REMARKS AND FORWARD LOOK

Insects are a viable option for supplying the growing demand for protein in the tropics, especially given the need to adapt to and mitigate climate change, potentially contributing to the UN's 2030 agenda. The advantages of insect farming in the tropics include a greater biodiversity, production throughout the year under stable environmental conditions and the contribution to at least 8 Sustainable Development Goals. This has led to the development of an emerging industry through initiatives based on black soldier fly production for fisheries in Kenya and Colombia. Organic residues and substrates, commonly used for this purpose, may, however, represent a hazard for both fishery and human health. We propose a new approach for insect-based value chains by integrating tropical forage-based diets in edible insect production systems, given the yet untapped forage diversity in international gene banks and on farms. Compared to commercial diets, tropical forages are a low-cost feed source for insects, with high dietary versatility, that provide opportunities for the transition to sustainable, circular economies. We found the main bottlenecks in the lack of specific regulations, the dependence on few species for large-scale industrial insect production and consumer food safety.

Further studies should focus on assessing several species of tropical forages to be included in the diets of commonly farmed insects Also, studies comparing the ease of using tropical forages as insect feed against that of conventional feed (commercial diets or organic waste) need to be performed. There also exists a need to further harmonize rearing, mass production, genetic diversity and harvesting of insects with consumption practices and strengthening of value chains and legislations. Knowledge from communities traditionally using insects as feed and food need to be considered since they can provide valuable insights. The synergies of these approaches will help the development of alternatives to feed both humans and livestock in a nutritious, secure and sustainable way.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

PE, LH, SB, and JC: conceptualization, methodology, and resources. PE and LH: formal analysis. PE, LH, SB, NP, and JC: writing the original draft and review and editing. SB and JC: supervision, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

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Tapping Into the Environmental Co-benefits of Improved Tropical Forages for an Agroecological Transformation of Livestock Production Systems

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Livestock are critical for incomes, livelihoods, nutrition and ecosystems management throughout the global South. Livestock production and the consumption of livestock-based foods such as meat, cheese, and milk is, however, under global scrutiny for its contribution to global warming, deforestation, biodiversity loss, water use, pollution, and land/soil degradation. This paper argues that, although the environmental footprint of livestock production presents a real threat to planetary sustainability, also in the global south, this is highly contextual. Under certain context-specific management regimes livestock can deliver multiple benefits for people and planet. We provide evidence that a move toward sustainable intensification of livestock production is possible and could mitigate negative environmental impacts and even provide critical ecosystem services, such as improved soil health, carbon sequestration, and enhanced biodiversity on farms. The use of cultivated forages, many improved through selection or breeding and including grasses, legumes and trees, in integrated crop-tree-livestock systems is proposed as a stepping stone toward agroecological transformation. We introduce cultivated forages, explain their multi-functionality and provide an overview of where and to what extent the forages have been applied and how this has benefited people and the planet alike. We then examine their potential to contribute to the 13 principles of agroecology and find that integrating cultivated forages in mixed crop-tree-livestock systems follows a wide range of agroecological principles and increases the sustainability of livestock production across the globe. More research is, however, needed at the food system scale to fully understand the role of forages in the sociological and process aspects of agroecology. We make the case for further genetic improvement of cultivated forages and strong multi-disciplinary systems research to strengthen our understanding of the multidimensional impacts of forages and for managing agro-environmental trade-offs. We finish with a call for action, for the agroecological and livestock research and development communities to improve communication and join hands for a sustainable agri-food system transformation.

Keywords: tropical forages, improved forages, cultivated forages, agroecology, mixed crop-tree-livestock systems, environmental co-benefits

IMPORTANCE OF SUSTAINABLE LIVESTOCK PRODUCTION SYSTEMS

Even though the role of animal based proteins as part of a sustainable twenty-first century food system is a highly debated topic (Meybeck and Gitz, 2017), the livestock sector currently plays a key role in food and nutrition security, particularly in developing countries. Livestock products (meat, milk and eggs) contribute 15% and 31% of the global per capita calorie and protein supply, respectively (Godde et al., 2021). Large regional differences characterize the nutritional contributions of livestock, with low intakes of animal-source food in the Global South compared with excesses in the Global North (Meyfroidt, 2018). Livestock are kept by more than half of rural households (FAO, 2018, 2021), with more than 844 million people worldwide receiving some income from agriculture, and the livestock sector contributing about 40% of the value-added in agriculture (Gontijo de Lima et al., 2015).

In general, family farming—often by smallholders cultivating less than two hectares—is still the predominant form of livestock production in the global South, in terms of numbers as well as occupied area (Lowder et al., 2021). On these family farms, livestock production mainly occurs in mixed crop-livestock systems (Herrero et al., 2010), where livestock has a multitude of functions, ranging from the provision of food, nutrition, income and risk reduction to farmers as well as the contribution of essential nutrients and draft power to reduce drudgery and improve crop productivity. The farms are further connected to—mostly local, regional, and national—markets where they generate a plethora of other jobs along livestock value chains (Lie et al., 2017; Bravo et al., 2018; Enciso et al., 2018).

In response to increasing demand for livestock products, these traditionally mixed systems increasingly intensify and are thereby replaced by specialized livestock production systems with spatially decoupled crop and livestock production and high levels of resource depletion and/or environmental pollution (Garrett et al., 2017a; Jin et al., 2020). For instance, about 51% of total feed nitrogen (N) in China was imported in 2015, greatly increasing energy requirements for transport, greenhouse gas (GHG) emissions abroad, and causing nutrient surpluses in China (Du et al., 2018; Zhang et al., 2020). The spatial decoupling of crop and livestock production is further associated with smaller fractions of manure returned to cropland and larger losses of manure N to surface and ground waters and GHG emissions (Bai et al., 2018). Hence, specialized crop production systems increasingly rely on synthetic fertilizers, and have higher environmental costs per unit of crop product (Zhao et al., 2017). Lastly, the proportion of grain-based feed ingredients and thus direct competition with human nutrition typically increases in the specialized livestock production systems. At the same time, their dependence on antibiotics and growth promoters is harmful for public health (antibiotic resistance, foodborne, and zoonotic diseases) (Peterson et al., 2020).

Globally, the livestock sector has a huge environmental footprint. It is responsible for emitting 14.5% of the total anthropogenic GHG emissions (Adegbeye et al., 2020), 33% of the total reactive nitrogen emissions (Mueller and Lassaletta, 2020), and is utilizing 30% of the total ice-free land area (Havlík et al., 2012). While large regional differences exist, many of the current livestock production systems in the tropics are responsible for undesirable environmental effects. Expansion of grazing land for livestock is a major driver for deforestation especially in Latin America, leading to about 57% of pasture land replacement with forests over the last decades (Graesser et al., 2015). Overgrazing in pasture and rangelands has resulted in severe soil degradation through compaction and erosion (Martinez and Zinck, 2004), especially in the drylands, with SOC losses creating a large carbon deficit in soils globally (Sanderman et al., 2017). In addition, livestock production is associated with biodiversity loss and high water use (Alkemade et al., 2013; Heinke et al., 2020) Among the most recognized and studied side effects of livestock production related to environmental damage in the tropical areas are: GHG emissions contributing to global warming, deforestation, biodiversity loss, high water use, and land/soil degradation (Martinez and Zinck, 2004; Alkemade et al., 2013; Chirinda et al., 2019; Boddey et al., 2020; Butterbach-Bahl et al., 2020). Widely publicized recent reports, such as EAT-Lancet (Willett et al., 2019), prompted a wave of media outreach arguing that one of the main solutions to the climate change and human health crises, globally, is to eat no or little animal-source foods (Paul et al., 2020a). Although we concur that the growing demand for livestock products presents a threat to environmental sustainability, we question the notion that stopping livestock production altogether is the most suitable or feasible option. Firstly, the political will is lacking and the necessary behavioral change of the majority of consumers is unlikely to occur (Winders and Ransom, 2019). Under these circumstances, it is important to have complimentary strategies that do not eliminate livestock but instead transform its production to reduce the environmental damages from the livestock sector. Secondly, livestock is not only of vital importance for low-income societies in socio-economical terms, but—when managed well—also plays various complex and often positive environmental and social benefits (Paul et al., 2020b). To reduce the consumption of animal source food could be a valid option for the Global North where diets show an excess in protein and energy consumption, but not for low and middle income countries where most people are under recommended nutrition standards. There, it is, thus, critical to identify sustainable management strategies. These strategies should be applicable to the local context, socially-acceptable, economically viable and avoid the environmental degradation that in the long-term undermines their existence.

Agroecology has been put forward as a solution to modern crises such as climate change and malnutrition, contrasting with the dominant industrial agricultural model based on the use of external inputs (Wezel et al., 2020), while improved forages have been proposed as an important entry point for the sustainable intensification of livestock production systems (Rao et al., 2015). This paper takes a closer look at and links up both these proposed solutions. It explores the benefits of including improved forages in integrated crop-livestock-tree systems and investigates the role of such forage-based systems in agroecological transformation. We thereby specifically focus on mixed cropping systems and cultivated forages in the tropics, i.e., crops that are specifically grown as animal feed, be it for grazing or cut-and-carry purposes; and exclude from our analyses the native and naturalized pastures and rangelands.

Based on a review of literature and expert opinion, we aim to demonstrate the importance of cultivated tropical forages, with their emerging environmental co-benefits, for ensuring sustainable livestock production based on agroecological principles. In section The Agroecological Framework, it starts by briefly introducing agroecology as (i) a science, (ii) a practice and (iii) a movement supporting the application of 13 principles—and their underlying values—to the design of farming and food systems. The next section, section Ensuring System Sustainability Through Integrating Improved Forages in Mixed Crop-Tree-Livestock Systems in the Tropics, summarizes how cultivated forages have been put into practice by farmers in the global south and how this provides benefits across different sustainability domains and barriers to further adoption at scale. Section Contributions of Improved Cultivated Forages to Agroecological Transformation proceeds by (i) outlining through which pathways and mechanisms this practice is in line with each of the agroecological principles and (ii) assessing to which extent applying these principles is covered in the scientific literature about forage-based livestock production systems in the tropics. Based on field experience and literature review, we summarize our understanding of the mechanisms and pathways through which the integration of forages in animal production systems can contribute or has shown to contribute to each of the 13 agroecological principles. Based on this understanding, search strings were developed for agroecology as a whole and separately for each principle. They were combined with a general search string capturing the integration of cultivated forages in smallholder mixed crop-tree-livestock systems in the tropics (see **Supplementary Material**). We report the number of hits in Web of Science as a metric for the availability of evidence of this contribution from the perspective of the scientific community. After reviewing the science at the forage-agroecology nexus, section Future Outlook finally identifies critical knowledge gaps and recommends the next steps for scaling up the contribution of cultivated tropical forages to the agroecological transformation of agri-food systems.

THE AGROECOLOGICAL FRAMEWORK

The principles of agroecology have evolved in history, from agriculture-centered to a holistic food system approach (Gliessman, 2018; Wezel et al., 2020). The most common definition of agroecology, "the application of ecological concepts and principles to the design and management of sustainable agroecosystems, or the science of sustainable agriculture," has recently evolved into an integrated concept bringing the three dimensions of sustainability—ecological, economic, and social—to all parts of the food system. The approach is grounded in ecological thinking where a holistic, systems-level understanding of food system sustainability is required (Gliessman, 2018). An agroecological perspective on agri-food systems links the nutritional value of food and dietary choices to the environmental and social impacts of food production (Lamine and Dawson, 2018). Hilbeck et al. (2015) write that "agroecology is neither a defined system of production nor a production technique. It is a set of principles and practices intended to enhance the sustainability of a farming system, and it is a movement that seeks a new way of food production. Scholars thereby agree that the term incorporates three components (IFOAM EU, 2019). First, it is a scientific discipline, studying the ecology of agricultural systems. Second, it has evolved into a set of agricultural practices. Finally, it has turned into a movement that incorporates social justice, food sovereignty and the preservation of cultural identities (Méndez et al., 2013). As such, it operates at different levels and engages different stakeholders ranging from scientists to farmers and communities in the context of the sustainable agri-food systems.

As happens with multi-dimensional concepts, operationalization often ends up focusing on one or a few components and fails to maintain a holistic approach. While promoting unidimensional agroecological practices, often mainly technical, still contributes to an agroecological transformation, these approaches are less sustainable as they often lack the sociopolitical support needed e.g., to reverse the power balance with conventional agriculture (Le Coq et al., 2020). Practically, neglecting the multidimensionality of the agroecology concept results in confusion with other concepts like organic agriculture, conservation agriculture, nature-positive agriculture or the more recent regenerative agriculture. Organic and conservation agriculture are based on simple principles around soil fertility management at plot level, aiming at avoiding the use of agrochemical and protecting the soil through permanent soil cover. The two differ in their market orientation, with organic agriculture strongly driven by product certification. Regenerative agriculture proposes a more holistic approach, trying to reconcile agroecology and sustainable intensification under the same banner, but seems to generate just more confusion (Giller et al., 2021). Nature-positive solutions, in turn, are less specific and englobe anything where nature works to address societal challenges, in agriculture or other sectors (Seddon et al., 2021), which includes the agroecology concept. The difference would be that nature-positive agriculture focusses on practices, whereas agroecology focusses on processes. But a common feature between all these different concepts is their meager integration of the livestock component. Until 2015, only 5% of indexed studies concerning agroecology dealt with livestock (Soussana et al., 2015).

As the concept gains prominence as a way to sustainably transform agriculture and food systems, particularly in a post-COVID world (Altieri and Nicholls, 2020), attempts to recognize all its dimensions and make it operational have culminated recently with the development of a clear framework and evaluation tool (FAO, 2018, 2021; Mottet et al., 2020). The framework is composed of ten interlinked and interdependent elements: (i) diversity, (ii) synergies, (iii) efficiency, (iv) resilience, (v) recycling, (vi) co-creation and sharing of knowledge, (vi) human and social values, (vii) culture and food traditions, (viii) responsible governance, (ix) circular, and (x) solidarity economy. The first five describe common characteristics of agroecological systems, the sixth and seventh describe foundational practices and innovation approaches, and the last three describe context features and enabling environment (FAO, 2018, 2021). These 10 elements imply a series of requirements for farming system management that can be articulated in 13 principles: recycling, input reduction, soil health, animal health, biodiversity, synergy, economic diversification, co-creation of knowledge, social values and diets, fairness, connectivity, land and natural resource governance, and participation (Wezel et al., 2020). A farming system that scores high in these principles can be seen as transitioning toward a sustainable food system via agroecological transformation. **Figure 1** presents a schematic overview of the different agroecological principles at play in a mixed crop-tree-livestock farm.

In section Contributions of Improved Cultivated Forages to Agroecological Transformation, we assess the role of improved tropical forages as a potential catalyst for enabling livestock systems to contribute to the 13 principles and support an agroecological transformation. As a background, the next section defines improved forages, summarizes documented uptake, the multi-dimensional impacts of this uptake and barriers to more wide-spread uptake.

ENSURING SYSTEM SUSTAINABILITY THROUGH INTEGRATING IMPROVED FORAGES IN MIXED CROP-TREE-LIVESTOCK SYSTEMS IN THE TROPICS

Livestock production in the global South takes place in a variety of livestock production systems. The grassland-based systems, in which crop-based agriculture is minimal, cover the largest areas (Robinson et al., 2011), while most production (i.e., meat, milk, eggs) occurs in mixed crop-livestock systems (Herrero et al., 2010). Cultivated forages include a wide variety of sown or planted grasses, herbaceous legumes, trees and shrubs (mostly legumes) that are integrated in a variety of mixed systems, including intensive or extensive mixed agricultural systems with grazing or cut-and-carry systems, agro-pastoral and silvo-pastoral systems (Rao et al., 2015). In Latin America and the Caribbean, permanent pastures are the most common use of forages, while in Sub-Saharan Africa and Southeast Asia cut-and-carry systems prevail.

There exists a large diversity of forages allowing adaptation to various production contexts. The so-called genetic improvement of tropical forages is relatively recent and was for several decades relying heavily on the agronomic selection of wild relatives. The agronomic/genetic evaluation of forages has been focused not only on productivity and feed quality but also on tolerance to biotic (insects, diseases) and abiotic (low soil fertility, aluminum toxicity, drought, waterlogging) stress factors. Through this selection from the wild it was possible to identify superior germplasm which resulted in substantial and sustainable productivity gains (per head and per unit area) as well as enhanced resilience (e.g., Peters et al., 2013; Rao et al., 2015; Schultze-Kraft et al., 2018). Recently the importance of bred forages has increased (Jank et al., 2014) and this has allowed attention to specific constraints, where diversity in the natural populations reached limitations in identifying productive, nutritive and stress-tolerant materials. For example, in well-drained environments in Latin America and the Caribbean with a wide distribution of *Urochloa* (previously known as Brachiaria; Cook et al., 2020) decumbens, resistance to a major insect, spittlebug, became an issue to be addressed by the breeding efforts, while for waterlogged environments there remains a scarcity of high-quality forages (Argel et al., 2007). Bred forages with a combination of desirable traits (e.g., productivity, quality and resistance to biotic and abiotic factors) are also attractive to seed suppliers for targeting specific agro-ecological niches, allowing a greater market differentiation providing incentives for development of the forage seed sector. For example, in the case of crop-livestock systems in Latin America and the Caribbean (LAC), we see expanding demand for forages requiring soil fertility management and greater attention to environmental concerns. There is also an increasing demand for shade-tolerant forages for silvopastoral systems with high resilience to vulnerable climates with extreme and unpredictable weather conditions. Throughout the rest of this paper we will use the term "improved forages" when we refer to forages that have gone through a process of agronomic selection from wild relatives or breeding and selection leading to genetic gain in desirable traits.

At first sight, such improved forages seem similar to the high yielding crops such as wheat and rice, widely promoted by the international agricultural research centers in the 1960s and 1970s and adopted as part of the Green Revolution (Byerlee and Lynam, 2020). We do, however, not expect the well-documented drawbacks, such as high input prices, environmental pollution and increased inequality, of the green revolution to re-occur with improved forages. First, the technology in itself differs

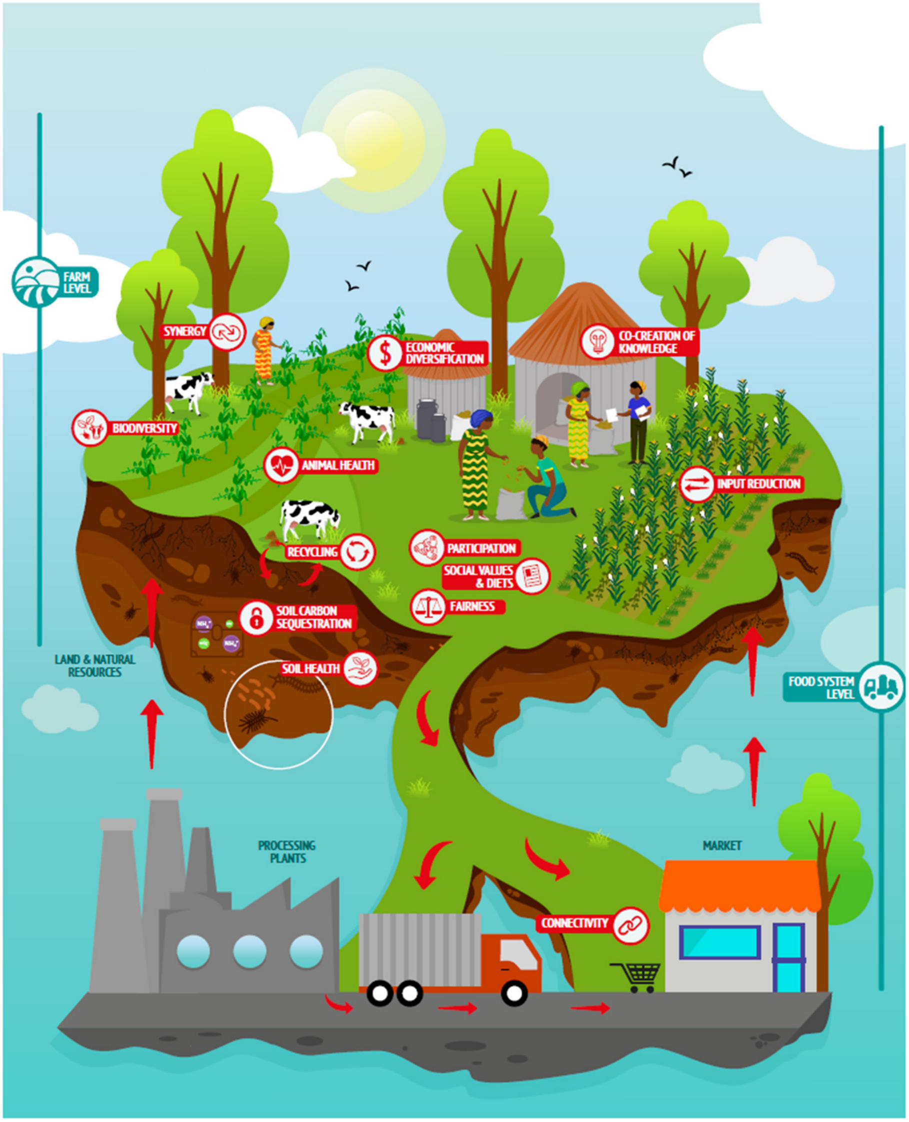

FIGURE 1 | The agroecological principles at work in a forage-based mixed croptree-livestock systems.

significantly, with the improved forages not requiring intensive application of pesticides, herbicides and synthetic fertilizers. On the contrary, many have been selected or are specifically bred for their capacity to perform well in marginal areas facing climate variability and change, low fertility or acid soils, water logging, and for pest and disease resistance. In addition, they are being promoted as a component of mixed cropping systems to improve the overall system performance and efficiency in using local resources. Finally, a wide variety of forage species and varieties, including indigenous trees and so-called neglected or orphan crops, are considered for system improvement.

Decades of efforts to promote cultivated forages for their productivity and environmental benefits have contributed to widespread adoption, particularly grasses in LAC (White et al., 2013; Baptistella et al., 2020, REDE ILPF ref). It is worthwhile to have a closer look at some successful scaling examples. Maass et al. (2015) estimated that the adoption of hybrid Urochloa cultivars in East Africa was about 1,000 hectares (20,000 households). Labarta et al. (2017) and ISPC (2018) reported that adoption of improved *Urochloa* cultivars in Colombia, Peru, Nicaragua, Costa Rica and Honduras occurred on approximately 7.9 million hectares. According to White et al. (2013), Stylosanthes varieties (from the CGIAR genebank) have been adopted on at least 200,000 hectares. Valentim and Andrade (2005) estimated the early adoption of *Arachis pintoi* for the Amazon region of Brazil to have reached 1,000 cattle producers and to have generated a gross profit of US$ 4,000 per year per producer. Wunscher et al. (2004) and Lascano et al. (2005) reported a successful early adoption of *Arachis pintoi* in Colombia and Costa Rica.

The benefits of integrating improved forages in livestock production systems have previously been described as part of the LivestockPlus concept (Rao et al., 2015). The authors describe how the sustainable intensification of forage-based systems, combining genetic, ecological and socio-economic intensification processes, increases the efficiency of the systems, has the potential to improve livelihoods, and yields a range of environmental co-benefits—including improved soil health, reduced erosion, reduced GHG emissions and improved GHG balances (emissions vs. carbon accumulation/life cycle), and improved adaptation to climate variability and change. **Figure 2** illustrates how forages can be integrated in mixed crop-tree-livestock systems and summarizes how this positively impacts on livelihoods and the environment.

The relatively wide adoption of improved tropical forages in LAC has convincingly demonstrated their capacity to increase productivity while reducing livestock-related GHG emissions per unit product. On one side, their ability to increase soil carbon sequestration has been demonstrated (Fisher et al., 1994) while the ability of certain grasses (e.g., Urochloa and Megathyrsus) to modulate the rhizosphere interactions through biological nitrification inhibition has proven to reduce soil-borne N_2O emissions up to 60% (compared to similar genotypes without this ability) either after fertilization or urine deposition (Subbarao et al., 2009; Byrnes et al., 2017). Another strategy is the improvement of cattle diets through supplementation with forage legumes, which has the potential to reduce up to 67% cattle enteric CH4 emissions based on a legume (i.e., Leucaena) inclusion proportion of 36% when compared to a grass alone diet (Gaviria-Uribe et al., 2020; Montoya-Flores et al., 2020).

In addition to these environmental co-benefits there is a huge body of evidence about their economic benefits. Zooming into forage grasses, the implementation of improved forage-based cattle production systems in Latin America, for example, increases the Internal Rate of Return (IRR)[1] by 10–100% compared to traditional grazing systems (Seré and Estrada, 1982; Seré et al., 1993). The implementation of improved *Urochloa brizantha* cultivars in Colombian beef cattle systems is expected to reduce the producer's risk of obtaining economic losses and lead to economic benefits of US$ 11.3 million at the national level (2022–2048) from which 62.5% would fall on the producer and 37.5% on the consumer. Supplementation by 35% with the forage oats (*Avena sativa* AV25T cv. Altoandina) in a Kikuyu grass dairy system increases the net present value (NPV)[2] by >100% when compared with a Kikuyu monoculture and leads to an IRR of 49.9% (Rivas and Holmann, 2000). The implementation of spittlebug-resistant Urochloa hybrids was estimated to have potential benefits equivalent to 43% of Colombia's beef and dairy production volume of 2003 (Rivas and Holmann, 2004a,b). The implementation of different planted forages in West Africa during the period from 1977 to 1997 was estimated to result in an social internal rate of return[3] on investments of 38% over 20 years (Elbasha et al., 1999).

Examples also abound around the dual economic-environmental benefits associated with forage legumes. The introduction of forage legumes in the crop-livestock systems of Nicaragua has proven benefits to tackling degradation and restoring land and soil health. When introduced into the smallholder traditional crop-livestock production system of the Nicaraguan hillsides, *Canavalia brasiliensis* derived on average 69% of its N from the atmosphere by symbiotic N_2-fixation, and increased the soil N balance when used as green manure (Douxchamps et al., 2010). In this case, 12% of the N from *Canavalia* was recovered in the subsequent maize crop (Douxchamps et al., 2011). However, when used as forage to increase milk yields and annual net income, *Canavalia* bears the risk of triggering soil N depletion, unless animal manure is recycled. Therefore, biophysical and socioeconomic trade-offs must be carefully balanced at the farm level to maximize nutrient use efficiency and ensure a sustainable farming system intensification (Douxchamps et al., 2014). Pastures on highly weathered soil in forest margins in Caquetá, Colombia increased dry matter and N/protein yield in farmers pastures containing legumes; because of additional N input via symbiotic N_2 fixation; greater P uptake in productive grass-legume than grass-alone pastures in spite of low plant available P in soils, which likely resulted in greater P recycling (Villegas et al., 2020). Furthermore, the inclusion of the legume *Arachis pintoi* in grass-legume associations in the same study area doubles beef and milk production and leads to an IRR of between 19.3 and 21.1%, which is significantly higher than for a traditional production system (Rivas and Holmann, 2000). For Costa Rica, grass-legume associations with *Arachis pintoi* and *Cratylia argentea* (Rivas and Holmann, 2000) lead to an estimated 30% reduction in production costs per kilogram of milk (Peters et al., 2001). Profitability evaluations in Costa Rica, Michoacán (Mexico) and the Colombian Caribbean region report an IRR that oscillates around 33% for a *Leucaena leucocephala*-grass association (Jimenez-Trujillo et al., 2011; González, 2013; Murgueitio et al., 2015). The inclusion of *Leucaena diversifolia* in a *Urochloa brizantha* cv. Cayman hybrid production system in Colombia is financially profitable and improves all risk and performance indicators when compared with Cayman as a monoculture. This legume increases the Net Present Value (NPV) and the

[1]The IRR is a financial indicator for estimating the profitability of potential investment projects. Although the IRR calculations are based on the same formula used for estimating the Net Present Value (NPV) of an investment project, it does not estimate the actual dollar value of the project but the expected annual return. Those potential investments with the highest IRR are generally the ones most desirable.

[2]The NPV is an economic indicator that describes the difference between the present values of cash in- and outflows over a defined period of time and is used in investment planning for analyzing the profitability of a potential investment. The NPV considers the time value of money, is used to compare different investment alternatives, and relies on a discount rate related to the cost of required capital for making the investment. Investment options with a negative NPV are most likely not profitable and should be neglected.

[3]The social IRR is a financial indicator that refers to the costs and benefits to society of a potential investment. It considers the opportunity costs of people not participating and the full cost of a potential investment for society, which makes it different from the general IRR indicator which only considers costs at the individual level. Apart from potential productivity increases derived from a potential investment, the social IRR also considers a broad range of possible non-economic benefits, such as better nutrition or a higher availability of end products.

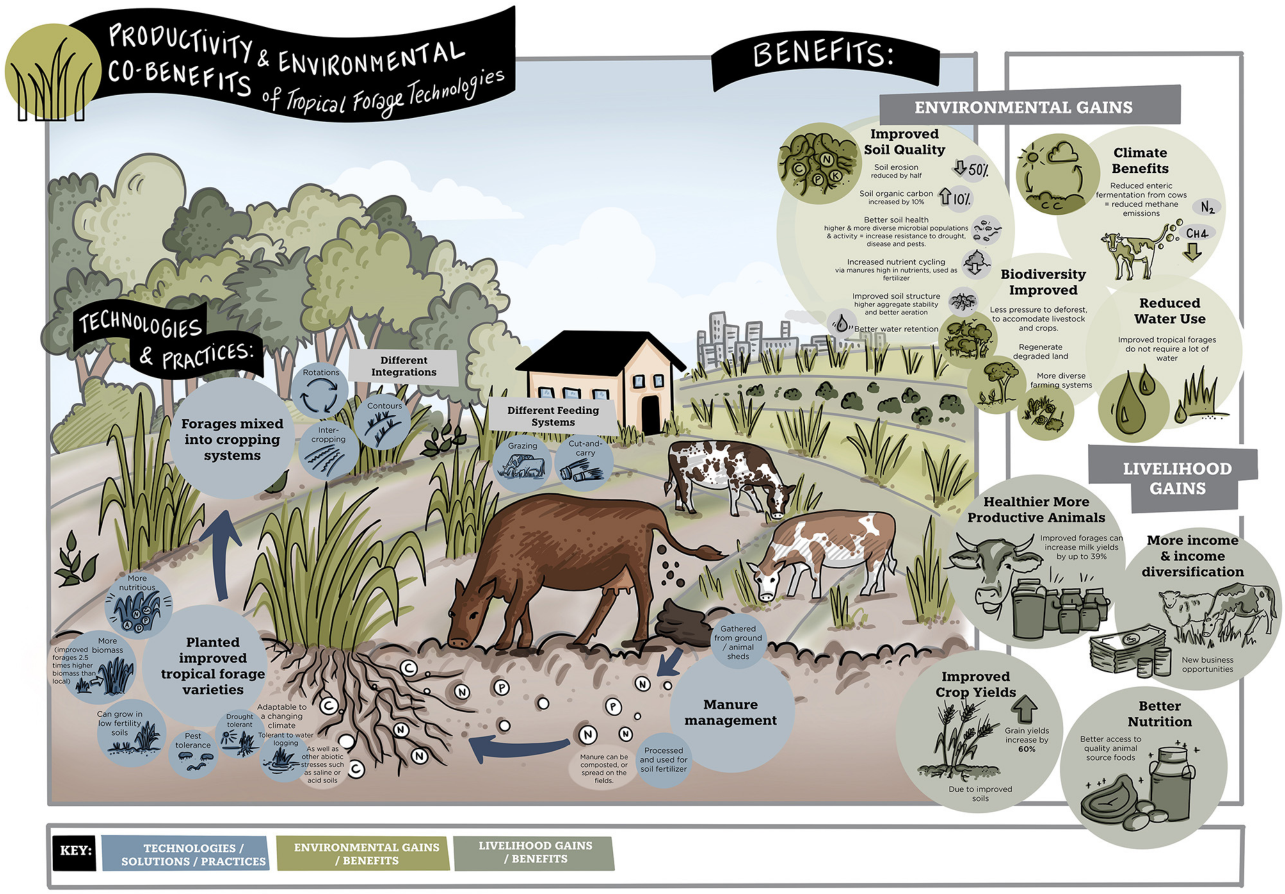

FIGURE 2 | Productivity and environmental co-benefits of tropical forage technologies.

IRR and decreases the minimum area required for generating two basic salaries, the payback period and the risk of obtaining economic loss (Enciso et al., 2020). Also in south-east Asia, forage legumes have proven to play multiple roles, supporting at farm level an increase of N recycling intensity, of N balances and of land productivity. However, the magnitude of the effects there depends strongly on the type of farming system, with more important effects where potential for improvement was high (Epper et al., 2019). While in Queensland, Australia, *Leucaena leucocephala* has been identified as the most productive and profitable legume, doubling the gross margin (expressed per unit of area), when compared with perennial grasses. At the regional level, economic benefits from the adoption of *L. leucocephala* have been estimated to be more than US$ 69 million/yr for 2006 in a planted area of 150,000 ha (Shelton and Dalzell, 2007; Bowen et al., 2016).

Also tree-based forage species have been demonstrated to have multiple benefits. Pilot sites in Mali, Burkina Faso and Niger, for example, show that more successful restoration outcomes are achieved when combining slow-growing indigenous trees or shrubs with fast growing native fodder species for livestock (Sacande and Berrahmouni, 2016). Fodder species have been used to incentivise restoration for example in Burkina Faso (Vinceti, 2020) leading to more resilient restoration outcomes and great adoption of restoration by farmers. Dry forest species can provide critical reserves during extreme drought offering important food and fodder for communities (Valette, 2019). Early effects of silvopastoral systems with improved forages also show improved soil health and increased abundance and diversity of soil macrofauna as documented by e.g., Barros et al. (2003), Lira et al. (2020), and Vazquez et al. (2020). Mixed systems with a strong tree component are thus gaining prominence because of their true multiple environmental wins: increased soil quality, GHG emission mitigation, higher biodiversity and improved water use efficiency.

As a final example, cactus pear (*Opuntia ficus-indica*) is gaining increasing interest across the globe because of its unique features that could help alleviate hunger in arid regions thanks to its ability to survive in harsh conditions. This spineless species is not invasive and is used as livestock feed that can improve meat and milk production for cash earnings, while helping to reduce groundwater use through its high-water use efficiency (species with CAM photosynthetic pathway). Furthermore, its evergreen cladodes can provide "at any time of the year" high palatable green fodder with a high Ca to P ratio. Despite its low crude protein and fiber content, the cactus pear cladodes are high in water, sugars, ash and vitamins A and C representing a digestible energy-rich feed when incorporated into livestock diets (Rocha Filho et al., 2021). Because of their high-water content, cactus pears also reduce the need for livestock watering. In fact, cactus pear is a very versatile, resilient crop. It is very easy to establish and able to grow on lands where no other crops can grow. Cactus pear is a multi-functional plant that can be utilized to restore degraded land, control soil and water erosion, regulate climate through carbon sequestration, and its fruits and cladodes are consumed by humans (Inglese et al., 2018; Hassan et al., 2019).

Even though the research on gender and social benefits has started later, good evidence on positive impacts in that dimension of sustainability is also emerging. A case study from Kenya shows that the adoption of improved planted forages in dairy systems leads to additional roles of women in feed and dairy production and thus more control over the derived incomes from the production system, but also to higher labor burdens, which might affect technology adoption (Lukuyu et al., 2021). Ba et al. (2013) report an average of 50% reduction in amount of labor and time spent by smallholder farmers in supplying forages to their animals in south Central Vietnam. The adoption of Urochloa hybrids and other improved forages in Ugandan pig production systems has led to time savings among male and female farmers (reduced time for collecting feed) and thus made it possible for the producers to engage in other economic activities (e.g., farming, small-scale enterprises). It also changed the decision-making structures in the households and empowered women to join their husbands in the decision on which forage to adopt and how to grow and manage it (Lukuyu et al., 2020). In Ethiopia and Kenya, women and youth are increasingly starting to engage in forage businesses, from which they retain income, and which is a promising pathway for women's economic empowerment (Njuguna-Mungai et al., under review).

Despite the growing evidence on the multiple benefits of integrating cultivated forages in mixed crop-tree-livestock systems and some successful scaling examples, overall the adoption rates of improved forages remain relatively low, especially outside Brazil and Latin America. Many of the determining factors for the adoption of forage technologies have been studied and include risk factors (perception of risk about future returns from implementing the technology, risk aversion of the producer) (e.g., Marra et al., 2003; van Winsen et al., 2014; Trujillo-Barrera et al., 2016), the availability of commercial seeds, forage establishment costs, the availability of technical information on the establishment and management, the promotion and availability of knowledge about potential benefits and risks (CIAT, 2004; Wunscher et al., 2004; Lascano et al., 2005), labor requirements (Kaimowitz and Angelsen, 2008), farm size and farm management, the proximity to input markets (ISPC, 2018), the growth of output markets (Kaimowitz and Angelsen, 2008), as well as the general access to productive inputs (e.g., fertilizer, manure, pesticides), capital (e.g., credits, payments for ecosystem services, product differentiation) (e.g., Charry et al., 2019), and extension/technical assistance (Ruiz et al., 2016; Bravo et al., 2018; Enciso et al., 2018; Charry et al., 2019), social capital, and membership of farmer groups (Oulu, 2020). Likewise, structural conditions can influence the adoption of improved forages, such as the prevailing extensive nature of the cattle production systems, low land prices (which can lead to an expansion of area instead of intensification) (White et al., 2001), land tenure rights (Kaimowitz and Angelsen, 2008), land speculation (Smith et al., 1997), political violence and warfare (ISPC, 2018), and missing regulatory and monitoring frameworks. When it comes to promoting the adoption of forage technologies, it is also important to analyze and understand how livestock producers make their decisions and how their decision-making process is influenced by factors such as trust (in the information provided or in its sources), social networks and socio-cultural contexts (e.g., Jones et al., 2013; Martínez-García et al., 2013; Rossi Borges and Oude Lansink, 2016; Ambrosius et al., 2019; Hidano et al., 2019).

CONTRIBUTIONS OF IMPROVED CULTIVATED FORAGES TO AGROECOLOGICAL TRANSFORMATION

As partly demonstrated in the previous section, integrating improved forages in mixed crop-tree-livestock systems is associated with a wide variety of practice changes. These changes include agronomic and animal husbandry practice change, awareness creation, capacity building, and multi-stakeholder engagement approaches to actions associated with the broader food systems, such as waste reductions and dietary shifts. As amply described in the scientific literature (see **Table 1**), they thereby align well to all 13 agroecological principles.

The first principle, recycling, prescribes to use local renewable resources as much as possible and close as far as possible resource cycles of nutrients and biomass. Forages take up nutrients available in the system, including from deep soil layers, and make these available to livestock. This results in improved nutrient use efficiency. More options to close nutrient cycles through animal manure also exist. In terms of input reduction, the second principle, forages are associated with a reduced need for external inputs, such as feeds, agro-chemicals and water. First, they are associated with a reduction of the need for commercial feed/supplements/concentrates through higher feed efficiency and quality. Well-managed high-quality forages can eliminate or minimize the need for concentrates by moderate producing animals, because intensive utilization of forages (cutting or grazing at the right moment of the phenology) increases the production of metabolizable energy and protein per unit of area. Second, they often are associated with a reduction of the need for off-farm manure or chemical fertilizers. This is facilitated through symbiotic N_2 fixation by forage legumes and the use of forages (partly/fully) as green manure. In addition, there is higher availability of on-farm animal manure because of increased livestock productivity (through higher stocking rates and better-fed animals) and increased availability of crop residues for soil amendments as they can be replaced by forages in the feed basket. Third, the use of forages as a cover crop reduces the need for weeding and chemical weed control, while the use of forages with

TABLE 1 | Key references describing the contribution of tropical forages in mixed crop-tree-livestock (MCTL) systems to the 13 agroecological principles described by Wezel et al. (2020).

Recycling
Andriarimalala et al., 2013; Epper et al., 2019; Paul et al., 2019; Dias et al., 2020; Dahlin et al., 2021
Input reduction
A. Reduction of the need for commercial feed/supplements/concentrates through higher feed efficiency and quality:
Snijders et al., 2011; Lukuyu et al., 2013; Silva et al., 2017
B. Reduction of the need for off-farm manure or chemical fertilizers:
Nyambati et al., 2006; Douxchamps et al., 2010, 2014; Schultze-Kraft et al., 2018; Boddey et al., 2020
C. Decreased use of chemical weed and pest control:
Xuan et al., 2006; Njeru et al., 2020
D. Decreased water requirements:
Ríos et al., 2006; Nefzaoui et al., 2014; Mayer and Cushman, 2019; Rocha Filho et al., 2021
Soil health
A. Improved chemical soil health:
Fisher et al., 1994; Schultze-Kraft et al., 2018; Baptistella et al., 2020; Lira et al., 2020; Olaya-Montes et al., 2020; Vazquez et al., 2020
B. Improved physical properties:
Schultze-Kraft et al., 2018; Baptistella et al., 2020; Boddey et al., 2020
C. Increased below-ground biodiversity and biological activity:
Vazquez et al., 2020
D. Climate change mitigation:
Byrnes et al., 2017; Boddey et al., 2020; Vazquez et al., 2020
Animal health
A. Improved animal nutrition:
Hoste et al., 2012; Sousa et al., 2015; Améndola et al., 2016; Sordillo, 2016; Nwafor et al., 2017; Mangwe et al., 2019; Mayberry et al., 2020
B. Increased animal welfare:
García-Cruz et al., 2013; Cuartas et al., 2014; Lerner et al., 2015; Pezo et al., 2018
C. Positive indirect effects on human health:
Hoffmann et al., in review
Biodiversity
A. Increased biodiversity across the landscape:
Alkemade et al., 2013
B. Increased forage diversity:
Giraldo et al., 2011; Rivera et al., 2013; De Farias et al., 2015
C. Increased agro-ecosystem diversity compared to monocultures:
D'Annolfo et al., 2021
D. Habitats:
Harvey et al., 2006; Moreno and Pulido, 2010; Rivera et al., 2013; Montoya-Flores et al., 2020
Synergy
Khan et al., 2008; Descheemaeker et al., 2010; Peters et al., 2012; Cheruíyot et al., 2020; Wan et al., 2020; Zahoor et al., 2021
Economic diversification
A. Commercial livestock production:
Rivas and Holmann, 2000, 2004a,b; Peters et al., 2001; Shelton and Dalzell, 2007; Murgueitio et al., 2015; Bowen et al., 2016; Schiek et al., 2018; Charry et al., 2019; Enciso et al., 2019, 2020; Chizmar et al., 2020; Ruden et al., 2020
B. Forage businesses:
Pezo et al., 2007; Nakamanee et al., 2008; Gontijo de Lima et al., 2015; Negassa et al., 2016; Charry et al., 2019; Creemers and Alvarez Aranguiz, 2019; Harrison et al., 2019; Mwendia et al., 2019; Burkart and Urrea-Benítez, 2020; Ntakyo et al., 2020; Ohmstedt, 2020a,b; Dey et al., 2021; Neres et al., 2021
Co-creation of knowledge
Peters and Lascano, 2003; Pezo et al., 2007; Bautista Solís, 2012; Geng et al., 2017; Dumont et al., 2019; David et al., 2020
Social values and diets
Rudel et al., 2015; Gupta, 2016; Charry et al., 2019; Shapiro et al., 2019; Ruden et al., 2020
Fairness
Calle et al., 2009; Broom et al., 2013; Cibils et al., 2015
Connectivity
Chakoma et al., 2016; Lie et al., 2017; Lema et al., 2021
Land and natural resources governance
Kaimowitz and Angelsen, 2008; de Oliveira Silva et al., 2016; Garrett et al., 2017b; Tapasco et al., 2019
Participation
Ayele et al., 2012; Lie et al., 2017, 2018; Bravo et al., 2018; Enciso et al., 2018; Tapasco et al., 2019; Burkart and Urrea-Benítez, 2020

genetic tolerance against certain pests and diseases or the use of forages in the push-pull system replaces chemical pest control measures (e.g., against stemborer and striga). Fourth, forages are associated with decreased water requirements. Increased soil water retention and infiltration is observed as a result of forages used as a cover crop or green manure to improve soil structure and limit run-off and in the case of improved forages established in areas previously covered by degraded pastures. Drought-tolerant and water-saver forages reduce dependence on water for irrigation compared to currently used forages grown in similar conditions.

Integrating cultivated forages in the systems enhances different dimensions of soil health, the third principle. The chemical soil health is improved through root exudation or forages used as green manure, through the stimulation of nutrient cycling, soil organic matter (SOM) accumulation, increased soil carbon stocks and sequestration. The physical soil properties are improved as a result of increased soil aggregation, improved soil structure and aeration, increases in particulate organic matter in soil, roots remaining in soil after harvest/grazing, forages as green manure or cover crop, or the use of forages to prevent soil erosion. Below-ground biodiversity and biological activity is increased through increased soil microbial diversity and activity, presence of rhizobia. Diverse pastures (mix of various species) of diverse functions (secondary compounds, root system) improve the conditions for biological activity at deeper horizons, while increased use of tree-based forages can improve soil quality through improved mycorrhizal networks. The integration of forages, with their capacity to sequester and store carbon in the soil and to inhibit biological nitrification, finally, can significantly enhance the climate change mitigation function of the soil.

Different mechanisms are at play for improving animal health and welfare, the fourth agroecological principle. High-quality forages (incl. legumes) in the systems improve the overall quality and quantity feeding and thus animal health, amongst others through enhanced immunity and resistance to pathogens. The conservation of forages (e.g., hay, silage, pellets) thereby increases the availability of feed during seasons where scarcity of feed leaves the animals most vulnerable to disease. Forages from diverse pastures (a mix of various species) complement each other in their contents of critical nutrients for the animal and secondary compounds. Some can, for example, be more efficient in utilizing P or pumping Cu or Mg, providing balanced nutrients and secondary compounds (antibloat, antiparasite agents), while recent results indicate that bioactive tanniniferous plants represent a valuable option as an alternative to commercial drugs for the control of gastrointestinal nematodes. Animal welfare is increased in silvopastoral systems. The trees/shade create more favorable microclimatic conditions and reduce heat stress, which has in turn been associated with more stable social/hierarchical behavior. In addition to animal health and welfare, also positive indirect effects on human health have been documented. Improved plant health, including those of forages, under minimal use of agrochemicals improves animal and human health through reduced exposure to chemical residues. Well-fed animals require less antibiotics thus reducing the need for antibiotics and risk of antimicrobial resistance. Well-fed and healthy animals cause a lower pathogen load in manure that can be transmitted through the food chain and feeding healthy forages can reduce feeding of feeds with high aflatoxins such as maize in East Africa.

The fifth agroecological principle, biodiversity, can be enhanced by increasing biodiversity across the landscape. Enhancing land productivity, through high-yielding forages, can spare land for biodiversity conservation and prevent the need for further land conversion to agriculture. The introduction of alternative forage species increases the diversity of species and genetic resources at farm and landscape level as compared to grass monocultures or degraded/intensively-managed pastures. This can include the use (and *in-situ* conservation) of local/neglected species. The broader variety of forage species in combination with reduced use of chemical weed/pest control is likely to attract/maintain wider diversity of e.g., pollinators and below-ground fauna. such well-managed pastures increase the natural introduction of native plant species with desired feeding value and resilience to extreme environmental conditions. In silvopastoral systems, the presence of shrubs and trees has been demonstrated to have a positive impact on biodiversity by creating complex habitats for wild animals and plants and harboring a richer soil biota as compared to conventional grazing systems. Cultivated forages enhance positive ecological interactions and complementarities among system components at the interface between the system's soil, plant, and animal components and thus align well with the sixth agroecological principle of synergy. Using for example tree-based forages can help to increase on-farm above and below ground carbon storage, leading to additional climate mitigation benefits.

Different mechanisms contribute to economic diversification, the seventh principle. In first instance, forages enable further commercialization of livestock production. Feed represents the highest cost of production in any livestock system and cultivated forages can substantially reduce the feed input costs. In combination with enhanced productivity, this results in increased rates of return and opens opportunities for income diversification with cattle fattening or commercial milk production. Also the forages in themselves allow for income-diversification. Income-generating opportunities along the forage value chain include forage seed supply, marketing and distribution, the sale of hay, silage, pellets and timber or fruits in the case of forage trees.

Approaches that encourage co-creation of knowledge and horizontal learning used in research and development efforts around cultivated and improved forages include: on-farm variety trials and participatory monitoring and evaluation, capacity building and knowledge exchange activities such as field days and farmer exchanges. These approaches promote farmer-to-farmer contacts as well as more equal relationships between farmers and researchers. This encourages sharing knowledge and skills and triggers innovation in combination with encouraging community-level seed production and "passing on the gift," the existing technology (and associated management practices) scale out quickly.

In terms of social values and diets, principle number nine, animal sourced foods (ASF) are an important source of proteins and readily available micro-nutrients, especially important for improving the nutritional status of especially young children and pregnant and lactating women. Integrating cultivated forages in livestock production systems can increase both the quantity and quality of ASF production. The forages also enable the production of sustainably produced ASF, with simultaneous social, economic and environmental benefits.

Efforts to ensure the affordability of quality and environmentally-friendly animal products and the creation of opportunities for smallholders, including for women and youth align well to principle ten, fairness. Forages support dignified and robust livelihoods along the livestock value chains. In line with connectivity, the eleventh principle, local feed, seed, and ASF production allow re-embedding food systems into local economies. Actors along the forages and ASF value chains have more proximity and confidence and are better connected to markets. Principle twelve, land and natural resources governance, prescribes to strengthen institutional arrangements to improve, including the recognition and support of family farmers, smallholders and peasant food producers as sustainable managers of natural and genetic resources. Forages create a need for land-use planning and offer opportunities for development of new resource management strategies, for instance to mitigate soil degradation (e.g., fanya juu terraces). Participatory land-use planning processes can ensure the optimal use of land areas that would not be suitable for crops, use in rotation/intercropping/life barriers/under trees and at times promote land use options for carbon-neutral agriculture. In line with the last principle, participation, the Forage community has started to apply a wide array of participatory approaches. Through participatory system dynamics modeling and participation in multi-stakeholder innovation platforms or round-table discussions, farmers can be included in the design of livestock and forage sector strategies. These approaches promote equal relationships and balanced powers between farmers, researchers and policy makers.

Between 2005 and 2021, a total of 1,183 peer-reviewed publications addressed the use of cultivated forages in smallholder systems. The most studied principles concerning the forages are economic diversification, social values and diets, biodiversity, and recycling, all illustrated by more than 200 peer-reviewed publications, mostly at farm scale. Animal health renders 126 hits, then the other principles with less than a hundred. Connectivity was the least represented, with only five hits. These results show that the most evident agroecological impact of forages, according to the scientific community, can be observed in terms of market opportunities and income diversification. The high number of hits for social value and diets illustrates how high the topic of animal-source food and vegetarianism is currently on the global agenda. The principle of biodiversity includes particularly papers reporting options to include forages in rotation or intercropping with different types of systems and pastures' diversity. Finally, the capacity of forages to provide options to close nutrient cycles at the farm level was well-documented. The scientific community's interest in these topics has evolved: social values and diets are high on the agenda since 2012, recycling emerged a bit later in 2015, while economic diversification and biodiversity display a sawtooth but generally increasing interest (**Figure 2**). Besides connectivity and participation, which are both only sporadically addressed, the documentation of the other principles increased during the period 2005–2020, with some promising peaks for animal health and synergy. More research is needed at the food system scale to fully understand the role of forages in agroecology, particularly on sociological and process aspects, which are both at the core of the four principles less documented. This also indicates a yet to be filled opportunity for forage experts to engage more with the agroecological movement and make forages part of sustainable agri-food system transformation. The finding that despite the existence of scientific literature about cultivated forages and each of the agroecological principles, only 38 out of the 1,183 publications in our WoS search explicitly mention agroecology corroborates this action gap.

FUTURE OUTLOOK

As illustrated in sections Ensuring System Sustainability Through Integrating Improved Forages in Mixed Crop-Tree-Livestock Systems in the Tropics and Contributions of Improved Cultivated Forages to Agroecological Transformation, there is increased research interest and understanding of the economic, social and agroecological dynamics related to improved forages and their integration in mixed crop-tree-livestock systems. However, several knowledge and technology gaps still exist. At the actual technology level, it is important to continue the genetic improvement and identify or develop forage varieties tolerant to a wide range of biotic and abiotic stress factors. Supported by state-of-art genomics and phenomics, this can be

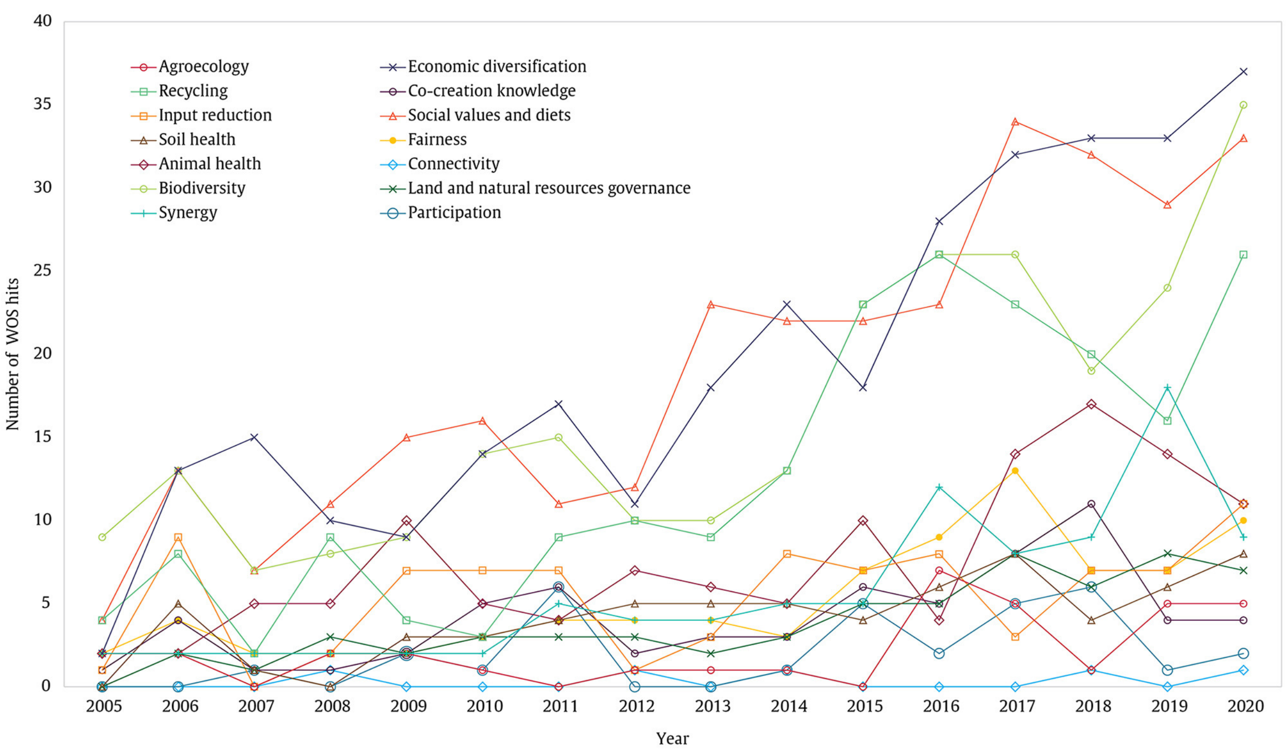

FIGURE 3 | Evolution of the interest of the scientific community for the different nexi between forages and principles.

done more efficiently and rapidly than before (Chang et al., 2019). Ensuring genetic diversity at forage level provides an insurance with respect to the impact of biotic and abiotic stress factors on yield and quality (Finckh, 2008). Livestock production, however, does not only take place in heterogeneous agro-climatic conditions, but also in a wide diversity in farm systems, and socioeconomic or policy contexts (Umunezero et al., 2016). To guide the choice of forage species and their integration into farming systems more systems agronomy is needed to produce robust socio-ecological niches for various systems that can be scaled (Paul et al., 2020c). This must be combined with increased research investments in the forages-soil health nexus which seem to have remained stable but low, with <100 WoS hits in total (**Figure 3**).

Further research is also required to strengthen our understanding of the multiple interacting impacts of improved forages at the food system level. An increased understanding of particularly the social dimension has a lot to offer, also in terms of understanding the drivers, underlying causes and impacts of changes linked to the productivity, economic, environmental and human dimensions (Rietveld et al., 2021), while our WoS search results show a low coverage of these issues in the scientific literature. Based on empirical data, foresight analyses and farming systems modeling can be used to estimate multidimensional impacts of forages and for reducing agro-environmental trade-offs (Groot et al., 2012; Paul et al., 2020c). In addition to developing context-specific data on the potential trade-offs associated with integrating forages in mixed crop-tree-livestock systems, a better understanding of what drives uptake of improved forages, especially within agroecological initiatives, is needed for guiding large-scale investments and supporting the decision-making processes around that.

At a more immediate action level, to ensure agroecological-based farming sustainability, there is a need for demand for the resultant products driven by sufficient public attention. To achieve the level of attention that results in changes in policy and consumer demand, there is a need for influential communication targeting policymakers and the different publics. Raising awareness at different decision-making levels should aim to differentiate, label and promote livestock products derived from agroecosystems based on agroecological principles. Concurrently, cultivated forages should be promoted as a versatile and multi-purpose crop through public campaigns (social media, workshops, leaflets, lobbying) (Louhaichi et al., 2018). However, from the literature search (**Figure 2**) these aspects seem to be understudied which would imply limited innovation in awareness raising. Yet, by highlighting the evidence-based benefits of integrating cultivated forages in agroecosystems, we can increase the visibility of crop-livestock systems and inform the flow of scaling-up investments. In addition, promotional and educational activities, along with results from further research involving farmer participation, in combination economic incentives, such as payments for ecosystem services and the development of inclusive business models, should be further explored (Schultze-Kraft et al., 2018).

CONCLUSION

The environmental and social consequences of the prevailing agri-food system have sparked a lively societal discussion on how to feed an increasing population in a socio-ecologically sustainable and equitable way. In response, agroecology has been presented as a practice, scientific discipline, and socio-political movement that applies ecological concepts in the sustainable management of agricultural systems. Although some literature highlights the important role livestock play in sustainable food systems and specifically agroecology, the prevailing narrative, especially so in the popular media, argues that one of the leading solutions to climate change and human health crises is to eat no or little animal-source foods.

In this paper, however, we point out that the narrow climate/diet framing misses the valuable role livestock can play, especially for family farmers in the south. Integrated systems present an opportunity to improve livestock production, support livelihoods, enhance and protect biodiversity, close nutrient loops etc. and forages play a key role in catalyzing this transformation. Scientific literature and documented practice change by farmers indicate that integrating cultivated forages in mixed crop-tree-livestock systems follows a wide range of agroecological principles and increases the sustainability of livestock production across the globe. We, therefore, have reason to believe that livestock production in the tropics based on improved forages can boost the sustainability indicators of this system, moving toward an agroecological transformation of the food system. It is, however, clear that a lot of this promise is yet to materialize and calls for an urgent coming together of the agroecological and livestock research and development communities. The specific role of the scientific community is therein to generate and use nuanced evidence on what is possible and what is not (taking multi-scale trade-offs into account). As part of the overall movement, they can help ensuring that forages gain more prominence in agroecological initiatives and that more investments are made in sustainable agri-food system transformation with explicit livestock and forage components.

AUTHOR CONTRIBUTIONS

AN, SD, JA, and BP: conceptualized the study and did a general literature review. DV: conducted the quantitative literature search in Web of Science. AN: led the writing of all drafts and revisions. MPe: contributed to forage breeding and agronomy and provided overall scientific oversight. CK: tree-based systems. MPu: study design. TR and MW: context and framing. EV, NT, and AO: soil health. SN: soil organic carbon. CP-P: adoption factors. NC: GHGe. SB: economics and adoption. All authors gaps and conclusions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.742842/full#supplementary-material

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Geographic Distribution of Colombian Spittlebugs (Hemiptera: Cercopidae) *via* Ecological Niche Modeling: A Prediction for the Main Tropical Forages' Pest in the Neotropics

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Spittlebugs (Hemiptera: Cercopidae) are the main tropical pests in Central and South America of cultivated pastures. We aimed to estimate the potential distribution of *Aeneolamia varia, A. lepidior, A. reducta, Prosapia simulans, Zulia carbonaria,* and *Z. pubescens* throughout the Neotropics using ecological niche modeling. These six insect species are common in Colombia and cause large economic losses. Records of these species, prior to the year 2000, were compiled from human observations, specimens from CIAT Arthropod Reference Collection (CIATARC), Global Biodiversity Information Facility (GBIF), speciesLink (splink), and an extensive literature review. Different ecological niche models (ENMs) were generated for each species: Maximum Entropy (MaxEnt), generalized linear (GLM), multivariate adaptive regression spline (MARS), and random forest model (RF). Bioclimatic datasets were obtained from WorldClim and the 19 available variables were used as predictors. Future changes in the potential geographical distribution were simulated in ENMs generated based on climate change projections for 2050 in two scenarios: optimistic and pessimistic. The results suggest that (i) Colombian spittlebugs impose an important threat to *Urochloa* production in different South American countries, (ii) each spittlebug species has a unique geographic distribution pattern, (iii) in the future the six species are likely to invade new geographic areas even in an optimistic scenario, (iv) *A. lepidior* and *A. reducta* showed a higher number of suitable habitats across Colombia, Venezuela, Brazil, Peru, and Ecuador, where predicted risk is more severe. Our data will allow to (i) monitor the dispersion of these spittlebug species, (ii) design strategies for integrated spittlebug management that include resistant cultivars adoption to mitigate potential economic damage, and (iii) implement regulatory actions to prevent their introduction and spread in geographic areas where the species are not yet found.

Keywords: ecological niche modeling, climatic change, pest distribution, future risk, *Aeneolamia*, *Zulia*, *Prosapia*, *Brachiaria*

INTRODUCTION

In the neotropics wide areas are planted in grasses, being *Urochloa spp.* P. Beauv. (syn. *Brachiaria* spp.) the most extensive forage monoculture (Ghimire et al., 2015; Worthington et al., 2021). Its economic impact is estimated at USD12.4 million in Mexico, Central America, Colombia, and Brazil, the largest contribution comes from *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Marandu in Brazil with USD 6.3 million (White et al., 2013). The major biotic stress affecting forage production and its quality in this region is caused by spittlebugs (Hemiptera: Cercopidae). A large group of species causes severe damage in susceptible grasslands (Cardona et al., 2004) with economic losses estimated at USD 840–2,100 million per year in all host crops (Thompson, 2004).

Although spittlebugs are found in most terrestrial ecosystems, the tropics are the most diverse ecozone harboring 70% of known species (Thompson, 2004; Dietrich, 2009). In the Neotropics, species are reported from the southeastern United States to northern Argentina (Peck and Thompson, 2008). Different spittlebug species coincide in each country. The main species that occur in Brazil are from the genus *Mahanarva* (Distant, 1909), *Notozulia* (Berg, 1879) and *Deois* (Fennah, 1949) (Resende et al., 2012). In Mexico, the species *Aeneolamia albofasciata* (Lallemand, 1939), *A. contigua* (Walker, 1851), and *A. postica* (Walker, 1858) are major pests of sugarcane and grasses (Cardona et al., 2004; Thompson and León González, 2005; Parada Domínguez et al., 2019). Whereas in Colombia the predominant species are *A. varia* (Fabricius, 1787), *A. lepidior* (Fowler, 1897), *A. reducta* (Lallemand, 1924), *Prosapia simulans* (Walker, 1858), *Zulia carbonaria* (Lallemand, 1924), and *Z. pubescens* (Fabricius, 1803) (Peck, 2001).

Climate change can modify the distribution of species by expanding their presence to new locations and disappearing from previously suitable areas (Hughes, 2000). Anthropic movement, land-use change, environmental degradation (e.g., habitat loss and fragmentation) and biotic interactions (e.g., competition, species introduction, and plant host distribution) produced by the on-going climate change are factors that influence this distribution (Wagner et al., 2021). Insects are well-known for being particularly susceptible to environmental changes of temperature, humidity, radiation, and resource availability driven by those factors (Larson et al., 2019). Processes that homogenize and simplify the landscapes as extensive agriculture, allow the growth of pests over native species (Cardoso et al., 2020). Several studies in recent years have warned about the decline of insect populations to extinction caused by changes in the seasonality and, consequently, in their life cycles. This reduction in the populations has great impact over the ecosystems as the loss of abundance and richness of species continue to occur (Hallmann et al., 2017; Goulson, 2019; Halsch et al., 2021).

Despite insect pest outbreaks are expected for the short term (Heeb et al., 2019; Liu and Shi, 2020), its severity may not be evenly increased due to the narrow environmental niche requirements, physiological tolerances of insects, and differential effects of climate variables on their life cycle (Lehmann et al., 2020). Previous models show an increase in suitable areas for pest species in Europe, e.g., *Helicoverpa zea* (Lepidoptera: Noctuidae*), Aleurocanthus spiniferus* (Hemiptera: Aleyrodidae), under climate change scenarios (Grünig et al., 2020). Thus, characterizing the effect of climate change in Colombian spittlebugs geographic distribution and identifying niches where these species would become key pests is important in the transition to more sustainable livestock systems.

In this context, ecological niche models (ENMs) provide an approximation to estimate potential geographical zones with environmental conditions that a species requires to maintain its populations (Peterson et al., 2011). This tool is widely used in insect pest management programs to anticipate unknown distributional areas, geographic potential of invasive species, and response to changing environmental conditions (Peterson and Soberón, 2012). ENMs can be built based on occurrence data (inductive or correlative niche models; Elith and Leathwick, 2009) or based on physiological data [deductive or mechanistic niche models; (Kearney and Porter, 2009)]. For spittlebugs associated with grasses, we identified only two studies focused on changes in suitability of geographical areas under climatic change scenarios. The first, based on physiological data of *Mahanarva spectabilis* (Distant) (Fonseca et al., 2016), and the second, based on occurrence data of four *Mahanarva* species (Schöbel and Carvalho, 2020).

This paper responds to the need to know whether *A. varia, A. lepidior, A. reducta*, *P. simulans*, *Z. carbonaria*, and *Z. pubescens* are potential key pests in new sites under climate change scenarios that consider the impact of human activities. Hence, spittlebug ENMs contribute to the development of adaptation strategies for tropical America climate-smart perennial grasslands, and sugarcane production, by addressing the need for shift toward more sustainable pest management practices (Macfadyen et al., 2018). For instance, adoption of cultivars with host plant resistance incorporated in high suitability predicted areas, or establishment of susceptible crops in low suitability sites, within intensive livestock and agriculture systems.

Our main objective was to determine the current distribution of these six species and estimate the potential distribution under two future climate scenarios via ecological niche methods based on presence-only data.

MATERIALS AND METHODS

Occurrence Data

Information about occurrence records of *A. lepidior, A. reducta, A. varia, P. simulans, Z. carbonaria*, and *Z. pubescens* were collected from a variety of sources: (1) human observations, (2) CIAT's Arthropod Reference Collection (CIATARC), (3) websites Global Biodiversity Information Facility (GBIF.org., 2020a,b,c,d,e) and speciesLink (https://splink.cria.org.br/), and (4) from extensive scientific papers revision (Hamilton, 1977; Avila de Moreno and Umaña, 1988; Peck, 1998; Sáenz et al., 1999; Cardona et al., 2000; Peck et al., 2001; Rodríguez Chalarca et al., 2002; Rodriguez Chalarca et al., 2003; Ferrer et al., 2004; Castro et al., 2005; Castillo, 2006; Valbuena, 2010; Figueredo et al., 2012; Matabanchoy Solarte et al., 2012; de la Cruz-Zapata et al., 2016; García-González et al., 2017; Paladini et al., 2018). Human observations data were obtained from CIAT historical records. These were captured by CIAT'S entomology department expert sampling in different locations. To georeference records from CIATARC without coordinates but with known location data, first, the geographic information available was verified and corrected according to National Statistics Offices (e.g., DANE to Colombia) and GeoNames (https://www.geonames.org/), second, coordinates were obtained via GoogleMaps (https://www.google.com/maps). A cleansing process was performed to this first base, removing the duplicates (i.e., more than one occurrence record in 10 km^2) and the records after the year 2000 to preserve the same temporal distribution between distribution data and climate data.

Climatic Data

Elevation layer and 19 bioclimatic layers (bio_1 to bio_19) were obtained from Worldclim from 1970 to 2000 using *raster::getData* function. For the current climate data, the Version 2 Bioclimatic variables with a spatial resolution of 2.5 min were selected (Fick and Hijmans, 2017) with the aim of maintaining the same spatial resolution of the species georeferenced (Sillero and Barbosa, 2021). To extract values from the bioclimatic layers, the *extract* function was used. Finally, the species names were combined with coordinates (latitude, longitude), bio_1 to bio_19, and elevation values into a single *data.frame*.

Ecological Niche Models

All analyses were performed in R studio version 4.1.0 (R. Core Team, 2021) according to Naimi and Araújo (2016) methodology (https://www.biogeoinformatics.org/), using the package sdm (Naimi and Araujo, 2019; R. Core Team, 2021).

Collinear Variables Removal

To prevent any multicollinearity-related bias in the models, a *collinearity test* among bioclimatic variables was performed using the *vifstep* function. Collinearity describes the situation where two or more predictor variables in a statistical model are linearly correlated (Alin, 2010). Therefore, it could inflate both the standard error and the confidence intervals, and prevent the determination of the significance of each variable on the dependent variable (Quinn and Keough, 2002). Variables with *VIF* (Variance Inflation Factors; Chatterjee and Hadi, 2006) values < 0.7 were selected for the subsequent analyzes. We created a *sdmData* object including species and previously selected variables, which means low collinearity, as predictors. Approximately 1,000 'pseudo-absences' points were randomly selected over the study geographical area for each species using argument method='gRandom'. Pseudo-absence refers to cells in which the species has not yet been recorded, not to cells in which the species is necessarily absent (Phillips et al., 2009).

Model Fitting

We used four species distribution models to predict the distribution of each spittlebug species under study. All models were based on presence and pseudo-absence data: Maximum Entropy (MaxEnt), Generalized Linear Model (GLM), Multivariate Adaptive Regression Spline (MARS), and Random Forest (RF) models. MaxEnt was used as default settings since it has shown the ability to achieve good performance as a default (Phillips and Dudík, 2008). Models are fitted with *sdm* function using two replication techniques (subsampling and bootstrapping) establishing 70% of the occurrence data as training data and 30% as test data. This process was repeated 3 times. As a result of our methodological procedure, a total of 24 different projections (4 models * 2 replication techniques * 3 repetitions) were generated for each species.

Model Prediction and Ensemble

We consider the accessible area of species under study as the entire neotropical ecoregion and that the species do not have restrictions since in this ecoregion there is a large pasture monoculture for livestock and it has a wide sugarcane planted area where cercopids can be established (Jank et al., 2014; Schöbel and Carvalho, 2021). The hypothesis was that climate change will impact or lead to an increase of future potential distributions of the species under study. Models obtained were used to estimate the current distribution in South America using the predict function from the *sdm* package. This function allows making a raster object with predictions from several fitted models (Naimi and Araújo, 2016). All 24 predictions were ensemble in one using the *ensemble* function which provides a consensus of multiple models. By combining projections from different models, errors tend to be canceled out thus aiding predictive accuracy (Diniz-Filho et al., 2010).

Model Evaluation

To evaluate model outputs, we used the receiver operated characteristics, analyzing the area under curve (AUC) (Fielding and Bell, 1997) and the true skill statistic (TSS) (Allouche et al., 2006). The AUC value is a standard method to assess the accuracy of predictive distribution models, AUC values below 0.7 were considered poor, 0.7–0.9 moderate, and >0.9 good (Araújo et al., 2005). TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. TSS values close to one denote an ideal prediction; values of zero or less denote a prediction that is not better than random (Allouche et al., 2006). For each species, the relative importance of bioclimatic variables selected based on multicollinearity analysis and AUC metric were plotted.

Future Distribution Model

To build future potential distribution, we used the BCC-CSM2-MR global climate model from the Coupled Model Intercomparison Project 6 [CMIP6; available for use in the WorldClim (https://www.worldclim.org/data/cmip6/cmip6climate.html); (O'Neill et al., 2016)] and two shared socio-economic pathways [(SSP); (1) SSP126: an optimistic scenario increasing shift toward sustainable practices with low greenhouse gas concentration levels and (2) SSP585: a pessimistic scenario that assumes an energy intensive, fossil-based economy with increasing greenhouse gas emissions over time (O'Neill et al., 2017; Riahi et al., 2017)] in a 2.5-min resolution. Habitat suitability was modeled using selected previously bioclimatic layers under each SSP scenario. In this study, only one time period was used for near future prediction: 2050 (average for 2041–2060). To quantify the change between current and future distribution, maps were converted from probability of occurrence to presence and absence. For this, the mean threshold (occurrence probability values) was used in the *ifelse* function which allows reviewing the probability values. If the probability values are greater than or equal to the average threshold, the new value assigned is 1 (presence) and if the probability value is less on the average threshold, the new value assigned is 0 (absence). Later, the current distribution raster was subtracted from the future distribution raster, as a result, possible extinction and invasion were plotted.

RESULTS

In total 590 occurrence records were obtained: 115 from human observations, 299 from CIATARC, 108 from GBIF, 24 from SpeciesLink, and 44 from literature review. After data cleansing, 48, 186, 19, 71, 55, and 120 points were used for *A. lepidior, A. reducta, A. varia, P. simulans, Z. carbonaria*, and *Z. pubescens,* respectively. Maps showing the occurrence records, estimation of current distribution and future potential distribution (2041–2060) under SSP126 - SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585) are presented in **Figures 1–6**. Suitable areas and suitability values as well as bioclimatic layers selected based on multicollinearity analysis differed according to the species in the study (**Figure 7**). Consequently, probability of occurrence (i.e., suitability) in the niches of each species as a function of two most representative biovariables (**Figure 8**) varied according to species. In general, the ensembled models reached acceptable values for metrics used to evaluate ENMs accuracy (see **Supplementary Table S1**). The most used, AUC and TSS metrics, showed high scores for all species under study indicating robust performance (**Figure 9**).

A. lepidior occurred in southern and central Costa Rica, central Panama, and northern Colombia. The ENM estimated a suitable area in central and north Colombia and some areas of Venezuela (AUC 0.97 ± 0.05, TSS 0.80 ± 0.1) (**Figure 1**). Bioclimatic layers with high contribution were isothermality (bio_3) and temperature seasonality (bio_4), showing high suitability with high values of bio_3 (>70 %) and low values of bio_4 (<77.45%) (**Figure 7**). Averages of AUC and TSS (± SD) were 0.97 ± 0.05 and 0.80 ± 0.1, respectively (**Figure 9**, **Supplementary Table S1**). A considerable increase in suitability is expected for large areas of Amazonas ecoregion of Peru, Venezuela, and the north of Brazil even in the optimistic scenario, with possible invasions in those sites and western Ecuador, northeastern Peru and northern Bolivia (**Figure 1**). Also in Panama, Costa Rica, and, in the pessimistic scenario, in Guatemala and Belize. Small areas in a few sites of the Pacific coast of Central America and tropical South America show a decrease in suitable areas for this species.

A. reducta occurred in Costa Rica, central Panama, and central and northern Caribbean Colombia. Fewer records were obtained in northwestern Venezuela and northern Brazil. The ENM estimated a suitable area in southern Costa Rica and Panama, as well as Eastern Ranges and Caribbean coast in Colombia, and Andean Venezuela (**Figure 2**). Bioclimatic layers with high contribution were minimum temperature of coldest month (bio_6) and isothermality (bio_3), showing high suitability with high values of both bio_6 and bio_3 (**Figure 7**). Average of AUC and TSS (± SD) was 0.94 ± 0.01 and 0.88 ± 0.05, respectively. An increase in suitable areas and possible invasions are expected for the future optimistic scenario in Colombian and Venezuelan Llanos and Colombian Caribbean region. In the pessimistic scenario, Amazonas ecoregion of Peru and Brazil, along with some sites in southern Costa Rica, Panama, Dominican Republic, and Mexico are predicted to be susceptible to new invasions (**Figure 2**).

A. varia occurred in central and southwestern Colombia and northwestern Venezuela. The ENM estimated a suitable area in Amazonas ecoregion of Colombia, Venezuela, and northern Brazil, and a smaller region in northern Peru (**Figure 3**). Bioclimatic layers with high contribution were precipitation of the coldest quarter (bio_19), temperature seasonality (bio_4), and precipitation seasonality (bio_15) (**Figure 7**). Average AUC and TSS (± SD) was 0.97 ± 0.01 and 0.89 ± 0.05, respectively. A decrease in suitable areas is expected for future scenarios compared to the same sites in current sites. Also, extinction is predicted in a few areas of Colombian and Venezuelan Llanos (**Figure 3**).

P. simulans was the most widespread species in this study. Occurrence records were obtained mostly from North America (Mexico) and Central America, with fewer records in western Colombia (**Figure 4**). Bioclimatic layers with high contribution were precipitation of the wettest month (bio_13) and precipitation of the coldest quarter (bio_18), showing high suitability with values <1,060 of bio_18 and values between 468 and 900 of bio_13 (**Figure 7**). Average of AUC and TSS (± SD) was 0.91 ± 0.06 and 0.73 ± 0.12, respectively. ENMs showed more habitats in South America and a small area in the Pacific Coast of Central America but with low suitability. An increase in suitability and possible invasions for small areas of Brazil Cerrado in both scenarios, along with Venezuelan Llanos in the optimistic scenario, and a noticeable decrease in Costa Rica is expected (**Figure 4**).

Z. carbonaria has been recorded only in western Colombia, across central Andes. The ENM estimated higher suitability in Colombian and Ecuadorian Andes (middle tropic) and the Amazonian Piedmont of Colombia, decreasing its values to zero in Colombian and Venezuelan Llanos (low tropic) (**Figure 5**). Bioclimatic layers with high contribution were isothermality (bio_3) and precipitation seasonality (bio_15), showing high suitability with values close to 40 of bio_15 and high values of bio_15 (**Figure 7**). Average of AUC and TSS (± SD) was 0.99 ± 0.02 and 0.93 ± 0.07, respectively. A decrease in suitability for the Amazonian Piedmont of Colombia and the Andes is expected (**Figure 5**).

Finally, *Z. pubescens* occurred widely in western and central Andes of Colombia, northern Ecuador and western Brazil, including Amazon and Cerrado biogeographic zones. Fewer records were obtained in southern Peru and northern Suriname

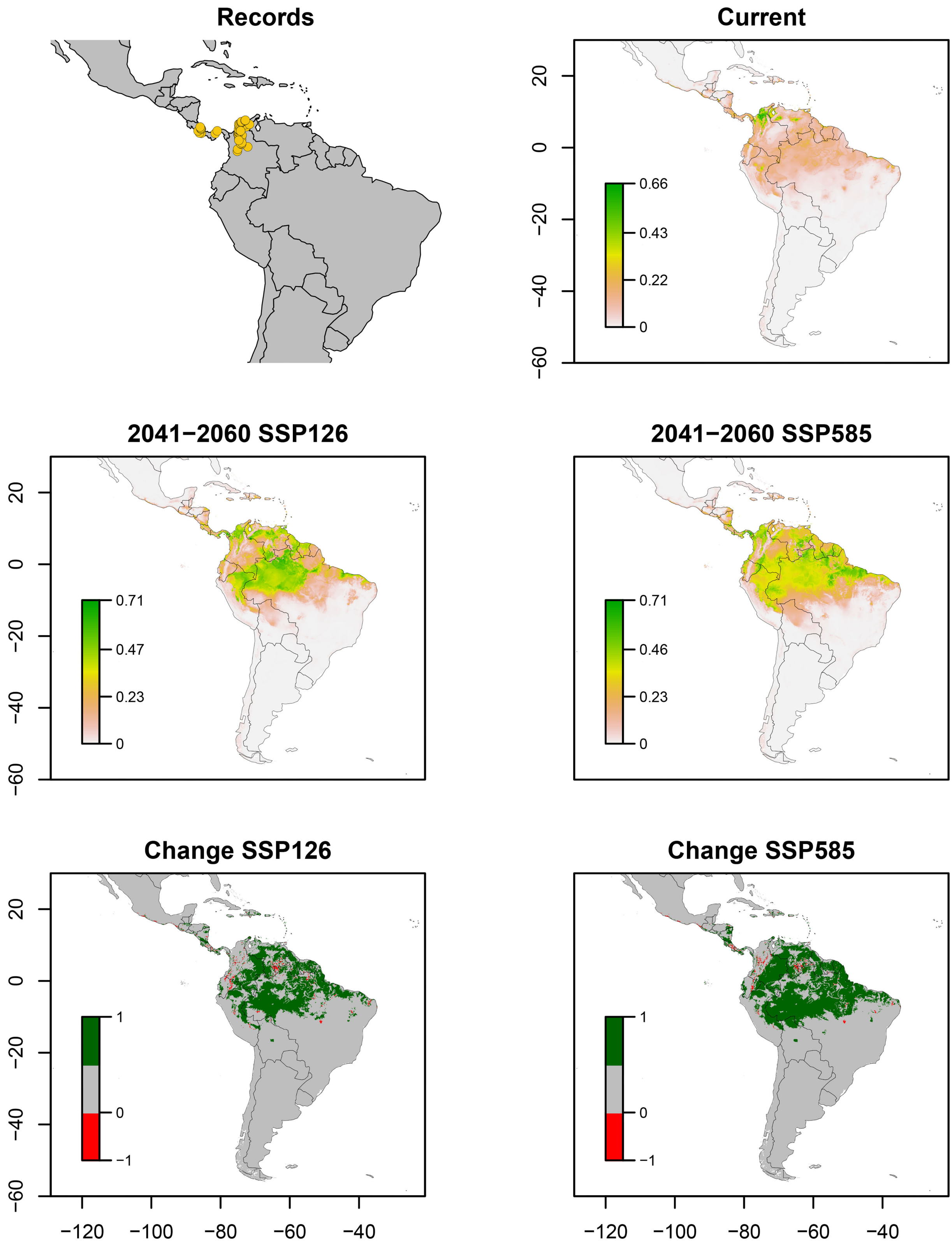

FIGURE 1 | Ecological niche models of *Aeneolamia lepidior.* Distribution records, current potential distribution, future potential distribution (2041–2060) under SSP126 and SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585). The scale shows the habitat suitability being 1 = higher suitability. Scale in change maps −1 = possible extinction and 1 = possible invasion.

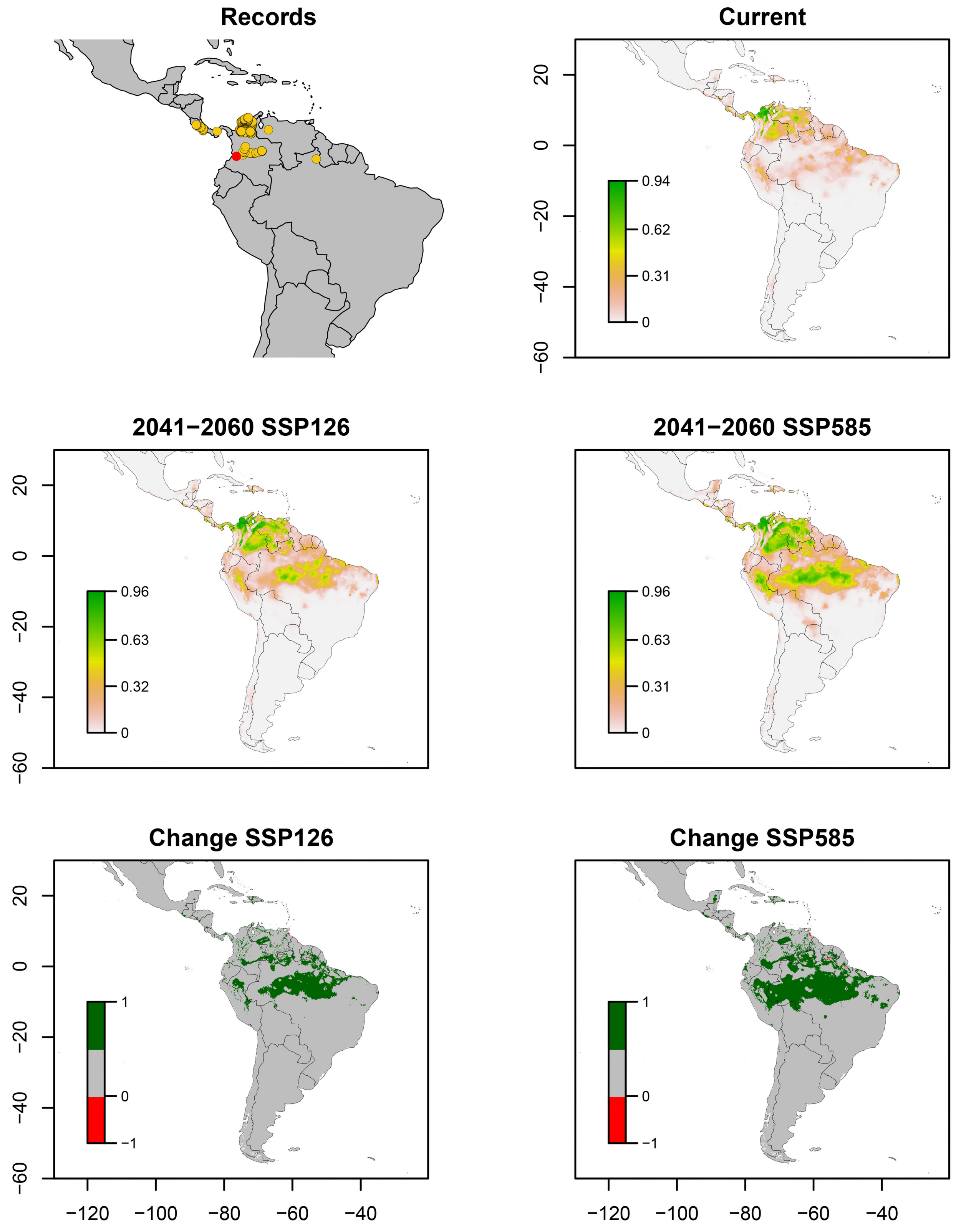

FIGURE 2 | Ecological niche models of *Aeneolamia reducta.* Distribution records (red point indicates the most recent report in a new niche), current potential distribution, future potential distribution (2041–2060) under SSP126 and SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585). The scale shows the habitat suitability being 1 = higher suitability. Scale in change maps −1 = possible extinction and 1 = possible invasion.

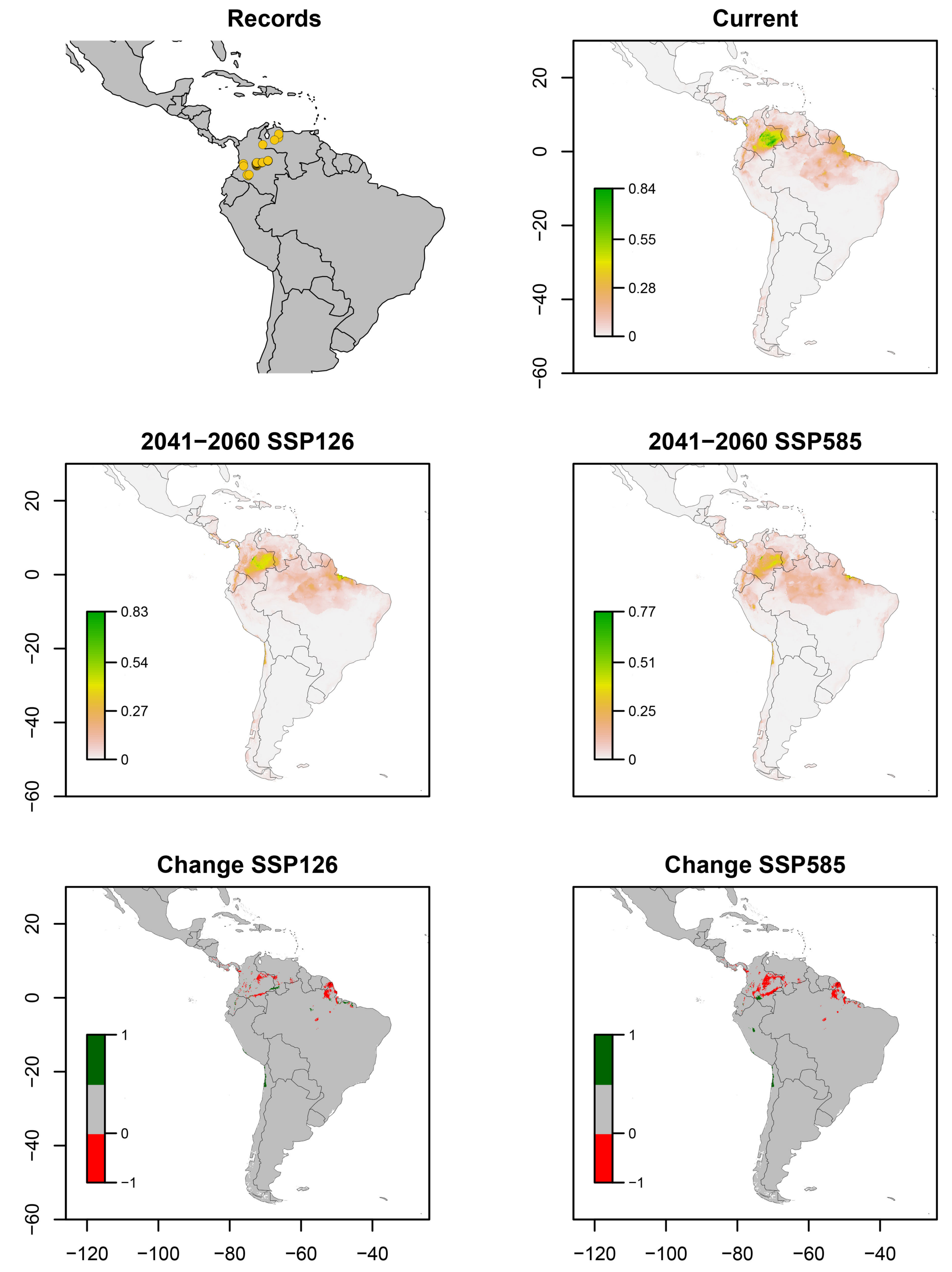

FIGURE 3 | Ecological niche models of *Aeneolamia varia*. Distribution records, current potential distribution, future potential distribution (2041–2060) under SSP126 and SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585). The scale shows the habitat suitability being 1 = higher suitability. Scale in change maps −1 = possible extinction and 1 = possible invasion.

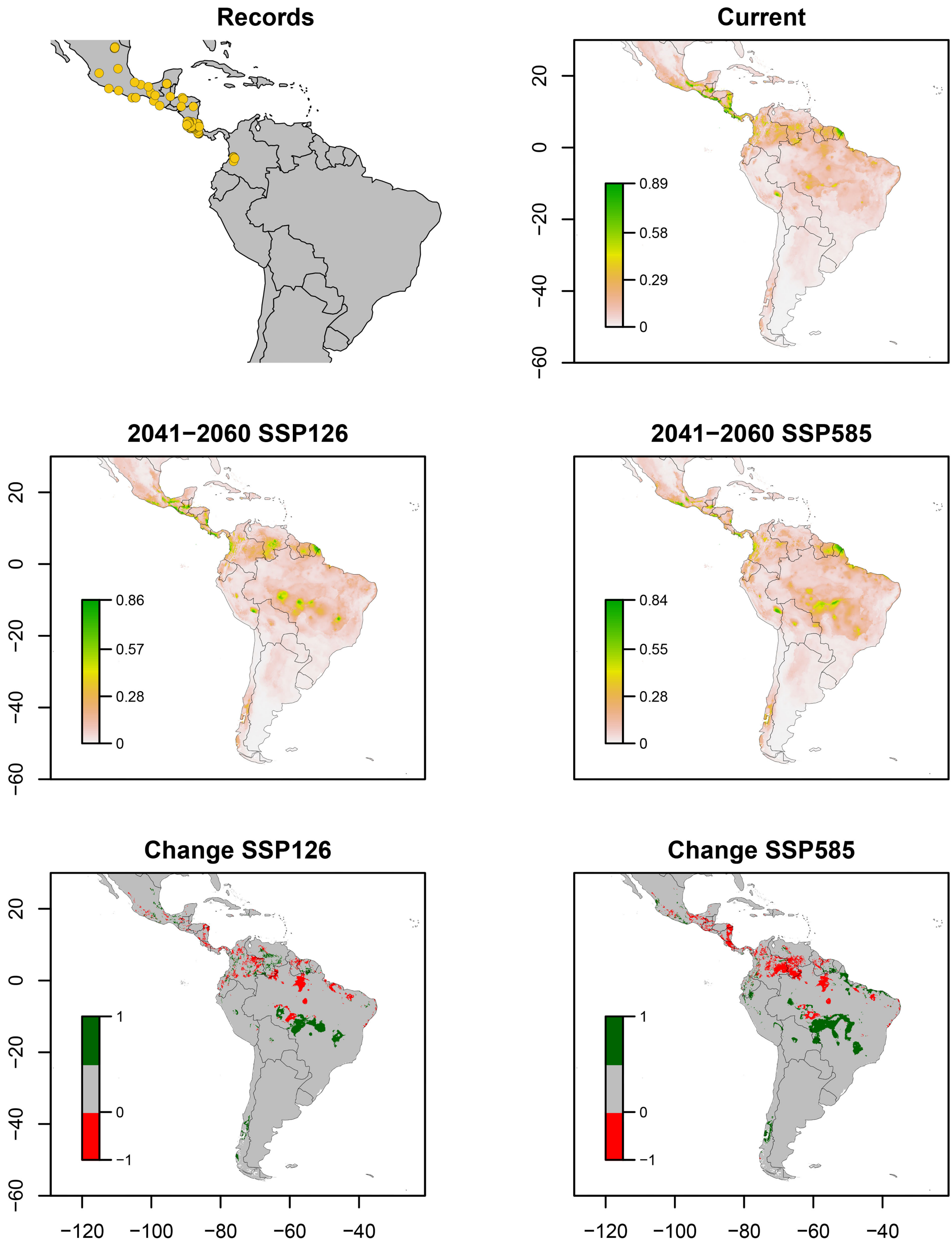

FIGURE 4 | Ecological niche models of *Prosapia simulans*. Distribution records, current potential distribution, future potential distribution (2041–2060) under SSP126 and SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585). The scale shows the habitat suitability being 1 = higher suitability. Scale in change maps −1 = possible extinction and 1 = possible invasion.

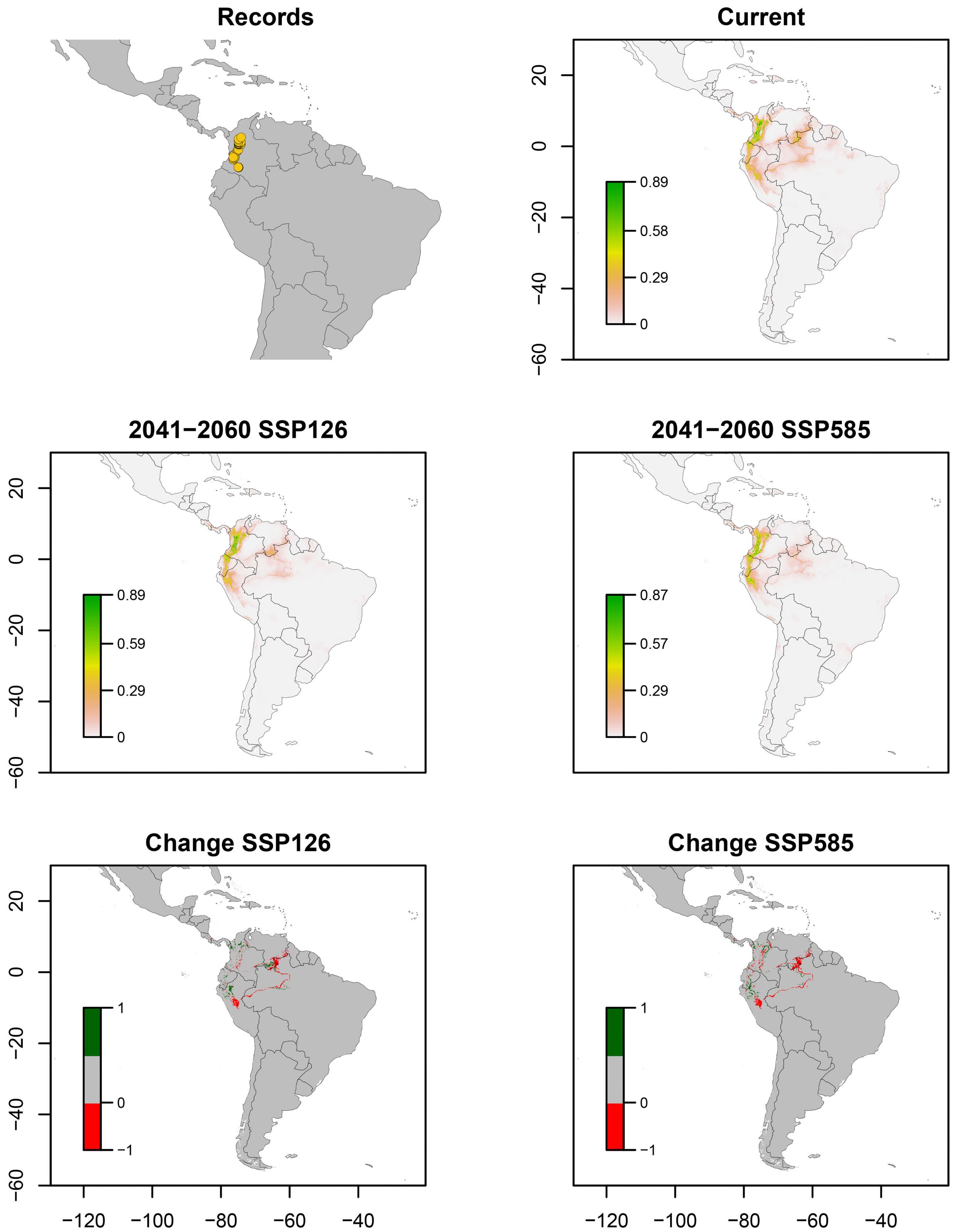

FIGURE 5 | Ecological niche models of *Zulia carbonaria*. Distribution records, current potential distribution, future potential distribution (2041–2060) under SSP126 and SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585). The scale shows the habitat suitability being 1 = higher suitability. Scale in change maps −1 = possible extinction and 1 = possible invasion.

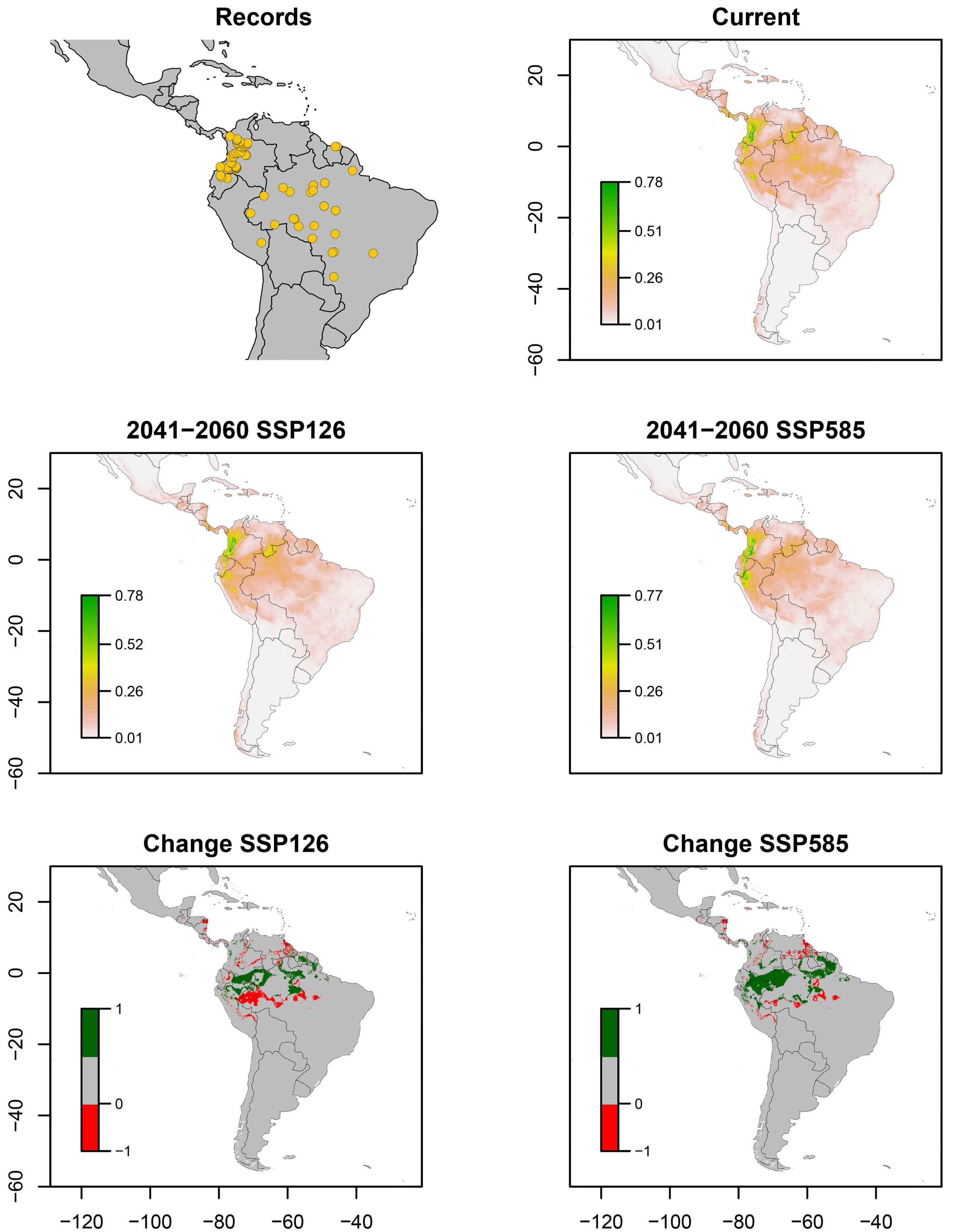

FIGURE 6 | Ecological niche models of *Zulia pubescens.* Distribution records, current potential distribution, future potential distribution (2041–2060) under SSP126 and SSP585 scenarios, and comparison between current and future scenarios (change SSP126 and SSP585). The scale shows the habitat suitability being 1 = higher suitability. Scale in change maps −1 = possible extinction and 1 = possible invasion.

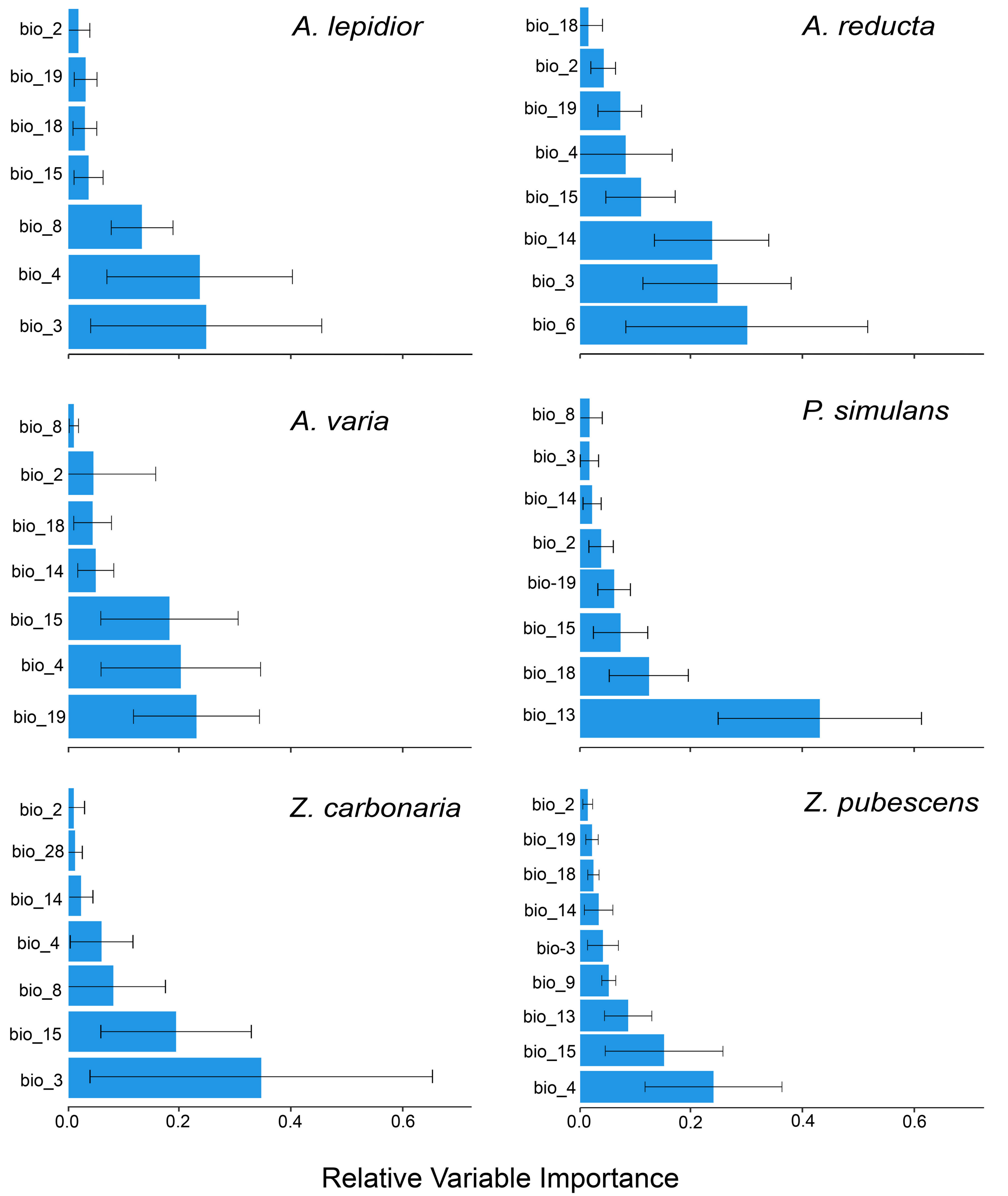

FIGURE 7 | Relative variable importance in modeling the ecological niche of each species of spittlebugs. Error bars represent the standard deviation of all 24 models. The graphs show only bioclimatic layers selected based on multicollinearity analysis for each species.

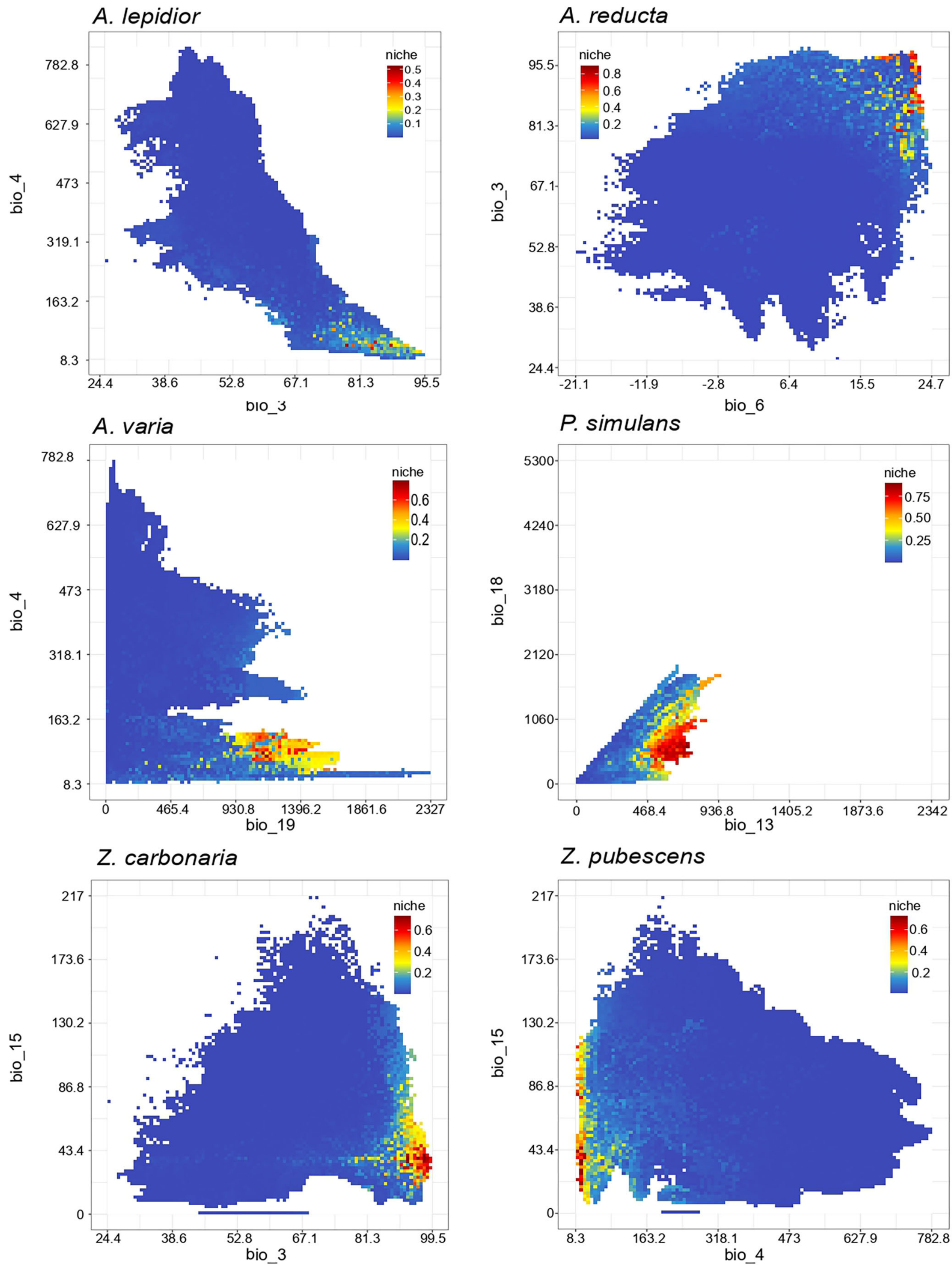

FIGURE 8 | Observed niche of Colombian spittlebugs as a function of two most representative biovariables. The scale shows occurrence probabilities.

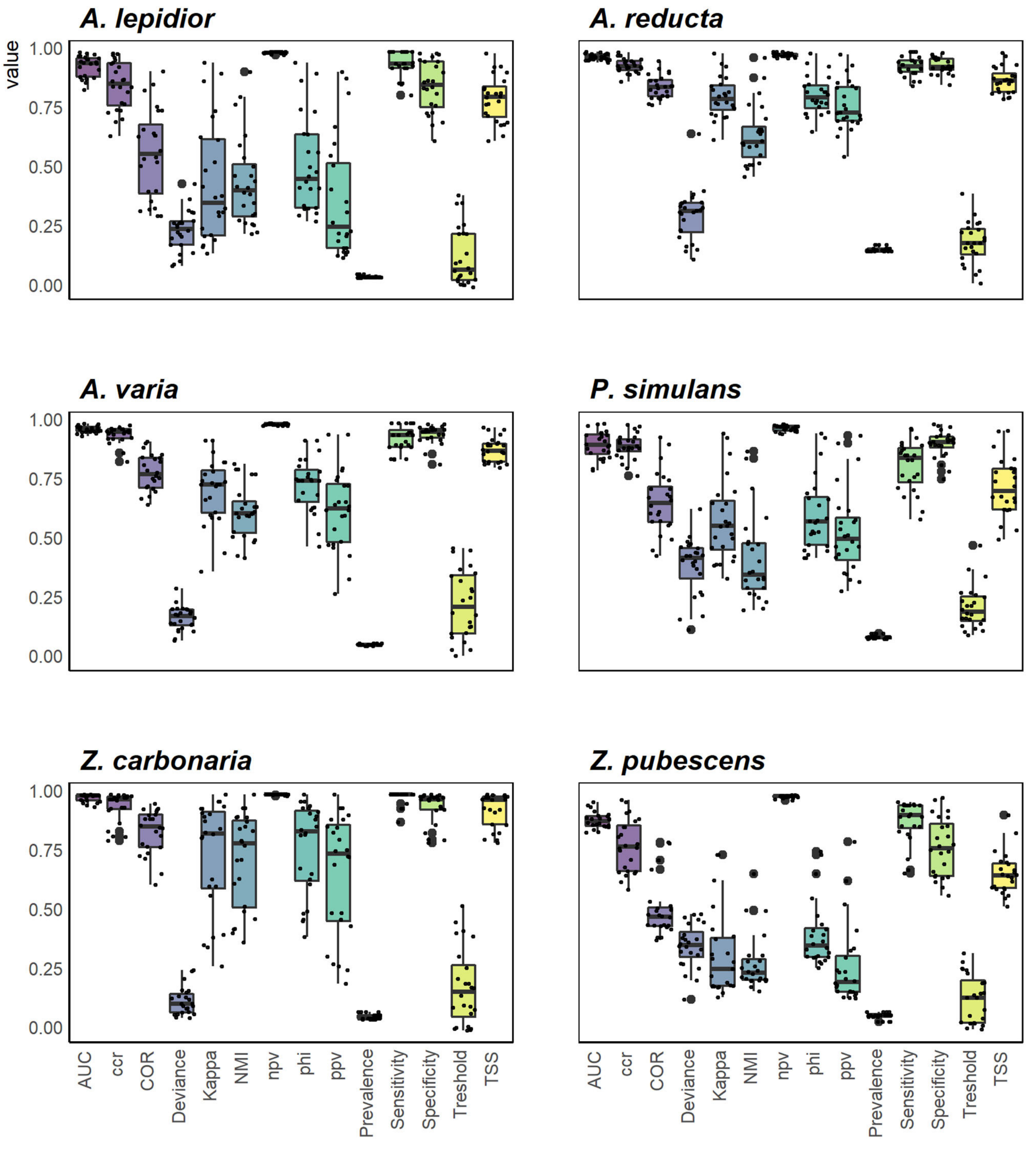

FIGURE 9 | Evaluation of ecological niche models of spittlebugs species across different metrics obtained from 24 model by each species.

(**Figure 6**). Bioclimatic layers with high contribution were temperature seasonality (bio_4) and precipitation seasonality (bio_15), showing high suitability with low values of bio_4 (<10) and values close to 40 of bio_15 (**Figure 7**). Average of AUC and TSS (± SD) was 0.89 ± 0.03 and 0.66 ± 0.09, respectively. An increase in suitability is expected for some areas of Ecuador, Peru, and Brazil in both climate change scenarios, being greater in the pessimistic scenario (**Figure 6**).

DISCUSSION

In our study ENMs of the occurrence data had a high grade of accuracy given the sample size of five species, except for *A. varia*, for modeling (>25 records) (van Proosdij et al., 2016; Schöbel and Carvalho, 2020). Despite small sample sizes methodologies based on calculation p-values through Jackknife are implemented in the SDM R package used in this study (Naimi and Araújo, 2016), more records may increase the model accuracy (van Proosdij et al., 2016). Low records for spittlebugs were previously reported for Mahanarva in Brazil (Schöbel and Carvalho, 2020) being underrepresented in occurrence databases. This phenomenon was also observed for the six species studied as most of the records were obtained from CIATARC collection and expert's reports through the years (human observation).

The ENMs also revealed differences in the distribution and ecological niche of the six spittlebug species in South America showing that these species ecological niche varies widely in the Neotropic, and has the potential to invade large areas, where livestock systems coincide. *A. reducta* y *A. lepidior* have great potential to impact grassland mainly in Colombian and Venezuelan Llanos where susceptible pastures (e.g., Urochloa decumbens) and sugarcane are planted in large areas. Another ecoregion where these two species have high suitability is the Amazonian ecoregion in Colombia and Brazil, where livestock extensive systems are increasing indiscriminately.

The evidence showed that *Z. pubescens* is distributed in a wide altitudinal range (8–3225 m.a.s.l) but with a local reduced temperature seasonality. Elevation has been reported as the most important variable with the highest contribution in the ENMs in other spittlebugs (Schöbel and Carvalho, 2020). Few species have such a wide altitudinal range, which allows us to propose two hypotheses: (1) *Z. pubescens* presents extreme thermal limits and (2) the species presents geographically separated populations. A case of biotypes is observed for the spittlebug *Calitettix versicolor* in China, which diverged in two lineages consistent with biogeographical regions separated by Hengduan Mountains (Yang et al., 2016). Similarly, this could be happening with *Z. pubescens* influenced by the Colombian Andes. Although the species is reported in Brazil (27 occurrence records; average of 400 m.a.s.l), the suitability values are lower than in Colombia and Ecuador (93 occurrence records; average of 1079 m.a.s.l.). The higher number of records in the highlands of Colombia and Ecuador could be causing an overestimation of the occurrence probability at these areas over the records of Cerrado places in Brazil, this would explain the current potential distribution estimated, and also could be reflecting the possible existence of, at least, two populations with different ecological niches.

The position of a species within an ecosystem is determined by the interactions with their biotic and abiotic environment (Polechová and Storch, 2019). Tropical spittlebugs have a seasonal dynamic strongly synchronized with rainfall patterns. For instance, *Z. carbonaria* and *A. reducta* in Colombia, *P. simulans* in Colombia and Venezuela, *D. flavopicta* in Brazil, as well as *A. contigua* and *A. contigua* in Mexico, reduce diapause rates and a higher abundance of nymphs is observed after rain season start (Peck et al., 2001, 2002; Sujii et al., 2002; Olán-Hernández et al., 2016). Hence, a strong effect of the biovariables 12 to 19 in the models, related with precipitation, in the models was expected but in our estimations, the distribution of habitat suitability of these six species also involved environmental variables related to temperature suggesting that variables derived from temperature has a strong effect on the biology of these species. For *P. simulans*, precipitation was more important than temperature to determine its distribution with a relative importance over 0.4 for precipitation of the wettest month, thus, greater probabilities of occurrence happen in precipitation between 500 and 940 mm. In general, the habitat suitability estimated for two-dimensional niches was low as the biovariables' relative importance varied among all the species with values below 0.4 (**Figure 8**). Similar results were obtained by Schöbel and Carvalho (2020) in ENM of four *Mahanarva* species showing that most of the WorldClim variables did not contribute to their analysis and that for *M. fimbriolata* and *M. spectabilis* the biovariables had contribution percentages from 15 to 27%.

Regarding the climate change scenarios proposed, we found that these have a significant influence on the potential distribution of the species in study, increasing the suitability value and suitable area for some (mainly for *A. reducta* and *A. lepidior*) or decreasing them for others (*A. varia*). Previous studies showed a declining tendency in suitability for *Mahanarva* across Central and South America (Fonseca et al., 2016; Schöbel and Carvalho, 2020) and *Philaenus spumarius* in North America (Karban and Huntzinger, 2018). Global warming and longer drought periods contribute to accelerate this phenomenon as spittlebug biology is highly dependent on plant water status. Being xylem feeders, they require excessive amounts of sap which flow is subject to transpiration (Novotny and Wilson, 1997). Under water stress conditions transpiration rates decrease as well as food availability for spittlebugs, particularly in the nymphal stages. Besides, these conditions may affect nymph thermoregulation by foam or "spittle" production, composed mainly of excreted semi-digested plant fluid, fatty acids, carbohydrates, mucopolysaccharides, and proteins produced by Malpighian tubules (Rakitov, 2002; Tonelli et al., 2018). Since the six species are *Urochloa* spp. key pests, a future limitation of ecological niche in future scenarios in livestock production zones should be taken into account as improved resistant grasses to spittlebug attack and increase the number of forage species are considered a sustainable strategy for the livestock systems under climate change (Rao et al., 2016; Schiek et al., 2018). Competition can influence species future distribution as well. Despite reaching the spittlebug habitat's food limits is unlikely (Schöbel and Carvalho, 2020), the variation among species' life cycles may determine the success of one species over others. *A. reducta* was reported for the first time in 2019 in Cauca River Valley, Colombia (Hernandez et al., 2021) where *A. varia* is a key pest of sugarcane and *P. simulans* of signalgrass [*Urochloa decumbens* cv. Basilisk; (Rodriguez Chalarca et al., 2003; Gómez, 2007)]. In Colombian Caribbean coast, *A. reducta's* entire life cycle is shorter (45.2 days) compared with *A. varia* (62 days) or *P. simulans* (71.9 days) in Cauca River Valley conditions (Peck et al., 2002; Rodriguez Chalarca et al., 2003; Castro Valderrama et al., 2011). Thus, *A. reducta* can coexist or even displace these two species in sugarcane and signalgrass for potentially having more generations per year in the region where ∼208 thousand ha of sugarcane was harvested in 2018 (Asocaña., 2019).

The current study contributes to the ecological knowledge of spittlebugs, which will be useful in the development of prevention and control strategies for this pest in South America. Finally, we suggest carrying out studies of physiology and genetics of populations to determine the thermal limits of the species and to corroborate if there are genetic divergences between geographically separated populations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LH and PE contributed to the data collection, data curation, analysis and interpretation of maps, manuscript preparation, and supervision. DF contributed to data collection and manuscript preparation. VC contributed to manuscript preparation. JC contributed to interpretation and manuscript preparation. MG-J contributed to data collection, interpretation of maps, and manuscript preparation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.725774/full#supplementary-material

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In vitro Fermentation Profile and Methane Production of Kikuyu Grass Harvested at Different Sward Heights

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Highly digestible forages are associated with an *in vitro* low-methane (CH_4) rumen fermentation profile and thus the possibility of reducing CH_4 emissions from forage-based systems. We aimed to assess the *in vitro* ruminal fermentation profile, including CH_4 production, of the top stratum of Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) harvested at different sward heights (10, 15, 20, 25, and 30 cm). Herbage samples (incubating substrate) were analyzed for their chemical composition, *in vitro* organic matter digestibility (IVOMD), and morphological components. *In vitro* incubations were performed under a randomized complete block design with four independent runs of each treatment. Gas production (GP), *in vitro* dry matter digestibility (IVDMD), CH_4 production, total volatile fatty acid (VFA) concentration, and their acetate, propionate, and butyrate proportions were measured following 24 and 48 h of incubation. Herbage samples had similar contents of organic matter, neutral detergent fiber, and crude protein for all treatments. However, a higher acid detergent fiber (ADF) content in taller sward heights than in smaller sward heights and a tendency for metabolizable energy (ME) and IVOMD to decrease as sward height increased were found. Similarly, the stem + sheath mass tended to increase with increasing sward height. Amongst the nutrients, ME ($r = -0.65$) and IVDMD ($r = -0.64$) were negatively correlated with sward height ($p < 0.001$) and ADF was positively correlated with sward height ($r = 0.73$, $p < 0.001$). Both the GP and IVDMD were negatively related to the sward height at both incubation times. Sward heights of Kikuyu grass below 30 cm display an *in vitro* profile of VFAs high in propionate and low in acetate, with a trend toward lower methane production of CH_4 per unit of IVDMD. These findings are important to aid decision-making on the optimal sward height of Kikuyu grass and manage animal grazing with the opportunity to reduce CH_4 production.

Keywords: methane mitigation strategy, methanogenic potential, sward structure, tropical grass, forage nutritive value, grazing management

INTRODUCTION

Livestock is under fire of critics for its major share in the environmental impact of the agricultural sector. Total global greenhouse gas (GHG) emissions from livestock (animals, manure, feed production, and land-use change) are estimated to account for 14.5% of total anthropogenic emissions (Gerber et al., 2013). Among livestock production systems, grassland-based ruminants are the most controversial in the present-day literature (Teague et al., 2016; Gerssen-Gondelach et al., 2017). On the one hand, ruminants produce methane (CH_4) as a natural byproduct of microbial fermentation of feed in the rumen, contributing approximately 6% of the global anthropogenic GHG emissions (40% of all livestock emissions; Gerber et al., 2013; Beauchemin et al., 2020). On the other hand, grazed pastures which are the basis of those systems, when properly managed, potentially improve the sustainability of livestock production (Lobato et al., 2014; Elgersma, 2015; French et al., 2015), provide many social and environmental services (Werling et al., 2014; Mottet et al., 2017; Horrocks et al., 2019; Zubieta et al., 2020), and improve soil health indicators in tropical systems (Teutscherová et al., 2021). Hence, current grazing systems are being redesigned to link animal production with environmental management (Boval and Dixon, 2012; Carvalho, 2013) in light of current demands for sustainable agricultural production around the world (Herrero et al., 2010; Mottet et al., 2017).

The profitability and sustainability of forage-based dairy systems depend on efficient management (Herrero et al., 2000). In this regard, grazing management is of particular importance since when properly managed, it can improve the quantity and quality of herbage consumed by the animals and ultimately reduce CH_4 emissions (Congio et al., 2018; Savian et al., 2018, 2021). Previous studies have shown that the sward height is a useful and reliable tool to optimize pasture management (Carvalho et al., 2011; Kunrath et al., 2020). The literature suggests that under moderate- to low-intensity grazing management, animals ingest a diet with high nutritive value composed primarily of leaf lamina from the top stratum of the sward (Savian et al., 2018, 2020; Zubieta et al., 2021). Likewise, it is well known that diet digestibility declines from the top to the bottom of the sward, showing a vertical quality gradient of forages (Delagarde et al., 2000; Benvenutti et al., 2016, 2020). Moreover, as pasture matures, the sward height increases and the nutritive value decreases (Benvenutti et al., 2020). High forage digestibility is associated with a fermentation profile in the rumen that is unfavorable to CH_4 production (Hristov et al., 2013; Muñoz et al., 2016). Therefore, if grazed herbage is the main source of nutrients for animals, it is pivotal to offer a highly digestible forage that may have a high potential for mitigating enteric CH_4 emissions.

Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*), widely known as *Pennisetum clandestinum Hochst*, is a highly productive subtropical grass of African origin that is well adapted to the forage-based dairy systems of some countries of Latin and Central America (e.g., Colombia, Brazil, and Mexico) and Oceania [e.g., Australia and New Zealand; (García et al., 2014; Sbrissia et al., 2018; Marín-Santana et al., 2020)]. When managed correctly, Kikuyu grass is recognized for its moderate to good quality and high yield potential, especially in high-fertility soils (Reeves et al., 1996; Fulkerson et al., 2006; García et al., 2014). Commonly, grazing management goals of Kikuyu grass are based on plant characteristics associated with the regrowth age, phenological state, leaf stage, critical leaf area index, among others (Reeves et al., 1996; Fulkerson and Donaghy, 2001; Schmitt et al., 2019b). Currently, and for several forage species, including Kikuyu grass, the sward height is proposed as an easy-to-use grazing management criterion and a key performance predictor (Marin et al., 2017; de Souza Filho et al., 2019; Kunrath et al., 2020), as there is a strong relationship with the quantity and quality of the herbage that animals ingest. On the other hand, *in vitro* studies may predict enteric CH_4 production with reasonable accuracy and precision (Danielsson et al., 2017) and can help to identify promising strategies for *in vivo* studies oriented to reduce the environmental impact of livestock (Danielsson et al., 2017; Valencia Echavarria et al., 2019; Molina-Botero et al., 2020). Previous studies examined the effects of stage of regrowth on the nutritive value of whole plants of Kikuyu pastures and on the *in vitro* fermentation parameters (Ramírez et al., 2015; Vargas et al., 2018). Basic and key information regarding the sward height relationship with the nutritive attributes of Kikuyu grass and the main ruminal fermentation parameters, including CH_4 production, has not yet been established.

We hypothesized that the top stratum of the Kikuyu grass harvested at intermediate sward heights (15, 20, and 25 cm) has highly digestible leaves and displays an *in vitro* low-CH_4 rumen fermentation profile with similar chemical and sward structural characteristics. Thus, this study aimed to assess the effect of the sward height of Kikuyu grass from herbage samples of the top stratum (incubating substrate that reflects the potentially grazed stratum) on the *in vitro* ruminal fermentation profile. We also evaluated the *in vitro* CH_4 production and identified the sward heights that may offer the largest opportunity to mitigate enteric CH_4 production from grazing cattle fed with Kikuyu grass.

MATERIALS AND METHODS

Origin of Herbage Material

Herbage samples for the *in vitro* incubations were produced within a grazing trial with dairy heifers at the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), municipality of Lages, S.C., Brazil (27°47′10.5″S, 50°18′20.5″W, 937 m a.s.l.). According to Köppen's climate classification, the region is humid subtropical under oceanic influences. It has an annual average temperature of 17°C and annual average precipitation of 1460 mm (Alvares et al., 2013). The soil was classified as Humudept (with an umbric epipedon) according to the USDA Soil Taxonomy (Soil Survey Staff, 2014). The soil is developed from sedimentary rocks (sandstone and siltstone) and has an acidic pH, high aluminum content and low sum and base saturation (Rauber et al., 2021).

The grazing trial was carried out in a 5000-m^2 permanent pasture of Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) established in the early 1990s and grazed by dairy and beef cattle since then. The whole area was mowed homogeneously until 5 cm of height and divided into ten paddocks of 500 ± 5 m^2. Fertilizers were split into two applications depending on rainfall occurrence and considering a two-period evaluation. The pasture received one application of 250 kg/ha of fertilizer (N-P-K, 9–33–12) and 135 kg/ha of urea on 26 January 2017 (first evaluation period). On 22 March 2017, 67.5 kg/ha of urea was applied (second evaluation period). Due to the frost event and low temperatures in winter and sometimes in spring, the Kikuyu growth season is from the final period of spring and early autumn (Sbrissia et al., 2018); therefore, the herbage collection in both periods lasted from 28 Feb to 15 Apr 2017.

Treatments and Experimental Design

Treatments consisted of herbage samples from the top stratum of Kikuyu grass harvested at five sward heights (10, 15, 20, 25, and 30 cm). The grazing trial was conducted in a randomized complete block design with two spatial (paddocks) and two temporal (morning or afternoon) replicates. The blocking criterion was the time of day due to differences that may exist in the herbage chemical composition and dry matter yield within a day (Delagarde et al., 2000; Gregorini, 2012). Each sward height of the Kikuyu grass was randomly assigned in two paddocks, each one evaluated once in the morning and once in the afternoon (two periods of evaluation), in an alternated scheme with random start. Once target sward height was achieved after the initial mowing and before to start a grazing assessment, herbage sampling was performed (i.e., in the morning, period one). After that, the sward was mowed again to half of the treatment sward height (residuals were retired), and when it reached the set sward height again, a second herbage sampling was conducted (i.e., in the afternoon, period two). A total of four herbage samples from the top stratum per treatment were collected for *in vitro* incubations.

The *in vitro* incubation experimental design was carried out through four independent runs of each treatment, two ruminal liquids from steers (unmixed), and two independent sets corresponding to 24 and 48 h of incubation. In addition, four blanks (no substrate) for each incubation time were included.

Sward Measurement and Herbage Sampling

The sward height was measured at 150 random points per paddock using a sward stick (Barthram, 1985). When the treatment sward height of individual paddocks was confirmed, metallic quadrants (0.25 m^2) were placed at three random sites; average sward heights were calculated from five readings taken inside the quadrants with the sward stick to perform herbage clipping at half of the canopy height (samples representing the grazing stratum). Half of the herbage samples were separated into morphological components (leaf lamina, stem + sheath, and dead material) and dried in a forced-air oven at 55°C for 72 h. The dry weights of morphological components were used to calculate total herbage mass (kg DM/ha) as the sum of each component's mass. The other half was also dried and then pooled per paddock and time of the day for chemical analysis and *in vitro* incubations.

Chemical Composition and *in vitro* Organic Matter Digestibility

The herbage samples were analyzed in duplicate for dry matter (DM, method 930.04; AOAC, 2016), ash (method 930.05; AOAC, 2016), and for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991) by using an Ankom 200 fiber analyzer without heat-stable alpha-amylase. ADF and NDF procedures are not ash-free. Samples were also characterized for N content by the Kjeldahl digestion. The crude protein amount was calculated as N × 6.25 (N, method 984.13; AOAC, 2016). The two-stage Tilley and Terry (1963) technique (incubation with rumen fluid followed by acid-pepsin digestion) was used to estimate the *in vitro* organic matter digestibility (IVOMD). The total digestible nutrient (TDN) concentration of the simulated grazing samples was estimated as a percentage of IVOMD (Moore et al., 1999). The metabolizable energy (ME) were estimated using the following equations of NRC (NRC, 2001): DE (Mcal/kg) = 0.04409 × TDN (%), and ME (Mcal/kg) = 1.01 × DE (Mcal/kg) −0.45.

In vitro Ruminal Fermentation

Procedures involving animals were carried out in accordance with the relevant guidelines, regulations, and requirements of Colombian law No 84/1989 and the following protocol, approved by the Ethics Committee of the International Center for Tropical Agriculture (CIAT).

The *in vitro* incubations were conducted according to Theodorou et al. (1994) in the Forage Quality and Animal Nutrition Laboratory (certified by the FAO-IAG proficiency test of feed constituents 2017 including *in vitro* gas production) at CIAT located in the Valle del Cauca department, Colombia (3°29′34′′N, 76°21′37′′W, 965 m a.s.l.). Rumen fluid was collected at 7:30 am from two rumen-fistulated *Bos indicus* Brahman steers with an average body weight of 720 ± 42 kg, which were grazed on *Cynodon plectostachyus* (star grass) pasture, with free access to water and mineral salts.

The rumen fluid was filtered using a 250 μm nylon pore size cloth, dispensed into two thermal flasks prewarmed to 39 ± 0.5°C, and immediately transferred to the laboratory. The time between rumen fluid collection and inoculation did not exceed 30 min. Five-hundred milligrams of each herbage sample (DM basis) was incubated in 160 mL glass bottles, prewarmed in an incubator at 39°C, with 20 mL filtered rumen fluid mixed with 80 mL rumen medium in a 1:4 ratio (Menke and Steingass, 1988), and dispensed with continuous flushing of CO_2. The bottles were slightly stirred, sealed with rubber stoppers and aluminum caps, and incubated in a water bath at 39°C in two different sets corresponding to incubation times of 24 and 48 h. Four blanks of rumen medium (bottles without substrate that contained only inoculum and medium) per each set were also incubated. The gas production was measured at 3, 6, 9, 12, 24, and 48 h using a pressure transducer (Lutron Electronic Enterprise Co. Ltd., Taipei, Taiwan) connected to a digital wide-range manometer (Sper Scientific, Arizona, USA) and a 60 mL syringe through a three-way valve (Theodorou et al., 1994). After each measurement, the gas of the bottles was released to avoid partial dissolution of CO_2 (Tagliapietra et al., 2010) and possible disturbance of microbial activity (Theodorou et al., 1994). Cumulative pressure values were converted into volume (GP, mL) from measured pressure changes at incubation times and after correction for blank pressure values using the ideal gas law and expressed per unit of dry matter incubated (DMi) and *in vitro* dry matter degraded (IVDMD) (López et al., 2007).

In vitro Methane Production and Calculations

Methane (CH_4) analyses were carried out in the Greenhouse Gas Laboratory CIAT. A gas sample in the headspace was collected into a 5 mL vacuum vial (Labco Ltd., High Wycombe, England) at 24 and 48 h. The CH_4 concentration was determined using a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan) equipped with a Hayesep N packed column (0.5 m × 1/8" × 2 mm ID) and flame ionization detector (FID). The operating temperatures of the column, detector, methanizer, and valves were 80, 250, 380, and 80°C respectively. Ultrahigh purity 5.0-grade N was used as the carrier gas with a linear velocity of 35 mL/min. The CH_4 concentration was calculated using a standard of 10% CH_4 balanced in N (Scott-Marrin Inc., Riverside, CA) and corrected for the CH_4 blank values. The volume of CH_4 (mL) produced at the end of each incubation time (24 and 48 h) was calculated as a product of the total gas produced (mL) multiplied by the concentration of CH_4 (%) in the analyzed sample, as described by Lopez and Newbold (2007).

Volatile Fatty Acids and *in vitro* dry Matter Digestibility

Following 24 and 48 h of incubation, the fermentation was stopped by dipping the bottles in cold water with ice and then processing to determine volatile fatty acids (VFAs) and the *in vitro* digestibility of dry matter (IVDMD). Ruminal fluid samples (10 mL) were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (1.6 mL) was transferred into a 2 mL Eppendorf tube, and 0.4 mL of metaphosphoric acid (25% w/v) was added for VFA analysis. Samples were then stored frozen at −20°C and later analyzed for acetate, propionate, and butyrate concentrations by high-performance liquid chromatography (HPLC) with an SPD-20AV UV-VIS detector (SHIMADZU, Prominence UFLC System) fitted with a BIO-RAD Aminex HPX-87H, 300 × 7.8 mm Ion Exclusion Column. The total VFA concentration was calculated as the sum of the individual VFA concentrations in the ruminal fluid and was corrected for the blank values. Based on the obtained results, the proportion of each VFA in the total VFA amount was calculated. The acetic: propionic ratio was also calculated. All contents remaining in the bottle were finally filtered through preweighed sintered glass crucible pore number 1 (Pyrex®) and dried in a forced-air oven at 105°C for 24 h to determine the IVDMD.

Statistical Analysis

All statistical analyses were performed using R 3.5.3 (R Core Team, 2018). Herbage chemical composition and sward characteristics were analyzed with ANOVA in a randomized block design: $Yijk = \mu + \alpha i + \beta j + \varepsilon ijk$, where: $Yijk$ is the response variable, μ is the overall mean, αi treatments (herbage samples from the top stratum), βj is the effect of the block (time of the day), and ϵijk is the residual error. HSD Tukey's test was used to compare means among treatments; significance was declared at $p \leq 0.05$ and tendencies at $0.05 < p \leq 0.10$. The nutritive value (NDF, ADF, CP, ME, IVDMD) and *in vitro* fermentation parameter (GP, acetate, propionate, and butyrate) results were submitted to Pearson's correlations and visualized using the R package corrplot (Wei et al., 2017).

The *in vitro* fermentation data were analyzed as linear ($Y = \beta 0 + \beta 1SH + \varepsilon$), quadratic ($Y = \beta 0 + \beta 1SH + \beta 2SH^2 + \varepsilon$), and a double linear function of sward height ($Y = f\{p + a1 \times (SH - v), p + a2 \times (SH - v)\}$), where Y is IVDMD, GP, *in vitro* CH_4, VFA (acetate, propionate, and butyrate), f is the min or max function, v and p are the coordinates of the crossing point of sward height, SH are the observed values of sward height, and a1 and a2 are the slopes of the component lines adapted from Mezzalira et al. (2017). Linear and quadratic regression models were fitted by using R lm{stats} function and double linear models were fitted by deviance minimization with the optim{stats} function. After fitting a regression model, the residual plots were checked and the Shapiro–Wilk test was carried out using the R function shapiro.teststats. The best model was selected by the smaller value of Akaike's information criterion (AIC). The objective of the regression analysis was to understand how the nutritive value of the top stratum of Kikuyu grass, harvested at different sward heights, influences the *in vitro* ruminal fermentation profile.

RESULTS

Sward Characteristics and Chemical Composition of the Herbage Incubated

The sward heights obtained were close to the nominal treatment heights and different between treatments ($p < 0.001$, **Table 1**). Herbage mass in 10 cm swards was less than in the 30 cm swards but did not differ among the other sward heights. The 25 and 30 cm sward heights resulted in a higher green leaf mass than the 10 cm sward height ($p < 0.01$) but did not differ between 15 and 20 cm ($p > 0.05$, **Table 1**). The stem + sheath mass tended to increase with increasing sward height ($p = 0.09$, **Table 1**).

No differences were found for OM, NDF, and CP contents ($p > 0.05$, **Table 2**), however, the ADF concentration was greater at 30 cm sward heights than at 10 cm sward heights, but not different from other sward heights ($p = 0.02$, **Table 2**). The IVOMD and ME tended to decrease with increasing sward height ($p = 0.16$ and $p = 0.10$, respectively; **Table 2**).

Relationship Between Sward Height, Chemical Composition, and *in vitro* Fermentation Parameters

The correlation values among the sward height, nutritive value and *in vitro* fermentation parameters at 48 h are presented in **Figure 1**. The sward height showed a moderate negative correlation with IVDMD ($r = -0.64$), GP ($r = -0.46$), CP ($r = -0.45$), and ME ($r = -0.65$). Conversely, a high and positive correlation ($r = 0.73$) between the ADF (g/kg) and

TABLE 1 | Sward characteristics of herbage samples from the top stratum of five Kikuyu sward height.

Item	Sward heights (cm)					*p*-value	SEM
	10	15	20	25	30		
Sward height (cm)	9.8^{e}	15.1^{d}	20.1^{c}	24.3^{b}	31.3^{a}	<0.0001	0.51
Herbage mass (kg DM/ha)	426.0^{b}	502.0^{ab}	796.0^{ab}	870.3^{ab}	950.3^{a}	0.01	107.2
Green leaf mass (kg DM/ha)	363.9^{b}	463.1^{ab}	737.4^{ab}	791.3^{a}	842.8^{a}	0.01	93.6
Stem + sheath mass (kg DM/ha)	31.9	24.25	52.94	73.88	91.1	0.09	18.0

Common superscript letters among the same row denote non-significant difference at 0.05 level, as determined by HSD Tukey's test. DM, dry matter; S.E.M, standard error of the mean.

TABLE 2 | Chemical composition and *in vitro* organic matter digestibility (IVOMD) of herbage samples from the top stratum of five Kikuyu sward heights.

Item	Sward heights (cm)					*p*-value	SEM
	10	15	20	25	30		
DM (g/kg of DM)	923.7	913.0	918.0	923.9	919.8	0.10	2.8
OM (g/kg of OM)	907.1	911.6	905.3	905.8	902.1	0.22	2.7
NDF (g/kg of DM)	535.9	541.9	543.1	541.1	545.6	0.98	11.0
ADF (g/kg of DM)	194.1^{b}	198.7^{ab}	210.9^{ab}	213.1^{ab}	218.8^{a}	0.02	3.8
CP (g/kg of DM)	316.8	301.8	305.0	302.9	281.3	0.22	8.0
IVOMD (g/kg of OM)	686.6	657.5	635.1	610.7	592.3	0.16	31.0
ME (Mcal/kg of DM)	2.3	2.2	2.1	2.0	1.9	0.10	0.1

Common superscript letters among the same row denote non-significant difference at 0.05 level, as determined by HSD Tukey's test. SEM, standard error of the mean; DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy.

sward height was observed (**Figure 1**). The GP exhibited a high positive correlation with IVDMD ($r = 0.74$) and ME ($r = 0.62$), and at the same time, IVDMD was highly and positively related to ME ($r = 0.84$) (**Figure 1**). The total CH_4 had a moderate and positive correlation with GP ($r = 0.39$); however, it was poorly related to the other variables evaluated. In addition, acetic acid had a strong negative correlation with propionic acid ($r = -0.79$, **Figure 1**). Pearson's correlation of dataset at 24 h (**Supplementary Figure 1**) and the correlation matrix at 24 and 48 h (**Supplementary Tables 1**, **2**, respectively).

The *in vitro* Fermentation Parameters

The GP, expressed as milliliters per unit of dry matter incubated (mL/g DMi), and IVDMD (g) linearly decreased with sward height at both incubation times (24 and 48 h are shown in **Figures 2A,B**, respectively). However, when the GP was expressed as milliliters per unit of *in vitro* digestible dry matter (mL/g IVDMD), it was not related to the sward height either at any incubation time (data not shown). There was no relationship between the total *in vitro* CH_4 production, expressed in terms of milliliters per dry matter incubated (mL/g DMi), and the sward heights studied at any incubation time (data not shown). However, after 24 h of fermentation, the *in vitro* CH_4 production expressed as milliliters per unit of *in vitro* digestible dry matter (mL/g IVDMD) fitted a double linear trend model ($p = 0.060$). The minimum value of CH_4 production at 24 h (15.4 mL/g IVDMD) occurred at 21.3 cm (**Figure 3A**). CH_4 production, first described a straight line slightly inclined but not different between 10 and 20 cm ($a1 = -0.22$ g mL/IVDMD/cm, $p = 0.32$), and then increased with the sward height ($a2 = 0.61$ g mL/IVDMD/cm, $p = 0.02$) (**Figure 3A**). Likewise, CH_4 production (mL/g IVDMD) at 48 h tended to increase linearly as a function of sward height (**Figure 3B**).

Meanwhile, the total VFA (mM/L) concentration did not differ between treatments for any incubation time, but it was close to double at 48 h relative to 24 h (data not shown). The main VFA proportions, acetate, propionate, and butyrate (mol/100 mol), were unrelated to sward height at 24 h (data not shown) but significant changes were found after 48 h of incubation. Overall, the acetate, propionate, and acetate: propionate ratio following 48 h of fermentation showed that the minimum methanogenic profile occurred below 30 cm (**Figures 4A,B,D**). The acetate and propionate molar proportions and the acetate: propionate ratio were well described by a double linear model (**Figures 4A,B,D**, respectively). The relationship between acetate (mol/100 mol) and sward height first described a straight line slightly inclined ($a1 = -0.09$ mol/100 mol/cm, $p = 0.06$) and after 28.4 cm tall, it showed a steeper line with a higher and more significant slope ($a2 = 1.55$ mol/100 mol/cm, $p < 0.0001$). Conversely, the propionate (mol/100 mol) first increased (increasing slope, $a1 = 0.20$ mol/100 mol/cm, $p = 0.002$) until 28.42 cm and then decreased (decreasing slope, $a2 = -1.34$ mol/100 mol/cm, $p < 0.0001$) with sward height. The butyrate showed a negative and linear fit as the sward heights increased ($p < 0.0001$, **Figure 4C**). The acetate: propionate ratio subtly decreased with sward height between 10 and 28.8 cm (decreasing slope, $a1 = -0.013$ units/cm,

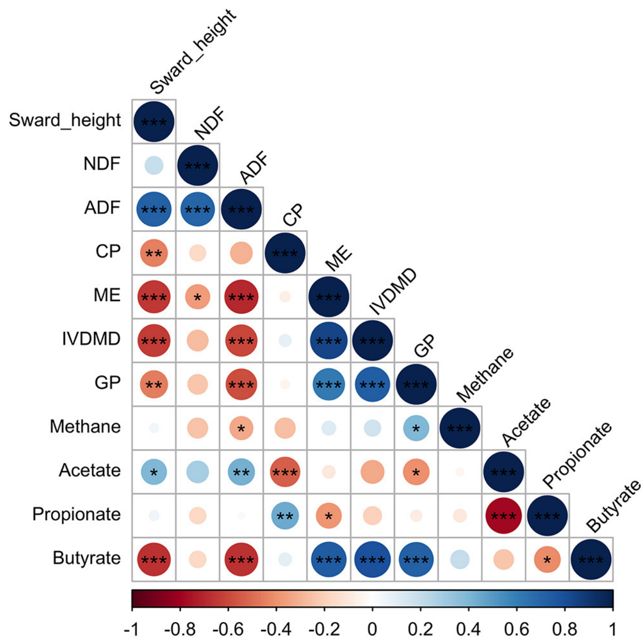

FIGURE 1 | Correlation plot between the sward height, nutritive value, and *in vitro* fermentation parameters at 48 h of Kikuyu grass harvested at different sward heights ($n = 36$). Positive and negative correlation coefficients are displayed in blue and brown scale, respectively. Sward_height, (cm); NDF, neutral detergent fiber (g/kg of DM); ADF, acid detergent (g/kg of DM), CP, crude protein (g/kg of DM); ME, metabolizable energy Mcal/kg of DM; IVDMD, *in vitro* dry matter digestibility (g); GP, Gas production (mL/ g DMi). DMi, dry matter incubated. Methane (total in vitro CH_4 production, ml), acetate, propionate, and butyrate (mol/100 mol). Significance level (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).

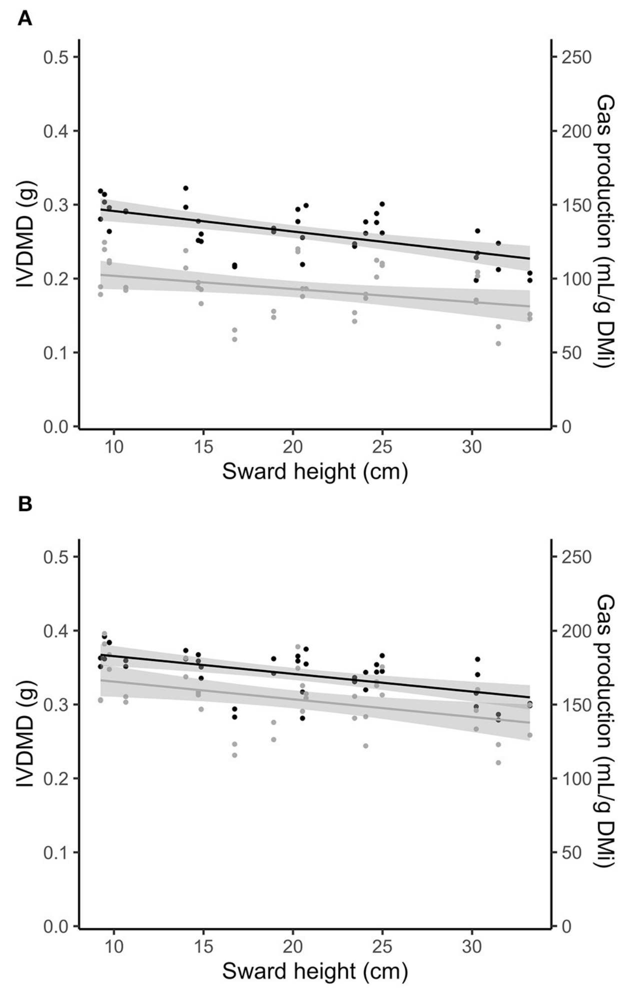

FIGURE 2 | Relationship between gas production (GP, mL/g DMi; gray dots) and *in vitro* dry matter digestibility (IVDMD, g; black dots) and sward height (SH, cm) of kikuyu grass. **(A)** include all data of GP and IVDMD at 24 h of fermentation ($n = 40$); equation for: GP = 110.74–0.90SH, $p < 0.01$), $R^2 = 0.12$. IVDMD = 0.32–0.002SH, $p < 0.0001$, $R^2 = 0.40$. **(B)** include all data of GP and IVDMD at 48 h of fermentation ($n = 40$); equation for: GP = 177.42–1.19SH, $p < 0.05$, $R^2 = 0.16$; IVDMD = 0.32–0.002SH, $p < 0.0001$, $R^2 = 0.32$. DMi, dry matter incubated.

$p = 0.004$) and then increased at sward heights taller than 28.8 cm (increasing slope, a2 = 0.14 units/cm, $p = 0.0001$, **Figure 4D**).

DISCUSSION

Moderate to low-intensity grazing management strategies favor animals to select bites of the top stratum of plants, whose diet is mainly composed of highly digestible leaves with high CP and low fiber content (Savian et al., 2018; Zubieta et al., 2021). This study assessed the effect of the sward height of Kikuyu grass from herbage samples of the top stratum on the *in vitro* ruminal fermentation profile and its relationship with the chemical composition and IVDMD. The key finding was that the sward heights of Kikuyu grass below 30 cm display a profile of VFAs high in propionate and low in acetate, with a trend toward lower CH_4 production per unit of IVDMD. Although the chemical composition between the treatments was similar, the tendency for stem and sheath mass to increase led to an increase in ADF contents and a tendency to decrease the IVOMD with sward height, shifting the fermentation profile toward an *in vitro* rumen environment more favorable to CH_4 production at sward heights above 28 cm.

Sward Characteristics and Chemical Composition

The chemical composition of herbage from the top stratum of the Kikuyu grass showed many similarities between the sward heights. The overall tendency to decrease IVOMD and increase ADF contents with sward height is consistent with the changes in the relative proportions of the leaves and stems + sheath within the top stratum as the sward height increases. In swards of *Cenchrus clandestinus*, Schmitt et al. (2019a) observed that NDF and ADF contents of herbage samples from the upper stratum did not change between 10 and 25 cm heights. Previous studies on the vertical distribution of chemical composition and digestibility of a perennial ryegrass sward showed little variation

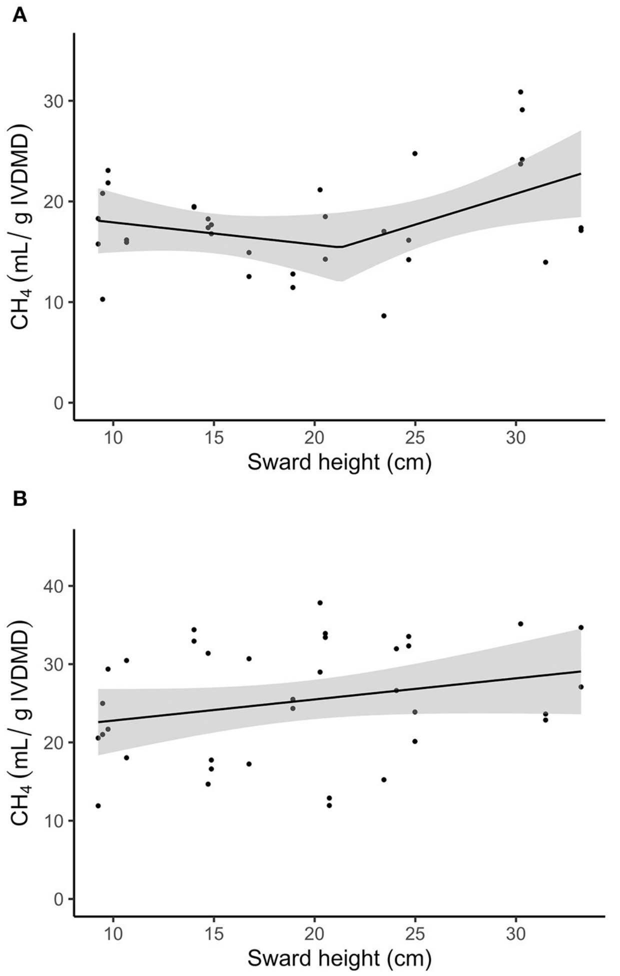

FIGURE 3 | Relationship between *in vitro* CH_4 (mL/g IVDM) and sward height (SH, cm) of kikuyu grass. Equation for: CH_4 = min [15.4–0.22 (SH−21.3)], [(15.4+ 0.61 (SH−21.3)], $p < 0.06$, $R^2 = 0.11$, following 24 **(A)**; and CH_4 = 20.1–0.26SH, $p < 0.12$, $R^2 = 0.04$, following 48 **(B)**.

in NDF and organic matter digestibility at different regrowth ages and at different times of the day (Delagarde et al., 2000). Regardless of the regrowth age, leaves were located mainly in the top stratum, while steams were present mainly in the bottom stratum of Kikuyu pastures; consequently, CP decreased, and NDF and ADF increased with age of regrowth and from top to bottom of the swards (Benvenutti et al., 2020). For a given stratum of the sward, the differences between regrowth age are commonly more marked between vegetative and reproductive stages (Schmitt et al., 2019a; Benvenutti et al., 2020). In the vegetative stage, the nutritive value differs little among plant parts (Laca et al., 2001; Benvenutti et al., 2020).

The results concerning the NDF, ADF, CP, ME, and IVOMD are consistent with those values found from the upper stratum of the Kikuyu sward (Benvenutti et al., 2020). However, CP exhibited higher values than usually reported for the whole plant (Correa et al., 2008; García et al., 2014) or the upper stratum of this species (Schmitt et al., 2019a). Nonetheless, when the nutritional value was evaluated by strata through the vertical distribution, the observed CP values were consistent with the CP content of the upper layer of the plant (Benvenutti et al., 2020). Previous studies have shown that the CP contents of leaves change significantly with anatomical characteristics along the length of leaf blades (Garcia et al., 2021). In addition to the high CP content of the upper stratum due to green leaves, the higher N levels due to fertilization could have influenced the results. According to Correa et al. (2008), the higher CP content (true protein and nonprotein nitrogen (NPN)) in highly fertilized Kikuyu swards is closely related to the higher amounts of ruminal ammonia ($N\text{-}NH_3$) and lower N use efficiency. Even though high N fertilizer rates are common for Kikuyu ryegrass pasture systems, animal excreta on pasture can negatively affect the Nitrogen efficiency of the cows (Marais, 2001; Viljoen et al., 2020) and contribute to nitrous oxide (N_2O) emissions (Maire et al., 2020).

Relationship Between Chemical Constituents and *in vitro* Fermentation Parameters

The strong and positive correlation between GP and the IVDMD at 48 h and the high and positive correlation between ME and GP and IVDMD were expected once GP was directly related to the amount of OM fermented by rumen bacteria, which is consistent with the principles of the *in vitro* gas production technique (Theodorou et al., 1994; Mauricio et al., 1999). It is widely known that GP can be a good index of forage ME content and provides an effective method for assessing the nutritive value of the feeds (Menke and Steingass, 1988). On the other hand, the negative correlation between sward height and GP and chemical components such as ME, IVDMD, CP and at the same time the positive correlation between sward height with the ADF is an interesting result; since the sward height has a consistent correlation with herbage mass and it is a practical and reliable indicator to optimize grazing management (Carvalho et al., 2011; Kunrath et al., 2020).

The chemical composition of forages is influenced by several factors, including sward structure, stage of maturity, season of harvest, and stratum harvested (Benvenutti et al., 2020; Marín-Santana et al., 2020). In general, the correlations between pasture chemical components and *in vitro* fermentation parameters in this study are consistent with previous studies with tropical grasses (Bezabih et al., 2014; Kulivand and Kafilzadeh, 2015), and with other studies using different types of feeds and forages (Getachew et al., 2004). However, unlike expected, CH_4 production had a poor and negative relationship with NDF and ADF content. This discrepancy is probably due to the high variability of CH_4 data at both incubation times. The highly significant correlation between ME and butyrate and the negative relationship between ADF and butyrate indicate the contribution of these components to VFA production (Ungerfeld, 2015).

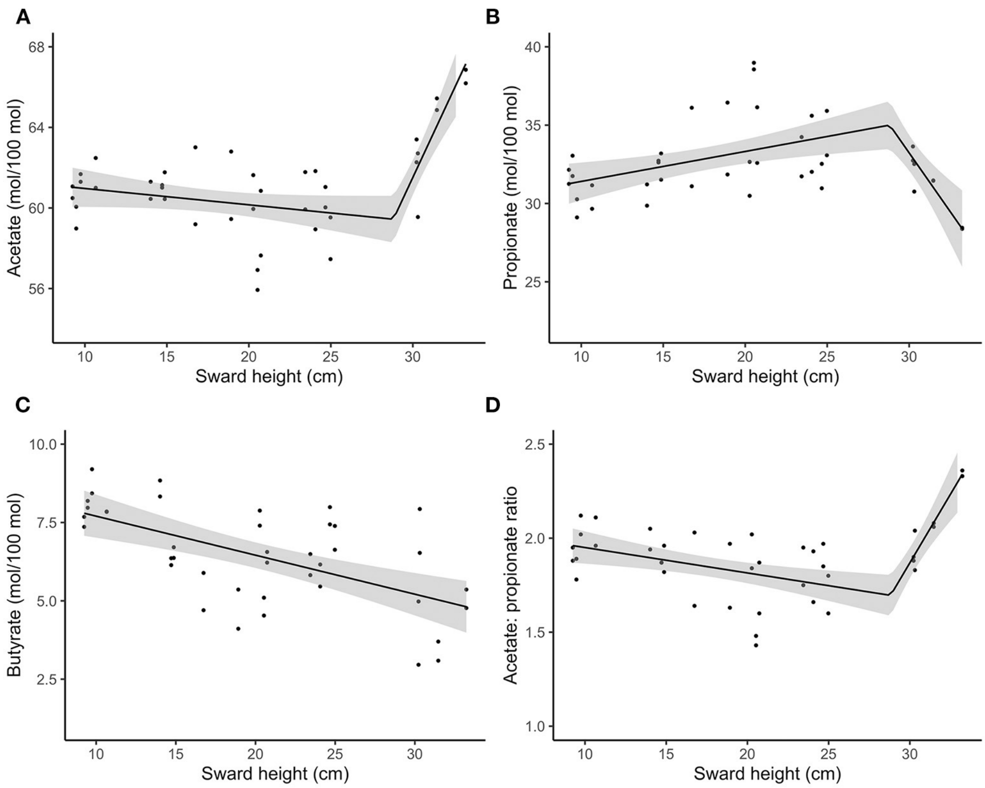

FIGURE 4 | Relationship between VFAs (mol/100 mol) and acetate to propionate ratio with sward height (SH, cm) of kikuyu grass following 48 h of fermentation. Equation for: Acetate = min [59.33–0.09 (SH−28.4)], [59.33 + 1.55 (SH−28.4)], $p < 0.0001$, $R^2 = 0.54$, **(A)**; Propionate = min [35.04 + 0.20 (SH−28.42)], [35.04–1.34 (SH − 28.42)], $p < 0.001$, $R^2 = 0.30$, **(B)**; Butyrate = 8.94–0.12SH, $p < 0.0001$, $R^2 = 0.34$, **(C)**; and Acetate: propionate ratio = min [1.70–0.013 (SH−28.8)], [1.70 + 0.14 (SH−28.8)], $p < 0.0001$, $R^2 = 0.44$, **(D)**.

In vitro Fermentation Parameters

The sward height of Kikuyu grass influenced its nutritive value and *in vitro* rumen fermentation profile. Since the stems + sheath mass tended to increase and IVOMD tended to decrease as a function of sward height, the GP and IVDMD also decreased. As stated above, *in vitro* gas production is a suitable indicator to predict the carbohydrate degradation of forages (Menke et al., 1979; Theodorou et al., 1994; Danielsson et al., 2017). It is widely accepted that the higher the IVDMD is, the higher the GP (Durmic et al., 2010; Meale et al., 2011). Consistently, taller sward heights (>28 cm) displayed a higher methanogenic profile than shorter (10 cm) and intermediate (15, 20, and 25 cm) sward heights due to the changes in morphological components and chemical composition, which resulted in a higher acetate: propionate ratio at 48 h of fermentation. The highest methanogenic profile of sward heights of Kikuyu grass above 28 cm, is due to the tendency of more stems + sheath with the sward height, and the tendency of the ME and IVDMD diminished with the sward height. CH_4 production in an *in vitro* gas system is strongly associated with the fermentation of structural carbohydrates. It has been previously reported that decreasing the digestibility of herbage and increasing the fiber content with advancing plant maturity influences not only total VFA production but also the molar proportions, with greater acetate and lower propionate, and therefore a higher acetate: proportionate ratio and higher CH_4 production per unit of degraded dry matter (Boadi et al., 2002; Beauchemin et al., 2008; Navarro-Villa et al., 2011; Purcell et al., 2011). In our study, the GP reduction as a function of sward height may reflect a higher structural carbohydrate content at taller heights than at shorter heights. Likewise, the trend toward lower *in vitro* CH_4 production with sward height is explained by the lower IVDMD as a function of sward height. Assessing the *in vitro* CH_4 output from different maturity stages of Kikuyu grass, other studies have shown a lower CH_4 production per unit of degraded organic matter (Vargas et al., 2018) and per gram of digestible dry matter (Ramírez et al., 2015), in the youngest forages than in the most mature forages.

The end products of *in vitro* ruminal fermentation, such as the acetate, propionate, and butyrate proportions, are consistent with the data published by other authors (Burke et al., 2006; Marín et al., 2014; Ramírez et al., 2015; Vargas et al., 2018) who also evaluated the *in vitro* fermentation of Kikuyu grass. The lack of differences found in the total VFA concentration and the molar proportions of the main VFAs measured at 24 h may be associated with subtle changes in the fermentation pathways during the first h of fermentation. In agreement with (Meale et al., 2011), batch culture *in vitro* fermentation has a low sensitivity to elucidate small differences between the same type of substrate (e.g., herbage) in the early fermentation. However, prolonged incubation in a closed system potentially favors VFA production changes and their proportions (Ungerfeld and Kohn, 2006), as observed at 48 h. The high molar proportion of acetate and the low of propionate in Kikuyu pastures harvested above 28 cm of sward height matched with a tendency toward more *in vitro* CH_4 output (mL/g IVDMD) and suggested a low *in vitro* rumen fermentation efficiency at tall sward heights. It is also widely known that forages that increase propionate and decrease acetate are often associated with reducing ruminal CH_4 production (Moss et al., 2000; Beauchemin et al., 2009; Meale et al., 2011). Nevertheless, the lower proportion of propionate at smaller heights was unexpected due to the similarities of the chemical composition and IVDMD at sward heights below 25 cm. A possible explanation of this finding could be related to the increase in butyrate concentration at the expense of propionate, as the sward height increases. In this study, the butyrate seems to have acted as an alternative H_2 sink (Moss et al., 2000; Ungerfeld, 2015), which is also in agreement with the trend toward lower CH_4 production per unit of IVDMD (mL/g IVDMD) at sward heights below 28 cm. Changes in the fermentation pathways could be associated with superior CP concentrations and, probably, with the higher nitrate concentration in the evaluated Kikuyu structures as a product of the high N fertilization of the Kikuyu, as suggested by Lovett et al. (2004). Nitrate is an alternative H_2 sink and an effective inhibitor of methanogenesis (McAllister and Newbold, 2008; Van Zijderveld et al., 2010; Yang et al., 2016; Patra et al., 2017). Other studies have suggested that the inclusion of nitrate in *in vitro* ruminal fermentation could increase the molar proportion of acetic acid and reduce the molar proportion of propionic acid (Navarro-Villa et al., 2011).

The similar chemical composition of herbage samples from swards heights of 10, 15, 20, and 25 cm in this study suggests an *in vitro* rumen environment less favorable to CH_4 production, therefore the possibility of flexible grazing management. However, Kikuyu swards managed with the 10 cm sward height target could result in low herbage and green leaf mass, which may affect herbage intake and animal performance (Marin et al., 2017; Schmitt et al., 2019b). Therefore, grazing managers must make strategic decisions considering a holistic management framework.

Another important consideration is that *in vitro* CH_4 production may not reflect the *in vivo* conditions and should be interpreted with care (McAllister et al., 2011; Klop et al., 2017). Therefore, it is recommended to carry out long-term grazing studies that include *in vivo* CH_4 and dry matter intake measurements (Yáñez-Ruiz et al., 2016).

CONCLUSIONS

We conclude that Kikuyu grass harvested below 30 cm displays an *in vitro* profile of VFAs high in propionate and low in acetate, with a performance less favorable to CH_4 production per unit of IVDMD. Our findings suggest that grazing management sward height targets of Kikuyu grass at intermediate sward heights (15 to 25 cm) may be a promising strategy to reduce CH_4 emissions. Further studies based on *in vivo* measurements may be necessary before practical application.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Procedures involving animals were carried out in accordance with the relevant guidelines, regulations, and requirements of Colombian Law No 84/1989 and following protocol, approved by the Ethics Committee of the International Center for Tropical Agriculture (CIAT).

AUTHOR CONTRIBUTIONS

AM, JB, AZ, and PF: conceptualization and methodology. AM and GC: performed the statistical analysis. AM: writing – original draft preparation. AM, JB, AZ, PF, GC, JA, and NC: writing – review & editing. JB, PF, and JA: supervision. JA: project administration. PF, NC, and JA: funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.682653/full#supplementary-material

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PERSPECTIVE
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Perspectives on Reducing the National Milk Deficit and Accelerating the Transition to a Sustainable Dairy Value Chain in Zimbabwe

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The Zimbabwean dairy industry is massively underperforming, as evidenced by a reduction in milk yield from 262 million liters in 1990 to <37 million liters in 2009 and a steady but slow increase to 82 million liters in 2021. The current demand for milk in Zimbabwe stands at 130 million liters, and there is a national capacity for processing 400 million liters per annum. This study used literature, stakeholder inputs and expert knowledge to provide a perspective on practical options to reduce the national milk deficit and, simultaneously, accelerate the transition to a sustainable dairy value chain in Zimbabwe. Following a discussion on the key barriers and constraints to developing the milk value chain, we explored opportunities to improve the performance of the underperforming smallholder and medium-scale dairy farmers. Specifically, we discussed innovative management, creative policy instruments and alternative technological options to maximize milk production in Zimbabwe. We also highlight the need for an inclusive and creatively organized dairy value chain to optimize stakeholder linkages and improve information flow and equity. Examples of crucial investments and incentive structures for upgrading the existing value chain and monitoring greenhouse gas emissions and carbon uptake are discussed. Furthermore, the socio-economic effects (i.e., profitability, women empowerment and employment creation), milk quality, safety and traceability issues linked to a better organized and performing dairy value chain are highlighted.

Keywords: greenhouse gas emissions, gender roles, employment creation, innovation, policy, milk productivity

INTRODUCTION

The agricultural sector in Zimbabwe supports the livelihoods of approximately 70% of the population and contributes approximately 17% of GDP (FAO, 2021). In a baseline survey conducted by Transforming Zimbabwe's Dairy Value Chain for the Future Action (TranZ DVC) (2019), income from milk and milk by-products were reported to contribute only 0.3% of the total GDP, and the milk processing component of the dairy value chain was reported to employ 282 male and 86 female youth (<35 years). Moreover, of the total number of jobs that offer a fair income and social protection (descent jobs), along the dairy value-chain, 39.5% and 23% were reported to be held by women and youth, respectively (Transforming Zimbabwe's Dairy Value Chain for the Future Action (TranZ DVC), 2019).

From the mid-90s, the dairy cattle herd decreased due to recurrent droughts, economic contraction, and the land reform programme that disrupted large-scale dairy operations responsible for >95% of the national milk pool (Kagoro and Chatiza, 2012). The land reform programme, which involved redistributing land from the large-scale commercial sector to households from the overcrowded communal areas, and the resultant lack of clarity in the security of land tenure were probably the most important factors that negatively impacted the dairy sector (Mzumara, 2012; Marecha, 2013). The difficult operational conditions created by the factors mentioned above resulted in a decrease in the number of registered commercial dairy farmers from 559 in 1987 to 165 in 2012 (SNV, 2012). Over the same period, 1987-2012, the dairy herd decreased from 113,006 to 27,400 resulting in the underperformance of the value chain, as evidenced by a reduction in milk yield from 262 million liters in 1990 to <37 million liters in 2009 (Dairy Services, 2020).

Although recent public and private sector interventions contributed to a steady but slow increase in annual national milk outputs, which stood at 80 million liters in 2019 (Dairy Services, 2020), these are below the national capacity for milk consumption which is 130 million and the capacity for the processing which is 400 million liters per annum (Ministry of Lands, Agriculture and Rural Resettlement, 2016). Since national milk demand stands at 130 million liters (Dairy Services, 2020), milk deficits are covered by importing milk and dairy products (TrendEconomy, 2020). Meeting this demand through local production instead of imports presents an opportunity to improve the welfare of producers and support sectors through increased income and employment generated along the value chain. This perspective article is aimed at exploring practical options for reducing Zimbabwe's milk deficit by improving the performance of smallholder (<200 liters per farm per day) and medium-scale (200–500 liters perfarm per day) dairy farmers. To achieve this objective, in early 2021, we reviewed existing literature (e.g., scientific articles, databases, gray literature) and sought inputs from key stakeholders and experts with knowledge on the dairy value chain in Zimbabwe (most of them involved as co-authors). With these inputs, we provide our perspective on (i) how milk production is organized in Zimbabwe, (ii) where and how milk is being processed and marketed, (iii) who the key stakeholders along the dairy value chain are, (iv) what the environmental impacts of dairy production are, and (v) the barriers and constraints for improving the performance of the dairy value chain. Based on this, we then provide a discussion where we suggest key interventions that could help improve the dairy value chain performance and improve the livelihoods of various value chain actors.

MILK PRODUCTION REGIONS AND PRODUCTION SYSTEMS

Zimbabwe is divided into five agro-ecological regions (AER) based on the amount of received rainfall. Large-scale commercial dairy production is mainly conducted in AER I (>1,000 mm, 1,100–2,600 masl), AER IIA and IIB (750–1,000 mm, 1,100–1,800 masl), AER III (650–800 mm, 1,100–1,200 m) [Marongwe et al., 1998; FAO, 2006a; Government of Zimbabwe (GoZ), 2013]. Mean annual temperatures in areas supporting large-scale dairy production range between 15–18°C, 16–19°C and 18–22°C in AER I, II and III, respectively (Mugandani et al., 2012). Smallholder dairy farmers are located in all AER, including the dry regions (<650 mm annual rainfall), AER IV (600–1,200 masl) and AER V (300–900 masl). A visual representation of the spatial distribution of the AERs is given by Kashagura (2014).

Smallholder farmers, with an average of three cows per farmer, generally practice dairying for household consumption and sales of excess production to informal markets (Kagoro and Chatiza, 2012). While milk production levels vary between different farms, low milk yields (<200 liters per farm per day) in the smallholder sector contribute to their small share of the national milk pool (∼2–3%) (Hanyani-Mlambo, 2000; Munangi, 2007). Therefore, while smallholder production is essential for food security, low milk yields partly due to reliance on low-yielding local breeds and cross-breeds (4–6 L per cow per day) result in their contribution to the national milk pool being largely invisible (Chinogaramombe et al., 2008; SNV, 2012). The contribution of medium-scale farmers (200–500 L per farm per day) to the national milk pool is variable as some of these farmers have a large number of animals with low milk productivity. This variability in production levels was one of the reasons that led to dairy farmers now being classified based on total milk yields per day rather than cattle numbers. Currently, natural grasslands and crop residues are the primary feed resources used by smallholder and medium-scale dairy producers (Gwiriri et al., 2016). Consequently, the low milk yields experienced in the smallholder and some medium-scale farms are partially due to low yielding cattle breeds, seasonality in the availability of quality and adequate feed resources (Ngongoni et al., 2006).

Large-scale commercial dairy producers (>500 L per farm per day) that contribute to >95% of the national milk pool are primarily located in AERs receiving relatively high (>650 mm) rainfall and relatively high (>1100 masl) altitude. The large dairy producers mainly use pure exotic cattle breeds (e.g., Holstein-Friesian breeds, Red Dane, Jersey, Guernsey), with a productivity range of 14–25 liters per cow per day (Mandiwanza, 2007; Matekenya, 2016). Besides high yielding cattle breeds, the high productivity of cattle in the large-scale producers is partially due to access to extensive grazing areas and financial resources to buy supplementary stock feeds during dry periods (Matekenya, 2016).

MILK MARKETS

Viable markets are crucial for incentivizing the increased competitiveness of any commercial enterprise. A major challenge that needs to be tackled in the dairy sector is that smallholder and medium-scale farmers (<500 L per day) are underperforming, thus not significantly contributing to the national milk pool. There are milk collection centers (MCCs) strategically located in the milk-producing regions for easy access to dairy farmers. Farmers deliver their milk to these centers, where it is tested for quality before being added into bulk milk tanks. In 2020, 17 operational farmer-owned MCCs were reported to have received milk from 386 farmers [Zimbabwe Dairy Industry Trust (ZDIT), 2021]. Several MCCs (e.g., Nharira and Honde Valley) have ventured into small-scale value addition producing products such as yogurts and cheese and increased their profitability (Kandjou, 2012). Otherwise, medium and large-scale (e.g., Dairibord) processors collect bulk milk from the milk collection centers and transport it to their processing factories. Smallholder farmers' contribution to the national milk pool was about 1.1 million liters (2% of national production) in 2012. In the same year (2012), only six smallholder producer associations were reported to have produced sufficient quantities of milk to deliver to a major milk processor (Kagoro and Chatiza, 2012). In 2019, a study conducted across 60 districts in the country's ten provinces reported monthly milk production levels of 1,703,666 liters per month and 5,020,034 liters per month in the large-scale commercial sector (Transforming Zimbabwe's Dairy Value Chain for the Future Action (TranZ DVC), 2019).

Milk processing is dominated by five out of the eight registered large-scale dairy processors (see **Table 1**) that are processing 85% of the milk [Zimbabwe Dairy Industry Trust (ZDIT), 2021]. On the other hand, 27 registered small-scale and 12 medium-scale processors correspondingly process 8% and 2% of the milk [Zimbabwe Dairy Industry Trust (ZDIT), 2021]. Dairibord Holdings (2019), a major dairy processor in Zimbabwe, reported that about 3.4 million liters of the raw milk processed in 2019 were collected from smallholders. The increase in quantities of smallholder milk annually sold on the formal market (i.e., 1.1 million liters in 2012 to 3.4 million liters in 2019) signify progress in overall milk production (SNV, 2012). However, relative to their current annual production levels (∼20 million liters), the amount of milk entering formal markets from smallholder and medium-scale dairy producers is still low.

TABLE 1 | Summary of Zimbabwe milk value chain actors.

Category	Main actors
Farmer representation	Organizations advocating for dairy farmer interests include Zimbabwe Association of Dairy Farmers (ZADF), Commercial Farmers Union (CFU), Zimbabwe Farmers' Union (ZFU).
Farmer extension and veterinary services	Departments in the Ministry of Lands, Agriculture and several NGOs, milk processors,
Research services (Research Institutes, NGOs and Universities)	The setting of research priorities is mainly done by the Zimbabwe Dairy Industry Trust, Research institutes and universities
Animal and milk traders	Cooperatives
Milk processors	Dairibord Holdings, Nestle, Kefalos, Dendairy, Prodairy, Kershelmar, Alpha Omega, Yomilk.
Input provision and financial support for farmers	Private sector dealers, banks and micro-credit providers.
Regulatory services	Government ministries and Parastatals, and civil society actors
Consumer protection	Organizations interested in consumer interests (i.e., quality and prices), including the Consumer Council of Zimbabwe (CCZ) and the Standards Association of Zimbabwe (SAZ)

ENVIRONMENTAL IMPACTS

Cattle production heavily relies on natural resources and has a substantial environmental footprint due to methane and nitrous oxide emissions from enteric fermentation and manure; ammonia loss during manure handling and storage; deforestation and biodiversity loss when clearing land for grazing; and degradation linked in review to poor pasture management, overgrazing and soil erosion (FAO, 2006b; Gerber et al., 2013). Studies on the environmental impacts of dairy production systems in Zimbabwe are limited. For example, we only found one study on greenhouse gas emissions from livestock systems in Zimbabwe. A drawback of the study was that Tier 1 (default) IPCC emission factors were used to quantify GHG emissions. These default emission factors are mainly determined using studies almost exclusively conducted in Western countries (Goopy et al., 2018), which have enormous uncertainties for African livestock systems. In the study by Svinurai et al. (2018), which covered 35 years, 58–75% of total annual emissions from livestock were estimated from the smallholder sector. The smallholder sectors' low productivity is associated with high GHG emissions per unit of milk. A study conducted in Kenya, under similar low intake dairy production systems, shows that increased feed intake increases milk production and the total GHG emissions from enteric fermentation (Ndung'u et al., 2018). If herd sizes grow to meet the demand and reduce the milk deficit, the total GHG emissions and water use are also likely to increase. To counteract this, herd growth needs to co-occur with productivity increases to reduce GHG emissions and water use (e.g., Douxchamps et al., 2021; Hawkins et al., 2021) per liter of milk. Increased productivity has to go hand-in-hand with increased land and water productivity (more animal nutrition per area of land and liter of water) and feed efficiency (more animal product per unit of feed), to avoid clearing of more land to produce feed, and enhance milk production per unit animal, water and land, respectively. A range of resource-use-efficient and climate-smart practices (e.g., forage production and conservation, water management, manure management) exist, but adoption is low due to various financial, communication and socio-economic factors (CIAT and World Bank, 2017).

Addressing productivity challenges should coincide with tackling the environmental impacts of the dairy sector. Land degradation, water scarcity and climate change should be addressed through pursuing management practices with environmental co-benefits. Generally, most technologies and practices that reduce GHG emissions have economic benefits as they often increase productivity (Gerber et al., 2013). In addition, Svinurai et al. (2018) showed that current livestock populations, production and emissions trends suggest that even if Zimbabwe's national livestock herd doubled in 2030, relative to 2014, methane emission intensities (per capita) would be similar to those observed in 1980. Therefore, there is potential to increase productivity and reduce the milk deficit without significantly increasing GHG emissions.

KEY STAKEHOLDERS

Several previous studies have mapped the key public, private and civil society actors along the dairy value chain (Marecha, 2009; Kagoro and Chatiza, 2012; Matekenya, 2016). Based on this already existing information, a summary of the roles different value chain actors play is presented in **Table 1**.

BARRIERS AND CONSTRAINTS TO OPTIMAL PERFORMANCE OF THE MILK VALUE CHAIN

It is unambiguous that the Zimbabwean dairy value chain is far from optimal performance resulting from multiple factors affecting local milk production. At the farm level, low milk yields and calving rates, late age at first calving and long calving intervals prevail and are directly related to nutritional aspects, the use of inappropriate breeds, poor farm management, limited disease control and poor extension (Smith et al., 2002; Ngongoni et al., 2006; Munangi, 2007). The already limited availability of suitable farmland and water are declining due to climate change and climate variations (Brown et al., 2012). Changing rainfall patterns, heat waves or droughts (e.g., 2015–2017) lead to poor pasture conditions, feed and forage seasonality, yield decreases and price increases (resulting in difficulties for animal breeding; Masama, 2013), and high susceptibility to pests and diseases—all having immediate adverse effects on milk yields and production costs. At the macro and value chain level, extreme climatic conditions are causing damages to infrastructure (i.e., water and energy supply), resulting in higher costs for milk cooling, disruptions in the transport of perishable goods such as milk (Chari and Ngcamu, 2017a), increased processing and transport costs, consumer prices, vulnerability and food insecurity (Chari and Ngcamu, 2019). In our view, the dairy sector requires strategic investments along the value chain to achieve its full potential, e.g., in cooling facilities, milking machines or road and transportation infrastructure. Zimbabwe, however, has high burdens (bureaucracy, complex procedures) for accessing financing (Hahlani and Garwi, 2014). In addition, credit providers are reluctant to lend money to farmers who do not possess collateral (Chari and Ngcamu, 2019); their credit rates are high (up to 14%; Commercial Farmers Union, 2014) and more oriented toward short-term investments. Long-term investment projects, such as establishing improved forages or purchasing milking machines, cannot be readily financed under these conditions (Chari and Ngcamu, 2017b), discouraging farmers from technology adoption.

Furthermore, productive inputs are expensive in Zimbabwe, affecting the dairy value chain. For example, both the purchase of heifers and on-farm breeding are costly (Hahlani and Garwi, 2014), forage seeds are often unavailable, high labor costs reduce returns along the value chain, and electricity is expensive and frequently disrupted, boosting the use of less efficient and more expensive energy sources for production and processing (SNV, 2012). Regarding policy-based constraints, Zimbabwe was facing a phase of instability from 1998 to 2000, followed by a fast track land reform program that affected the dairy sector. Large dairy farmers lost their farms, and land titles for the resettled farmers are still unclear (Marecha, 2013), and this, combined with unresolved land disputes between farmers, leads to low long-term investments in farm improvement plans (Marecha, 2013; Chari and Ngcamu, 2017a). Compared to other countries (e.g., South Africa, Kenya), raw milk prices are substantially higher in Zimbabwe (Kawambwa et al., 2014), probably due to the described production constraints and inefficiencies (Gadzikwa, 2013). The lack of infrastructure, technologies and adequate management affect milk quantity and quality, the latter being a major bottleneck for milk processing (Chari and Ngcamu, 2019). The situation is further aggravated by limited technical assistance schemes provided to dairy farmers (Smith et al., 2002). Gender inequality is a significant constraint in the development of the dairy value chain. Men, women and youth play essential roles in the livestock sector, but the level of participation differs significantly. Although the situation is gradually changing, men continue dominating livestock production, mainly for cultural reasons, overshadowing women's ownership of livestock, decision-making and control (Chawatama et al., 2005; Daniels, 2008; Mupawaenda et al., 2009). Gender roles are based on dynamic cultural beliefs for which the pace of change is determined by increased awareness and incentives. Thus, targeted social awareness campaigns, combined with appropriate policies and incentive mechanisms, can harness the perspectives and capacities of men, women, and youth to improve value chain performance and gender equity.

DISCUSSION ON KEY INTERVENTIONS TO IMPROVE THE MILK VALUE CHAIN IN ZIMBABWE

In **Table 2**, we present a range of interventions to improve the performance of the dairy value chain in Zimbabwe. Briefly, the interventions are disaggregated based on value chain links. While needed interventions are primarily known, the challenge is on ensuring that the needed actions for their actual implementation are taken. Taking the needed actions is not an easy task as smallholder dairy farmers, who include many underperforming farmers, are mainly resource-constrained and, at times, located in remote areas with limited supporting infrastructure. Post-land reform, the government of Zimbabwe has targeted the

TABLE 2 | Key interventions for improving the dairy value chain in Zimbabwe.

Value chain segment	Interventions
Inputs	• Availability and access to affordable improved forage seeds (including vegetative propagation) to increase the supply of forage/forage quality • Support local feed and forage seed production and seed distribution • Where necessary, support local businesses that import seeds of improved grasses and feedstock that cannot be produced locally due to physiological constraints • Accelerate the speed of input importation and the registration of new varieties • Feed conservation and associated business models • Access to regular and uninterrupted energy and water supplies • Installation of irrigation infrastructure • community-based animal health services, para-extension and artificial insemination
Production	• Improved availability of and access to in-calf heifers • Development of formal dairy training centers • Improved mechanization of dairy systems for improving efficiency in feed production, feed processing, cattle management, milking and milk processing. • Adoption of cattle breeds with high milk production potential (which need to go hand in hand with): ○ Good on-farm feed and animal management practices ○ Appropriate animal health measures • Improved farmer technical support, extension and education • Harmonization of efforts and concepts and training of technical assistants/extensionists among government agencies and NGOs
Processing	• Set up and rehabilitate processing infrastructure and quality assurance systems • Increase number of technical experts and their availability • Improve extension/training and access to inputs required for milk processing and value addition (e.g., cheese and yogurt production)
Distribution and marketing	• Improved distribution infrastructure (e.g., milk collection centers, road infrastructure) • Improve farmer access to information (e.g. price information systems; information fora, multi-actor platforms) • Support more collective actions, e.g., cooperatives, bulking of milk and guaranteed prices
Consumers	• Product differentiation and niche markets (e.g., denominated origin, quality attributes, environmental attributes, fair trade, animal welfare) • Consumer awareness campaigns on milk and milk products • Increase consumer promotional material
Financing	• Easy access to financing programs • Risk insurance • Affordable credit and general credit accessibility; credit lines for sustainable intensification efforts • International assistance, e.g., necessary assistance *vs*. reduction of dependence • Strengthening safety nets • Training on investment prioritization
Entrepreneurial support	• Local transformation and formalization • Increased number of local value addition and milk transformation plants (e.g., cheese, milk, yogurts) • Support of inclusive business models
Institutional, policy and regulatory support	• Better institutional coordination among value chain actors • Evidence-based policy support/legislation
Cross-cutting	• Women and youth empowerment (i.e., increasing women involvement in the dairy value chain) • Design interventions in the dairy value chain to allow women to change their lives (production of milk-based products value additions) • Strengthening collaboration among direct value chain actors but also with value chain framework • Access to in-depth education on the dairy industry (from a young age) • Organization and Training/capacity-building of Dairy farmers

dairy industry in its vision of transforming the nation into a middle-income country by 2030. Therefore, there have been several efforts to resuscitate the local dairy industry. For instance, in 2017, the government launched the Dairy Revitalisation Programme funded in review through the Dairy Resuscitation Fund and aimed to increase national production to 200 million liters per year by 2025. Also, in 2019, supported by the E.U., the government launched the Zimbabwe Agricultural Growth

Programme (ZAGP) to address weaknesses and gaps in livestock value chains. This programme aims to increase investments, propose institutional reforms and policy alignment to support the dairy sector [Zimbabwe Agricultural Growth Programme (ZAGP), 2019]. However, over-reliance on external funding to revive the dairy sector may not be a sustainable solution; shifting to more local and continuous investments may be a more prudent approach (Washaya and Chifamba, 2018). The Zimbabwean diaspora, estimated at four million [International Organization for Migration (IOM), 2015], presents a vast potential source of capital investment in the dairy sector (Madziva et al., 2018). However, the government may need to highlight challenges and investment opportunities along the dairy value chain, create proper incentives, and develop regulatory mechanisms to protect investments. In addition, by creating spaces for national discussions, including the diaspora, the country could also tap into their experiences and expertise to innovate along the dairy value chain.

It would be strategic for the public and private sector to increase research investments tailored to generate knowledge on technologies and practices that result in efficiency gains along the dairy value chain. For instance, due to high costs for feed, limited access to affordable finance and insecure land holdings, most farms have dairy animal herds below their potential [Zimbabwe Dairy Industry Trust (ZDIT), 2021]. Therefore, besides focusing on efficiency gains along the dairy value chain, investments need to increase the dairy herd in smallholder and medium-scale farms. For example, smallholder farmers with an average of 3 cows per farm (Kagoro and Chatiza, 2012), with each cow producing 5 liters per day (Chinogaramombe et al., 2008). Even if the average milk productivity per cow were to match the higher end of cows on large-scale farms (25 liters per day; Matekenya, 2016), their production levels would remain small-scale ($<$200 liters per farm per day). Therefore, to transition from a small to a medium-scale or a large-scale dairy producer, the initial focus should be on increasing dairy herd sizes per farm.

After increasing the dairy herd per farm, the next step would be to find creative, feasible and context based-solutions to overcome the low and seasonal supply of high-quality animal feed. Improved feed availability could be done by introducing and promoting improved forages tolerant to abiotic (excess and scarcity of water) and biotic (pest and diseases) stresses as the basis of feeding. Although the planting of improved forages is considered to be scale-neutral, meaning that the technology can be used by smallholders as well as medium- to large-scale producers, the private forage seed suppliers estimate that mostly smallholder to medium-scale livestock producers adopt them to sustainably intensify their production systems (Labarta et al., 2017; Fuglie et al., 2021). Forages compete less with human nutrition, e.g., grain crops, and have the co-benefit of maintaining soil fertility, enhancing carbon accumulation and improving GHG balances and Water-Use-Efficiency. However, this would require functional seed systems, ensuring seed availability, accessibility, and affordability (Peters et al., 2021).

With appropriate training and the proper incentive mechanisms, the estimated 8% of youth unemployed (World Bank, 2021) can be engaged to co-explore solutions to improve on-farm productivity. For instance, in the case of improving feed supply, a practical solution could be for the youth to receive support for establishing local seed supply systems (i.e., for forage legumes). The local seed supply systems could improve dairy farmers access to affordable, high-quality seed to sow on their private or communally owned pasturelands. This forage-based basal diet can be complemented by strategic supplementation with several crops grown in the rural areas (i.e., maize, groundnut, sunflower, pearl millet, sorghum and cowpea). Dependence on local crops presents farmers with an opportunity for cost-effective feed-level interventions that can improve market competitiveness and productivity of their systems (Murungweni et al., 2004; Ngongoni et al., 2006; Gusha et al., 2013; Mashanda, 2014; Gwiriri et al., 2016; Chifamba et al., 2018). To overcome periods of feed scarcity, high-quality forages and feed crops could also be conserved as hay or silage and become the basis of densified feeds; densification may allow an easier transfer from one region to another (Dey et al., 2021, unpublished).

Youth could establish feed processing businesses based on high-quality feed mixes based on local grains to provide dairy farmers with local high-value supplements or concentrates (Chifamba et al., 2018). We expect local sourcing to reduce feed costs and increase the profitability of dairy operations. In addition, youth can be trained as para-extension agents that can support artificial insemination programmes to improve the local breeds and veterinary services to support animal health (Kagoro and Chatiza, 2012). The engagement of youth (as local entrepreneurs) to supply improved seeds, deliver animal health services and improve cattle breeds will contribute to employment creation and the intake of quality feed by healthy and high yielding cattle breeds and ultimately improve milk supply and quality from smallholder and medium-scale dairy producers. Youth participation in the local economy may also prevent their migration to crowded urban areas.

Mhlanga et al. (2018) projected that without a global reduction in atmospheric CO_2 concentrations and the resultant high air temperatures would reduce feed availability and the area suitable for dairy farming and have devastating impacts on the local dairy industry. To maintain milk yield stability even during dry periods, dairy farmers may need to consider drought-tolerant forage crops that better use available moisture. One example of this is Cactus pear (*Opuntia* spp.), which efficiently converts water into dry matter (Galizzi et al., 2004). *Opuntia* species are known for developing physiological, phenological and structural adaptations (Guevara et al., 2011), making them productive in these drier environments (Nobel and Zutta, 2008). On average, the biomass production from cactus per unit of water is about three times as high as with C4 plants and five times as high as with C3 plants (Snyman, 2013), making *Opuntia* cladodes a valuable option for successfully balancing parts of the cattle diet (Einkamerer et al., 2009; de Waal et al., 2013). From a well-managed cactus pear plantation of 800 to 1000 plants/ha, around 10 t/ha cladode dry matter and 20 t/ha fruit biomass can be obtained, but values vary with genotype (Fouché and Coetzer, 2013). To improve the adoption of *Opuntia,* investments are needed in research and awareness-raising on its use and potential benefits. In addition, investments in technical support for establishing fodder banks with *Opuntia*, could stimulate its adoption as a feed option during dry and drought periods (Makumbe, 2010).

The smartphone penetration rate is 52 per 100 inhabitants (∼7.7 million users) (Econet Wireless Zimbabwe, 2020). However, considering that several inhabitants may have more than one smartphone, while the exact number of smartphone users is uncertain, it is probably lower than 52%. On the other hand, mobile subscriptions are very high (90 per 100 inhabitants; ∼ 13 million subscribers) (ITU, 2021). To support the complete transition toward digital agriculture, government and private sector actors need to innovate and improve smartphone affordability and reduce the cost of mobile data. These actions may incentivize the adoption of digital tools that will have cascading benefits across the dairy value chain. For instance, tools like smartphone applications and online platforms can help connect dairy value chain stakeholders and improve farmer participation, actor coordination, and information flow across the value chain. Other benefits include reducing the length of the value chain (by avoiding unnecessary intermediaries and associated costs), improving milk traceability and monitoring milk quality, using digital records to apply for credit, supporting decision-making, and optimizing farm operations (Born et al., 2020).

CONCLUSIONS

Several previous studies and reports have presented what needs to be done by the different actors to create a sustainable and inclusive dairy value chain, yet progress remains limited. While there are certainly no silver bullets, actions that support improved performance at different value chain stages are needed. Moreover, increased productivity in the dairy sector could return Zimbabwe to being a net exporter of dairy products and contribute toward meeting the ambitious national goal of transforming the nation into a middle-income country within a decade (by 2030). In our opinion, to sustainably solve challenges along the dairy value chain, more attention should be placed on the underperforming smallholder and medium-scale dairy farmers and supporting value-chain interventions that creatively balance investments, livelihoods, and profits within the local context.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

The introduction was written by NC, AW, and CM. The sections on milk production regions and production systems and milk markets were written by CM, JN, and AT. The section on environmental impacts was written by AN, MP, and NC. The section on key stakeholders was written by AW, CM, AT, and NC. The section on barriers and constraints to optimal performance of the milk value chain was written by SB, MP, AN, and AT. The discussions on key interventions to improve the milk value chain in Zimbabwe and conclusions were written by all the authors. All authors contributed to the article and approved the submitted version.

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Performance of *Urochloa* and *Megathyrsus* Forage Grasses in Smallholder Farms in Western Kenya

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Livestock productivity has remained low in sub-Saharan African countries compared to other places on the globe. The feeding component is the major limitation, in both quantity and quality. Among other inputs, feeding takes 55–70% of the costs involved. Livestock play a major role especially in smallholder mixed farms through provision of household nutrition and income through milk and meat. Equally, fertilization of cropland benefits from livestock manure, and livestock often act as insurance and savings by providing liquidity for unforeseen and urgent financial needs. Increasing livestock productivity would enhance the fore-mentioned benefits contributing to well-being and livelihoods. Toward this endeavor and with smallholder dairy farmers' participation, we undertook an evaluation of 10 selected forages from *Urochloa* Syn. *Brachiaria* and *Megathyrsus* syn. *Panicum* genus and compared them with Napier grass, i.e., *Cenchrus purpureus* Syn. *Pennisetum purpureum* commonly grown by farmers. For detailed and robust evaluation, we established the species in eight trial sites spread in four administrative counties in Western Kenya (Bungoma, Busia, Kakamega, and Siaya). In each site, the forages were established in plots in a randomized complete block design, replicated three times. Each site was linked to a group of farmers interested in dairy. For 2 years, dry matter production, plant height, and leaf-to-stem ratio was determined across all sites. Further, we guided farmers to generate participatory forage evaluation criteria, which they later administered across their respective forage demonstration sites individually on plot-by-plot basis to generate preference rating compared to what they normally grow—Napier grass. The results showed significant differences across the forage types within and between the sites. Cumulative dry matter yields ranged 13.7–49.9 t/ha over 10 harvestings across forage types and the counties, while values for crude protein were 1.85–6.23 t/ha and 110,222–375,988 MJ/ha for metabolizable energy. Farmer preferences emerged that highlighted forages with likely better chances of adoption with weighed scores ranging 5.5–7.6 against a scale of 1–9, across the counties. The observations provide additional and well-performing forage options for the farmers and possibly in similar production systems and ecologies. Awareness creation targeting livestock and dairy producers would be key, reaching, and informing them on alternative forage options, with potential to increase livestock productivity.

Keywords: leaf to stem ratio, farmer evaluation, forage quality, dry matter yield, forage grass

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INTRODUCTION

Tenacious low livestock productivity in sub-Saharan African (SSA) countries is by and large due to inadequate feeding (Alejandro et al., 2007). Feeds and forages account for up to 70% of costs in livestock production (Odero-Waitituh, 2017). Hitherto, meat and milk demands in SSA are growing at 3.4 and 2.9% annually, respectively (Latino et al., 2020). As such, the estimated consumers' demand of 35 and 83 billion tons for meat and milk, respectively, by 2050 (World Bank, 2014) will remain a challenge unless livestock feeding is addressed. Land as a production resource is limited especially in intensifying smallholder systems, and it is no longer possible to allocate land for free grazing. However, cultivated forage presents a realistic avenue to meet ruminant roughage requirements under such circumstances. Albeit extensive forage catalogs exist, efforts toward forage improvement through selection and/or breeding are limited compared to food crops globally. In SSA this has resulted in use of non-nutritious crop residues (FAO, 2018) and limited forage options developed decades ago. Use of low nutritious roughages in turn results in undesirable high emission of methane gas per unit of product, associated with global warming (Makkar, 2016).

Therefore, there is need to identify and deploy improved forage technologies in SSA to bolster livestock productivity. Use of grasses from genus *Brachiaria* (now *Urochloa*) and *Panicum* (now *Megathyrsus*) present realistic options toward quality and quantitative roughage production. For example, use of *Urochloa* hybrids has been successful in Latin America, supporting improved livestock productivity, especially beef (Rivas and Holmann, 2005). With temporal and spatial variations to environments, matching forage genotypes to biophysical environment and agricultural context remains unsatisfactory in SSA. We therefore set out to evaluate the performance of selected grass lines from *Urochloa* and *Megathyrsus* under farmers' context in western Kenya. Involving farmers who are the end users is desirable as participation brings to the fore farmers' perspective on attributes/characteristics they use on choice of forages to grow and therefore guide on forage breeding and selection in order to meet desired traits. The importance of participatory approaches have been underscored (Abeyasekere, 2001), and for example, Mwendia et al. (2017a) used the same to evaluate oat varieties for forage production in central Kenya. Largely, western Kenya is moving toward intensified livestock production owing to high and growing human population coupled with land subdivision over generations reducing areas of free grazing (Waithaka et al., 2002). As such, there is limited grazing on natural pasture and there is a buildup on cattle in confinement under cut-and-carry systems. The genotypes *Urochloa* and *Megathyrsus* trace their origin in tropical Africa and only improved through selection and/or breeding (Cook et al., 2020). Therefore, the forages stand a good chance in fitting under cut-and-carry intensified systems. We hypothesized variable performance of these grasses under different locations and varying farmers' preference, results that would have potential to influence wider scaling of these grasses in western Kenya and beyond.

MATERIALS AND METHODS

Site Selection

Four counties in western Kenya were selected based on their high bio-physical potential for dairy and commercialization, namely, Bungoma, Busia, Kakamega, and Siaya (**Figure 1**). Despite the areas being in mid-altitude 900–1,800 m, they differ agro-ecologically (Jaetzold et al., 2006). In addition, soils we analyzed from the specific trial sites showed significant differences in key soil attributes (**Table 1**). With a soil auger, we collected soil samples at 0–50 cm depth, and 3 samples along a replicate, hence 9 samples per site, and 72 samples from the 8 sites. In partnership with Send a Cow Kenya (SACK), a development partner, in these sites we linked up with farmer groups that have been engaged in SACK initiatives on improving human nutrition and incomes and selected two farmer groups with a keen interest in dairy per county, resulting in eight trial sites (**Figure 1**). Soil sample analysis was done at International Livestock Research Institute (ILRI), Nairobi, focusing on pH, total carbon, nitrogen, and phosphorus and contents of clay, sand, and silt.

Forage Technologies, Trial Design, Planting, and Management

At the start of the project, we sensitized the selected farmer groups on dairy improvement and the importance of animal feeding. Consequently, we offered them to try out several forage options with potential to grow well in the region. In the end, the groups offered land where we established demonstration trials. While the project provided forage seeds and technical advice, farmers agreed to provide labor for land preparation, planting, weeding, harvesting, and monitoring the performance of the grasses. We selected 10 forage grasses covering 3 hybrids and 4 cultivars from genera *Urochloa*. The hybrids include Cayman, Cobra, and Mulato II and the cultivars Basilisk, Piata, Xaraes, and MG4. Xaraes and MG4 are also known as Toledo and La Libertad, respectively. For *Megathyrsus* genera, we included cultivars Mombasa, Tanzania, and Massai. Napier grass (*Cenchrus purpureus* Syn. *Pennisetum purpureum*) from the farmers' farms was included as a control. The trial design was a randomized complete block design with three replicates per site and in eight sites. Farmers manually prepared the land by digging with hoes to about 0.2 m depth. To get sufficiently fine seedbed, farmers broke down big soil clods to the required soil tilth. Using wooden pegs, we marked out 15 m^2 plots (3 $\times$ 5 m) with 33 of them per site, to allow 3 replicates of the 11 grasses selected. Therefore, in the 4 counties we had 8 sites and 264 plots in total. Because of acidic soils in western Kenya (Kanyanjua et al., 2002), we applied lime at 2 t/ha prior to planting. At planting in May 2018, we randomly allocated the grasses to the prepared plots. We used the recommended seed rate for each genus, i.e., 6 kg/ha for *Urochloa* (Njarui et al., 2016) and 3 kg/ha for *Megathyrsus*, while for Napier grass we used splits spacing at 1 $\times$ 1 m grids (Mwendia et al., 2017a,b). We applied NPK inorganic MEA fertilizer® (NPK fertilizer 23:23:0) at the rate of 50 kg N/ha. Because of small seed size in *Urochloa* and *Megathyrsus*, shallow hills of about 0.02 m depth, 0.3 m

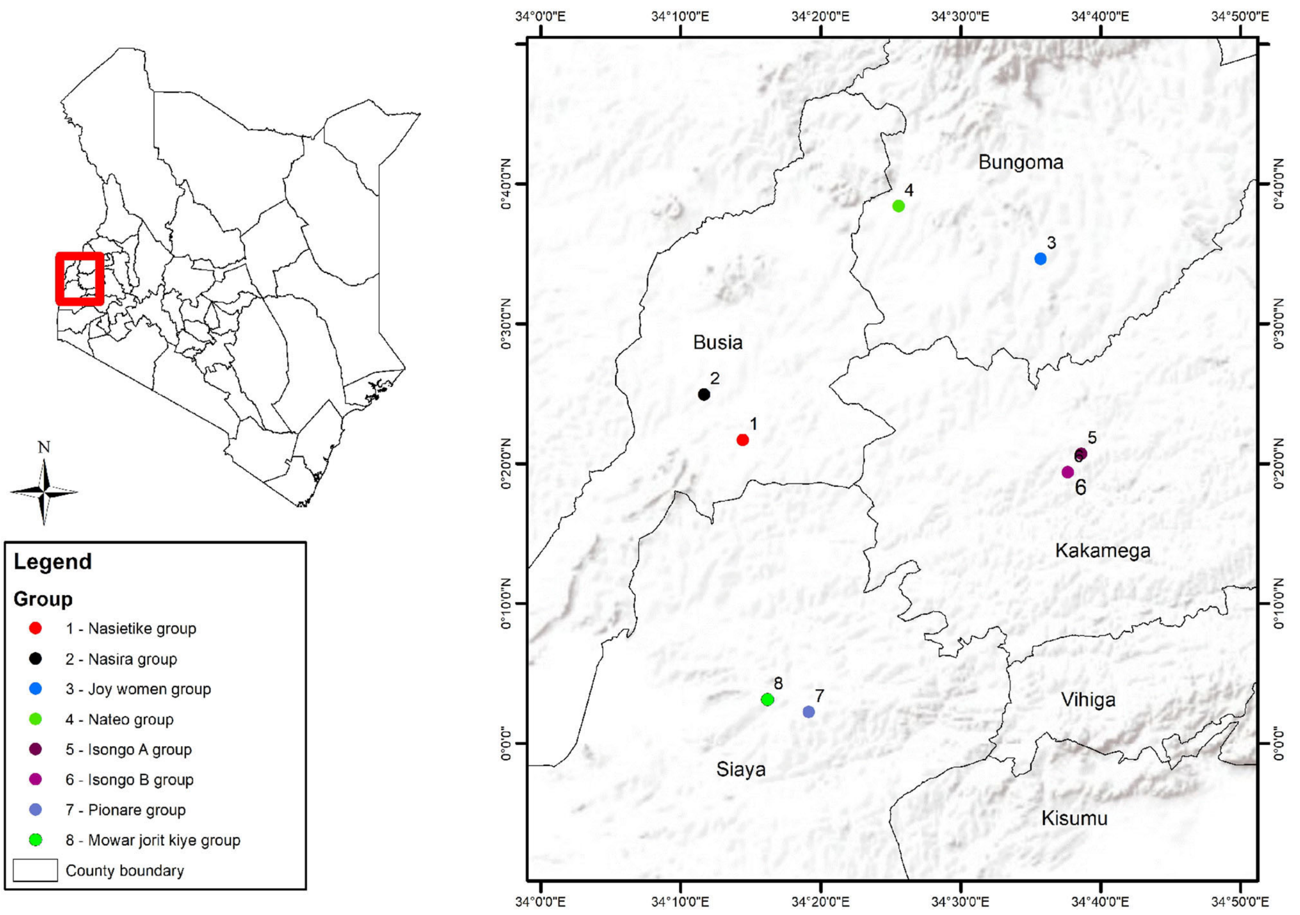

FIGURE 1 | Experimental sites in Busia, Bungoma, Kakamega, and Siaya counties in western Kenya indicating farmer groups linked to the sites during the experiment in 2019–20.

TABLE 1 | Summary of rainfall, altitude, agro-ecological zones, soil characteristics, and farmer groups selected in the trial sites in Bungoma, Busia, Kakamega, and Siaya counties in western Kenya.

Attribute	**Bungoma**	**Busia**	**Kakamega**	**Siaya**	
Precipitation (mm)	1,536–1,681	1,585–1,690	1,800	1,320	
Altitude (m)	1,433–1,829	1,200–1,440	1,300–1,550	890–1,020	
Agro-ecological zone	Low Midland 2	Low Midland 1	Low Midland 1	Low Midland 4	
Selected Farmer groups	**Joy, Nateo**	**Nasira, Nasietike**	**Isongo A, Isongo B**	**Pionare; Mowar Jorit Kiye**	
Soil characteristics					*lsd*
pH	5.6^{a}	5.4^{bc}	5.5^{ab}	5.3^{c}	0.13
Total C (%)	0.83^{c}	1.34^{a}	0.95^{b}	0.83^{c}	0.117
Total N (%)	0.073^{c}	0.11^{a}	0.075^{c}	0.082^{bc}	0.008
P (Mg/kg)	6.9^{b}	3.55^{c}	9.24^{a}	4.06^{c}	1.97
Clay (%)	28.2^{b}	45.2^{a}	27.2^{b}	43.9^{a}	5.88
Sand (%)	65.2^{a}	35.7^{c}	62.4^{a}	45.7^{b}	7.49
Silt (%)	6.7^{c}	19.1^{a}	10.4^{b}	10.4^{b}	1.93

For soil characteristics $n = 18$ per county and means with different superscript in a row differ $p < 0.05$.

between hills in a row, and 0.45 m row-to-row for *Urochloa* were used, and shallow furrows of about 0.02 m depth spaced at 0.3 m row to row for *Megathyrsus*. After planting, farmers manually maintained plots weed-free as necessary. The grasses took 3 months to establish, and standardization cut was done in September 2018.

Forage Participatory Evaluation and Dry Matter Yield Measurements

In each of the counties we selected one group (Nasietike, Joy, Mowar Jorit Kiye, Isongo B) to undertake participatory evaluation at the demonstration sites. The evaluations took place when the forages had established well and just before the third harvesting (described below). We guided each of the four farmer groups in developing criteria that describe the attributes they prefer in a forage grass. On a scale of 1–9, the farmers as a group scored each criterion where 1 = least important and 9 = most important (Mwendia et al., 2017a). Subsequently, each farmer was provided with a printed sheet containing 33 plots numbered serially in a column and the criteria developed by the group earlier along the topmost row. At the demonstration site, each farmer scored each plot across all the criteria, until all the plots were complete. We collected all data sheets for later weighted score analysis (Abeyasekere, 2001).

For dry matter yields the first harvest after standardization was January 2019. We allowed growth cycles of about 8 weeks (Njarui et al., 2016) after which the grasses were harvested at a stubble height of about 5–10 cm. Before cutting, we randomly selected and measured plant height of five tillers in each plot from the soil level to the tip of the topmost standing height. Fresh yield weight was measured with a digital weighing balance (KERN CH 50K50 with 10 g precision) and recorded on plot-by-plot basis each measuring 15 m^2. A sample of about 450 g per plot was randomly selected after mixing thoroughly the whole harvested biomass from each plot, for dry matter content determination. The sample as weighed and put inside a sample bag labeled and taken to the International Center for Tropical Agriculture (CIAT) sample processing room in Kisumu, western Kenya. Samples were manually separated into leaves and stems, labeled, and dried in an oven at 65°C for 48 h to determine dry matter content and leaf: stem ratio. Corresponding leaf and stem samples were combined back for further nutrition analysis (described below). The process was repeated for 10 consecutive cuttings, running in 2019 and 2020 except for nutritional analysis done only for the third harvest that had undergone rain season.

Forage Nutritive Value Determination

Dried samples were ground to pass through 1 mm sieve, packed in plastic zip-lock bags and sent for near-infrared-system (NIRs) analysis at Crop Nutrition Laboratory Services Ltd, Limuru, Kenya (https://cropnuts.com/service/animal-feed-analysis/). Analysis targeted metabolizable energy (ME), crude protein (CP), and *in vitro* organic matter digestibility (IVOMD).

Data Analyses

All data were managed in Microsoft Excel, and statistical analysis was carried out in GenStat 18th edition. We carried out repeated measures analyses of variance (ANOVA) where fixed variables included harvest number/time, site/location, and test forage grasses, while response variables included plant height, dry matter yields, leaf:stem ratio, ME, CP, and digestible organic matter, with the means separated by least significance difference (lsd). For the participatory evaluation we pooled individual scores by farmers and multiplied with the criteria scoring by the group, to generate weighted scores (Abeyasekere, 2001; Mwendia et al., 2017a) and subsequent ranking of the forages on county-by-county basis.

RESULTS

Analysis of Variance Summary Across Main Effects and Interactions

Significant differences were found in all traits for both county and forage grass type (**Table 2**). Where interactions were observed, we focused on their means for results and discussion.

Soil Characteristics and Dry Matter Yields

The soils were significantly acidic in Siaya ($p < 0.05$) than Bungoma and Kakamega (**Table 1**). Busia had greater carbon and nitrogen content than the other counties but had the least

TABLE 2 | Significance of main effects and interactions for cumulative dry matter yields, leaf:stem ratio, cumulative crude protein yield, metabolizable energy, and digestible organic matter.

Attribute	Main effects/interaction	*P*	Significance
Mean DM (repeated measures) (t/ha)	Time	<0.001	***
	Time × County	<0.001	***
	Time × group	<0.001	***
	Time × forage	<0.001	***
	Time × county × forage	0.008	**
	Time × group × forage	1	NS
Cumulative DM yield (t/ha)	Block/replicate	0.042	*
	County	<0.001	***
	Group	<0.001	***
	Forage	<0.001	***
	County × forage	<0.001	***
	Group × forage	1.00	NS
Leaf:stem ratio	Block/replicate	0.452	NS
	County	<0.001	***
	Group	0.928	NS
	Forage	<0.001	***
	County × forage	0.008	**
	Group × forage	1.00	NS
Cumulative CP yield (t/ha)	Block/replicate	0.202	NS
	County	0.019	*
	Forage	<0.001	***
	County × forage	0.002	**
Cumulative ME (MJ/ha)	Block/replicate	0.346	NS
	County	<0.001	***
	Forage	<0.001	***
	County × forage	<0.001	***
Cumulative digestible organic matter (t/ha)	Block/replicate	0.316	NS
	County	<0.001	***
	Forage	<0.001	***
	County × forage	<0.001	***

$P < 0.05$; $P < 0.01$**; $P < 0.001$***; NS, Not significant.*

phosphorus content, only similar to Siaya. By the proportions (%) of clay, sand, and silt, soil types in the sites were found to be as follows: sandy–clay–loam, clay, sandy–clay–loam, and sandy–clay for Bungoma, Busia, Kakamega, and Siaya, respectively. The mean dry matter per harvest showed significant differences across the harvests and interactions between sites and harvest, forage genotype and harvest, and sites and forage genotype (**Table 3**). The second and third harvests showed the least and greatest dry matter yields, respectively. In Bungoma and Busia sites, the second and fourth harvests presented the least and greatest dry matter yields, respectively, unlike in Kakamega and Siaya where the greatest biomass yield was in the third and seventh harvests, respectively. On forage genotype–harvest interaction, forage type producing the most dry matter yield varied across the harvestings. In the first harvest, Basilisk produced most, and Napier grass in second and third. From the fourth to the tenth harvests, Massai dry matter yield surpassed all the others except in the ninth harvest wherein Napier grass produced the most. On site–forage interaction, the most dry matter production was from Xaraes in Bungoma and Massai for Busia, Kakamega, and Siaya.

Cumulative dry matter yields over 10 cuttings showed interaction between the county and the grasses. Generally, across the counties the order of dry matter yield was Bungoma > Kakamega > Busia > Siaya (**Figure 2**). In Joy group site in Bungoma, Napier grass produced more biomass than Mulato II, MGA, and Basilisk but similar to the other grasses. This was different for Nateo group in the same county, where Napier grass only produced more than Mulato II but significantly less than Cayman, MG4, Xaraes, Piata, Tanzania, Mombasa, and Massai. In this site, Xaraes accumulated the most biomass significantly greater than all the grasses, except similar to Massai cultivar. In Busia County and at Nasietike group site, Napier grass produced the least biomass against all the other grasses. *Megathyrsus* cv Massai produced the most, significantly greater than all grasses, except similar to Basilisk and Mombasa. In Busia the second site, Nasira group, maintained the yield pattern for the grasses. Although Napier grass accumulated the least, it was similar to all the other grasses except for the three *Megathyrsus* species, Cayman, and Basilisk that produced significantly greater biomass. In Kakamega County and at Isongo A group site, Basilisk accumulated greater biomass than all grasses except for *Megathyrsus* cv Massai which had similar biomass. Among *Urochloa* hybrids, only Cayman had similar biomass to Napier grass. At Kakamega second site, Isongo B, Napier grass produced similar biomass to Mombasa and Massai, and the rest had

TABLE 3 | Mean dry matter yields (t/ha) per harvest over ten harvests and interactions for site × harvest, forage genotype × harvest, and site × forage genotype for 3 *Urochloa* hybrids (Cayman, Cobra, Mulato II) 4 *Urochloa* cultivars (Basilisk, MG4, Piata, Xareas), 3 *Megathyrsus* cultivars (Maasai, Mombasa, Tanzania) and Napier grass.

Attribute	County/forage type	Harvest 1	2	3	4	5	6	7	8	9	10	*P*	lsd
Harvest		2.69^{e}	0.98^{f}	4.29^{a}	4.05^{a}	3.74^{b}	3.15^{c}	3.60^{b}	3.42^{c}	3.20^{c}	2.97^{de}	<0.001	0.29
Site × harvest	Bungoma	3.85^{de}	0.42^{l}	5.04^{b}	6.22^{a}	4.18^{cd}	3.77^{e}	2.99^{gh}	4.04^{cd}	4.48^{c}	2.89^{gh}		
	Busia	2.98^{gh}	2.35^{ij}	3.46^{ef}	3.69^{ef}	2.58^{ij}	2.05^{jk}	3.17^{fg}	3.34^{ef}	2.67^{gh}	2.71^{gh}	<0.001	0.53
	Kakamega	1.76^{k}	0.64^{l}	5.08^{b}	3.52^{ef}	4.10^{cd}	3.14^{g}	3.71^{e}	2.82^{gh}	2.96^{gh}	3.67^{ef}		
	Siaya	2.16^{ijk}	0.50^{l}	3.56^{ef}	2.77^{gh}	4.11^{cd}	3.64^{ef}	4.50^{c}	3.49^{ef}	2.67^{gh}	2.59^{hi}		
Forage genotype × harvest	Napier	2.30^{gh}	1.19^{h}	4.85^{ab}	3.67^{ef}	3.19^{ef}	3.30^{ef}	3.05^{ef}	3.81^{cd}	3.98^{cd}	3.70^{ef}		
	Cayman	3.43^{ef}	1.10^{i}	3.98^{cd}	3.56^{ef}	3.23^{ef}	2.38^{g}	3.07^{ef}	3.04^{ef}	2.56^{g}	2.33^{g}		
	Cobra	2.68^{g}	1.05^{i}	3.97^{cd}	3.49^{ef}	3.72^{de}	2.77^{g}	3.21^{ef}	2.70^{g}	2.81^{ef}	2.33^{g}		
	Mulato II	1.15^{i}	0.76^{i}	3.24^{ef}	2.08^{gh}	2.74^{g}	1.99^{gh}	2.80^{ef}	2.34^{g}	2.57^{g}	2.59^{g}		
	Xareas	2.60^{g}	0.95^{i}	4.62^{cd}	3.95^{cd}	3.72^{de}	3.33^{ef}	3.63^{ef}	3.76^{cd}	3.49^{ef}	3.14^{ef}	<0.001	0.93
	MG4	2.61^{g}	0.82^{i}	4.71^{ab}	3.77^{cd}	3.69^{ef}	2.68^{g}	3.87^{cd}	3.03^{ef}	3.03^{ef}	2.59^{g}		
	Basilisk	3.62^{ef}	0.81^{i}	4.39^{cd}	3.64^{ef}	3.90^{cd}	3.39^{ef}	3.88^{cd}	3.29^{ef}	2.92^{ef}	2.84^{ef}		
	Piata	2.78^{fg}	1.00^{i}	4.76^{ab}	4.63^{cd}	3.49^{ef}	3.15^{ef}	3.71^{ef}	3.44^{ef}	3.18^{ef}	2.88^{ef}		
	Mombasa	2.70^{g}	0.95^{i}	4.00^{cd}	5.62^{a}	4.56^{cd}	4.12^{cd}	3.88^{cd}	4.11^{cd}	3.39^{ef}	3.39^{ef}		
	Tanzania	3.21^{ef}	1.00^{i}	3.90^{cd}	4.48^{cd}	3.47^{ef}	3.24^{ef}	3.76^{cd}	3.70^{ef}	3.59^{ef}	3.12^{ef}		
	Maasai	2.50^{g}	1.11^{i}	4.72^{ab}	5.64^{a}	5.46^{ab}	4.30^{cd}	4.68^{bc}	4.40^{cd}	3.64^{ef}	3.71^{ef}		

Site × forage genotype		Basilisk	Cayman	Cobra	Massai	MG4	Mombasa	Mulato II	Napier	Piata	Tanzania	Xaraes	*P*	lsd
	Bungoma	3.47^{cd}	3.87^{ab}	3.58^{cd}	4.13^{ab}	3.57^{cd}	4.07^{ab}	2.63^{ef}	3.89^{ab}	4.20^{ab}	3.87^{ab}	4.40^{a}		
	Busia	3.15^{cde}	2.97^{de}	2.96^{e}	3.68^{bc}	2.69^{ef}	3.42^{cd}	2.25^{g}	2.26^{fg}	2.57^{ef}	3.22^{cde}	2.72^{ef}	<0.001	0.65
	Kakamega	3.20^{cde}	2.77^{ef}	2.87^{ef}	3.92^{ab}	3.19^{cde}	3.31^{cd}	2.65^{ef}	3.46^{cd}	3.10^{cde}	2.88^{ef}	3.18^{cde}		
	Siaya	3.25^{cd}	1.85^{gh}	2.08^{g}	4.34^{a}	2.87^{ef}	3.89^{ab}	1.38^{h}	3.60^{cd}	3.34^{cd}	3.43^{cd}	2.98^{de}		

In Bungoma, Busia, Kakamega and Siaya counties in western Kenya in 2018–2021.
Means without common superscript within an attribute category differ significantly.

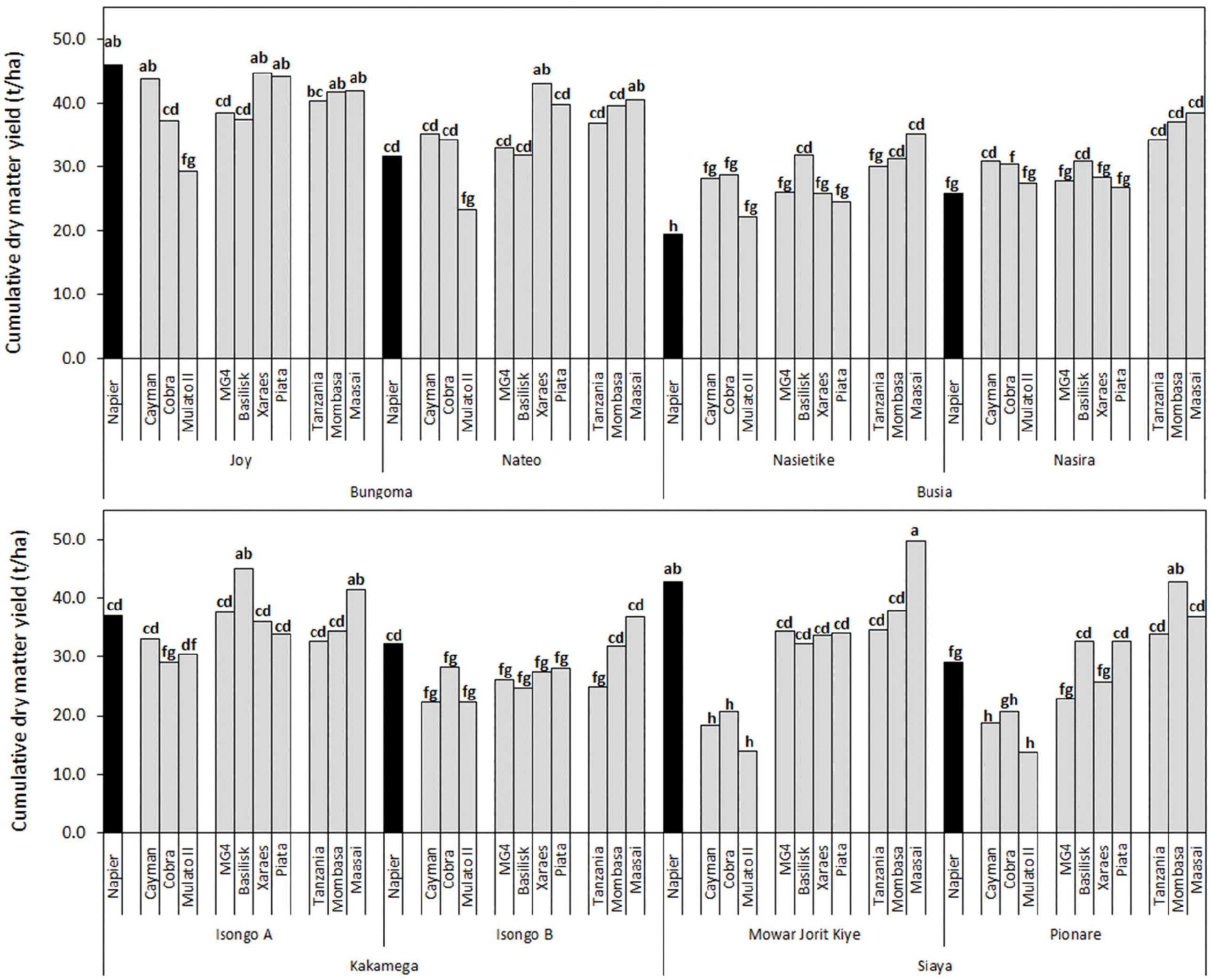

FIGURE 2 | Mean cumulative dry matter yield t/ha over 10 harvestings in 2 years, for 3 Urochloa hybrids, (Cayman, Cobra, Mulato II), 4 Urochloa cultivars (MG4, Basilisk, Piata, Xaraes) and 3 Megathyrsus cv (Mombasa, Tanzania, Maasai), compared to Napier grass in four counties, each with two farmer groups namely, Bungoma (Joy, Nateo), Busia (Nasietike, Nasira), Kakamega (Isongo A, Isongo B) and Siaya (Mowar Jorit kiye, Pionare) in western Kenya. Bars with different letter differ significantly $p < 0.05$.

significantly lower biomass (**Figure 2**). In this site, *Megathyrsus* cv Massai accumulated most dry matter significantly. In Siaya County and at Mowar Jorit Kiye farmer group site, *Megathyrsus* cv Massai accumulated the greatest biomass only similar to Napier grass but significantly greater than all the other grasses. The three *Urochloa* hybrids accumulated significantly low biomass than all the *Urochloa* cultivars, *Megathyrsus* cultivars, and Napier grass. In the second site of this county, *Megathyrsus* cv Mombasa accumulated greater biomass than all the other grasses, while the three *Urochloa* hybrids accumulated the least (**Figure 2**).

Plant Height, Leaf–Stem Ratio, Crude Protein, and Metabolizable Energy

Plant height significantly varied across counties and forage grasses (**Table 4**). Napier grass and Mulato II consistently had tall and short plants, respectively. However, the order was Napier grass > Mombasa > Tanzania > Massai > Xaraes > Basilisk ≈ MG4 > Piata > Cobra > Cayman > Mulato II. Leaf:stem ratio varied within and between counties. Across the counties, only Mulato II hybrid, Xaraes cultivar, and the three *Megathyrsus* attained leaf:stem ratio of 2. In Bungoma and Busia Counties, Mulato II attained the highest, *Megathyrsus* cv Mombasa in Kakamega, and *Megathyrsus* cv Massai in Siaya. The least leaf:stem ratio was by Napier grass in Bungoma and Basilisk in the other three counties.

CP yield (t/ha) varied across the grasses and within and between counties (**Table 4**). In Bungoma most of the grasses produced statistically similar CP yield including Piata, Massai, Mombasa, Tanzania, Xaraes, Basilisk, MG4, Cobra, and Cayman. Mulato II and Napier grass accumulated statistically low CP yield compared to Piata. In Busia County, there was a change in

TABLE 4 | Mean plant height (m), leaf to stem ratio, crude protein (t/ha), metabolizable energy (ME MJ/ha), and digestible organic matter (t/ha) for Napier grass, *Urochloa* hybrids (Cayman, Cobra, Mulato II), *Urochloa* cultivars (MG4, Basilisk, Xaraes, Piata), and *Megathyrsus* cultivars (Tanzania, Mombasa, Maasai) over 10 harvestings in 2019 and 2020 in western Kenya.

Attribute	County	Napier	Cayman	Cobra	Mulato II	MG4	Basilisk	Xaraes	Piata	Tanzania	Mombasa	Massai	*P*	lsd
Plant height (m)	Bungoma	1.23^{a}	0.40^{kl}	0.45^{ji}	0.27^{nop}	0.46^{jl}	0.46^{jl}	0.58^{hi}	0.50^{ij}	0.62^{fg}	0.67^{fg}	0.59^{hi}		
	Busia	0.80^{cd}	0.35^{mop}	0.37^{m}	0.30^{mop}	0.41^{jl}	0.45^{jl}	0.49^{j}	0.35^{mop}	0.56^{hi}	0.67^{fg}	0.60^{gh}	<0.001	0.09**
	Kakamega	1.00^{b}	0.35^{mop}	0.37^{m}	0.29^{mop}	0.38l^{m}	0.40^{kl}	0.51^{hi}	0.40^{kl}	0.56^{hi}	0.64^{fg}	0.55^{hi}		
	Siaya	1.14^{a}	0.34^{mop}	0.36^{mo}	0.26^{p}	0.50^{ij}	0.44^{jl}	0.51^{hi}	0.47^{jl}	0.78^{de}	0.71^{ef}	0.63^{fg}		
Leaf: Stem ratio	Bungoma	1.12^{g}	1.64^{de}	1.48^{ef}	2.20^{ab}	1.80^{cd}	1.17^{g}	1.99^{ab}	1.88^{cd}	1.90^{cd}	2.18^{ab}	2.12^{ab}		
	Busia	1.58^{ef}	1.95^{bc}	1.83^{cd}	2.28^{a}	1.99^{ab}	1.20^{g}	2.15^{ab}	1.78^{cd}	2.15^{ab}	2.13^{ab}	1.91^{cd}	0.007	0.30**
	Kakamega	1.25^{g}	1.37^{ef}	1.32^{fg}	1.84^{cd}	1.84^{cd}	1.12^{g}	2.01^{ab}	1.78^{cd}	2.15^{ab}	2.23^{ab}	1.90^{cd}		
	Siaya	1.15^{g}	1.62^{ef}	1.52^{ef}	1.85^{cd}	1.52^{ef}	1.05^{g}	1.87^{cd}	1.54^{ef}	1.72^{cd}	1.75^{cd}	2.05^{ab}		
Cumulative CP yield t/ha	Bungoma	3.28^{de}	4.07^{cd}	4.06^{cd}	2.99^{ef}	4.02^{cd}	3.75^{cd}	4.41^{cd}	4.74^{c}	3.64^{cd}	4.16^{cd}	4.59^{cd}		
	Busia	5.69^{abc}	4.46^{bcd}	4.54^{bcd}	2.83^{ef}	3.72^{cd}	5.36^{ab}	3.63^{cd}	3.94^{cd}	4.80^{bc}	4.74^{c}	4.29^{cd}		
	Kakamega	5.01^{ab}	4.18^{cd}	3.71^{cd}	4.32^{cd}	4.48^{cd}	6.00^{a}	3.87^{cd}	4.11^{cd}	3.49^{cd}	3.80^{cd}	4.92^{ab}	0.002	1.37**
	Siaya	4.44^{cd}	2.56^{ef}	2.78^{ef}	1.85^{f}	3.80^{cd}	4.29^{cd}	3.59^{cd}	3.85^{cd}	3.84^{cd}	4.79^{c}	6.23^{a}		
Cumulative Me MJ/ha	Bungoma	238778^{cd}	272997^{cd}	268066^{cd}	184435^{e}	260554^{cd}	255259^{cd}	327951^{ab}	326060^{ab}	275467^{cd}	293008^{c}	305066^{ab}		
	Busia	202349^{e}	234097^{cd}	229936^{cd}	169085^{ef}	213675de	236998^{cd}	204489^{e}	189494^{e}	250604^{cd}	293404^{bc}	288773^{c}	<0.001	71377.5***
	Kakamega	272373^{cd}	257685^{cd}	223583^{cd}	262288^{cd}	292982^{c}	359970^{ab}	270479^{cd}	261986^{cd}	238141^{cd}	253733^{cd}	309929^{a}		
	Siaya	322384^{ab}	145105^{ef}	165401^{ef}	110222^{f}	262374^{cd}	255720^{cd}	257754^{cd}	263601^{cd}	264696^{cd}	282990^{cd}	375988^{a}		
Cumulative digestible organic matter (t/ha)	Bungoma	17.02^{cd}	19.87^{cd}	19.46^{cd}	13.40^{ef}	18.84^{cd}	18.37^{cd}	23.58^{ab}	23.54^{ab}	19.75^{cd}	21.14^{cd}	21.88^{bcd}		
	Busia	15.09^{ef}	17.37cd	17.04^{cd}	12.33^{efg}	15.67^{ef}	17.95^{cd}	15.02^{ef}	14.12^{ef}	18.54^{cd}	21.46^{cd}	20.95^{cd}	<0.001	5.20***
	Kakamega	19.94^{cd}	18.89cd	16.39^{de}	19.18^{cd}	21.30^{cd}	26.28^{ab}	19.63^{cd}	19.10^{cd}	17.27^{cd}	18.43^{cd}	22.52^{ab}		
	Siaya	23.14^{ab}	10.61fg	12.07^{efg}	8.01^{g}	18.97^{cd}	18.56^{cd}	18.51^{cd}	19.00^{cd}	19.09^{cd}	20.69^{cd}	27.29^{a}		

Means with different superscripts within an attribute category are significantly different.
$^{**}P < 0.01$, $^{***}P < 0.001$.

the order. Napier grass produced the most that was statistically similar to those of Cayman, Cobra, Basilisk, Tanzania, Mombasa, and Massai. Only Mulato II, MG4, Xaraes, and Piata have statistically low CP yield compared to Napier grass. In Kakamega, cultivar Piata accumulated the most CP yield statistically greater than all the other grasses except for Napier grass and *Megathyrsus* cv Massai. In Siaya, *Megathyrsus* cv Massai yielded the most CP that was statistically greater than for all the other grasses (**Table 4**).

Cumulative ME yield (MJ/ha) varied cross the counties and among grasses (**Table 4**). In Bungoma, Xaraes accumulated the most that was statistically greater than all the grasses except for Piata and Massai. In Busia County, *Megathyrsus* cv Mombasa accumulated the most that was statistically greater than those of Piata, MG4, Mulato II, and Napier grass but similar to the other grasses. In Kakamega County, Basilisk accumulated statistically greater ME than all the grasses except *Megathyrsus* cv Massai. Hybrid Cobra produced the least in the county compared to other grasses. In Siaya County, *Megathyrsus* cv Massai accumulated statistically greater ME than all the grasses except Napier grass, while Mulato II produced the least.

On cumulative digestible matter in Bungoma County, Xaraes produced the most and statistically more than Napier grass and Mulato II (**Table 4**). Although Mulato II had the least, it was similar to that of Napier grass and Basilisk. In Busia County, the order was different. *Megathyrsus* cv Mombasa had the most digestible organic matter, statistically greater than those of Napier grass, Mulato II, MG4, Xaraes, and Piata. This was unlike in Kakamega County where Basilisk had the most and statistically greater than all the other grasses except for MG4 and *Megathyrsus* cv Massai. In Siaya County, *Megathyrsus* cv Massai accumulated the most and similar to Napier grass. The values for Mulato II were the lowest in this county and by 3.4 times compared to *Megathyrsus* cv Massai.

Participatory Evaluation

To connect biophysical performance of the grasses with end-users, we undertook farmers' participatory evaluation. Farmers from the counties and linked to the trial site's groups developed criteria that were closely related as follows. Nasietike from Busia identified disease tolerance, fast germination, fast regrowth, high germination rate, leafiness, more milk, softness, upright growth, drought tolerance, high biomass, and palatable as key considerations. This was similar for the other groups except Bungoma Joy group, which did not identify upright growth while Siaya's Mowar Jorit Kiye and Kakamega's Isongo B groups identified greenness that was not identified by Nasietike or Joy. Pooled ratings across the groups and by grass type varied (**Figure 3**). According to Nasietike group the order of preference emerged as Cayman >Xareas > Cobra ≈ Mombasa > Tanzania > Piata Massai ≈ Mulato II > MG4 > Napier ≈ Basilisk. For Joy group the order started the same as Nasietike for the first two but followed by interchange of the subsequent grasses. The order was Cayman > Xaraes > MG4 ≈ Mombasa > Piata > Basilisk > Cobra > Massai ≈ Tanzania > Mulato II > Napier. In Siaya by Mowar Jorit Kiye group the order sorted differently as Cobra ≈ Napier > Xaraes > Piata ≈ MG4 > Cayman > Massai > Mombasa > Mulato II > Basilisk > Tanzania. Kakamega by

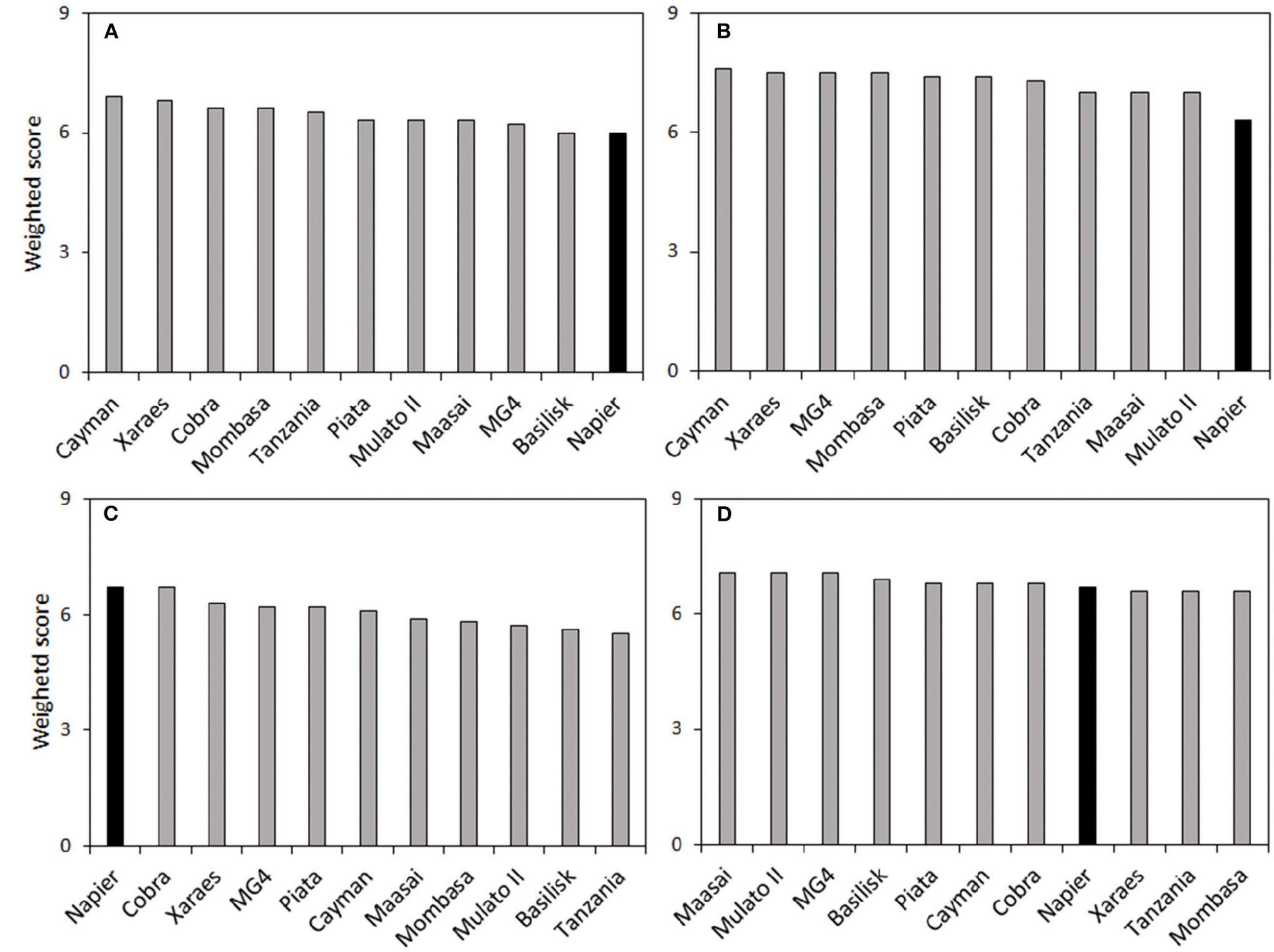

FIGURE 3 | Weighted Scores on 1–9 scale, where 1 = least important, 9 = most important against forage grass types for Nasietike farmer group **(A)**, Joy farmer group **(B)**, Mowar Jorit Kiye farmer group **(C,D)** Isongo B farmer group before third harvesting in 2019 in western Kenya.

Isongo B further presented a different order as MG4 ≈ Mulato II ≈ Massai > Basilisk > Cayman ≈ Cobra ≈ Piata ≈ Tanzania > Napier > Mombasa > Tanzania (**Figure 3**).

DISCUSSIONS

The overall objective of identifying performance of the different forages in different locations and engaging the end users was met. Indeed, in western Kenya with trial sites characterized by temporal and spatial differences, the sites equally showed variable performance (**Tables 2–4**) and farmers' ratings (**Figure 3**). The results present important information that would connect well with intensions of improving forage production in the region, to contribute to improved livestock productivity especially cattle under the smallholder mixed farming in the area. The importance of matching forage with biophysical environment and agricultural context is reported in previous research efforts (Tilman et al., 2011; Mwendia, 2015), and this work adds onto the basket of options toward this endeavor.

Dry matter yields realized in the study show the grasses and performance in the different sites. Clearly, a grass doing well in one location did not necessarily do so in another location. This is governed by grass genotype–environment interaction with environmental attributes including temperatures, soil type, and rainfall coming into play. Even within areas that are in close proximity, differences are likely to emerge because of transient conditions that may exist in one site and not the other. For example, while Napier grass at the Joy site in Bungoma accumulated significantly greater biomass than other grasses (**Figure 2**), this was remarkably reversed in Nateo site in the same county. The essence of placing the grass technologies in an agricultural context, therefore, serve to get the actual performance to inform recommendations, rather than providing generalized recommendations, but advise based on empirical evidence derived. As such, it would not be advisable to grow *Urochloa* hybrids in Siaya and other areas similar to the site, but the *Megathyrsus* or *Urochloa* cultivar stands a better chance. While Napier grass is the most grown fodder in the study counties (Khan et al., 2014), results here show that it does not produce well in Busia compared to the *Urochloa* and *Megathyrsus* varieties considered in this case study, indicating the latter two could successfully be used for livestock by producers in the area. However, in Joy and Mowar Jorit Kiye sites, Napier grass would be more advantageous especially on dry matter quantity than either Cobra, Mulato II, MG4, Basilisk, and all the other grasses except Massai for the two sites respectively. The suitability of the *Megathyrsus* and *Urochloa* grasses in the current study clearly emerged. Specifically, in Busia County, Cayman, Cobra, Massai, Tanzania, and Mombasa are better options than Napier grass, especially in Nasietike site. In Kakamega and similar ecologies to the study sites, Basilisk could be a grass of choice at Isongo A site and *Megathyrsus* cv Massai in Isongo B. Equally, Massai would also be a cultivar of choice at Mowar Jorit Kiye and Mombasa at Pionare site, both in Siaya County. Choice of cultivar could make a huge difference in bridging the forage quantity gap, which is often characteristic in intensified mixed smallholder systems in SSA (FAO, 2018). As observed in Busia, the cultivar Massai nearly doubled the biomass of Napier grass, which means providing roughage for nearly double the number of feeding days of Napier grass. Similarly, in Bungoma, Mulato II and Xaraes outperformed Napier grass (**Figure 2**). Any extra biomass production from the same unit of land is preferable, demonstrating improved resource-use efficiency, key especially in the face of global warming (Makkar, 2016). Extra feeding days for dairy producers translate into extra milk yield and a clear livelihood benefit.

While all the forage grasses in this study follow the C_4 photosynthetic pathway, being tropical grasses, their differences in performance could most probably be explained by physiology and/or adaptations that were not measured in the current study. For example, the grasses doing well in the relatively dry areas are likely to have better stomatal control when faced with limited soil moisture, exhibit osmotic adjustment, or may be accumulating greater root biomass to aid in nutrient and water exploration (Mwendia et al., 2013). Having greater leaf area index could also be beneficial in intercepting more light for photosynthesis and hence growth. Equally, some of the grasses have better nutrient and water use efficiency. This is an area worth investigating further in a physiological study to unravel key drivers responsible for the differences observed.

While plant height is positively correlated with biomass, and inversely with forage quality (Tessema et al., 2010), plant height also has implications especially where manual forage harvesting is practiced in smallholder farms (Mwendia et al., 2017a,b). For the 11 grasses evaluated, none exhibited prostrate growth habit, and all had upright tillers. Tall plants facilitate easier handling/grasping when cutting to the required stubble height. In this regard, Napier grass, the *Megathyrsus* and *Urochloa* cultivars, and the hybrids, in that order, would suit manual harvesting by farmers. However, there is a need to compromise and ensure forages are not allowed to overgrow as quality deteriorates. Although we did not report neutral detergent fiber, it is usually negatively correlated with organic matter digestibility (Roche et al., 2009); thus, the lower values for digestible organic matter (**Table 4**) suggest greater neutral detergent fiber.

Mulato II with slightly less than a foot height would be relatively difficult for proper hand grip during harvest, which may make it less attractive in smallholder cut-and-carry systems. However, Mulato II's leafiness, an attribute important in ruminants, as they select for leaves as opposed to stems (Mwendia et al., 2017b), is preferable. Short forages could fit better in systems where cattle graze directly without trampling that could lead to forage wastage/losses. Forage improvement, e.g., breeding, should therefore take into consideration the traits that fit under a given agricultural context as explained, in smallholder cut-and-carry systems.

Despite the low plant height for Mulato II, its great leaf:stem ratio compensates for its relatively low biomass yield, as most nutrients are in the leaves, and in effect the CP yield, ME, and digestible organic matter were similar to most of the grasses, e.g., in Kakamega and Bungoma. As such, Mulato II presents good quality also often a challenge in livestock production, and breeding for leafiness in forage would be preferable. While harvesting could pose a challenge to smallholder livestock producers dealing with Mulato II, its good quality should warrant investigating and devising cheap tools that could help in harvesting and make it friendly to grow.

The ratings by farmers (**Figure 3**) largely relied on what they could discern phenotypically, and it is interesting to note that this assessment is fully in line with the quantified physical and laboratory assessment. For example, in the Nasietike group from Busia where they ranked Cayman, Xaraes, Cobra, and Mombasa highly, we see that the same varieties also did well on leaf:stem ratio, plant height, CP and ME yields, and digestible organic matter (**Table 4**). This underscores the importance of including farmers' preferable traits in forage selection and breeding, to end with products that adapt to not only ecological niche but agricultural content under consideration. Participatory evaluation would indicate high chances of adoption, while good biophysical characteristics ensures that this adoption also has a positive impact on livestock productivity.

CONCLUSIONS

In situ evaluation of the forages revealed how the forages perform on biomass production, quality, and farmers' preferences. A mixed order of performance emerged from the study sites. While Napier grass is the prevalent forage grown across the study sites, evidence we show here reveals that there are alternative forage grasses that can be grown and provide great and quality roughages for ruminant production. In Siaya, which is relatively dry, the *Megathyrsus*, Napier grass, and *Urochloa* ecotypes are better suited. However, in Busia, Napier grass is least suitable with options of *Urochloa* hybrids (Cayman and Cobra) and the three Megathyrsus cultivars being better possibilities. All the grasses except Mulato II performed well in Bungoma, of which the farmers prefer Cayman, Xaraes, MG4, and Mombasa. In Kakamega, both the farmers' selection and agronomic performance indicate the virtuous grasses would be *Megathyrsus* cv Massai, *Urochloa* cultivars Basilisk and MG4, and *Urochloa* hybrid Mulato II. It is paramount that future forage selection and breeding take into consideration farmers' preferable traits in a given agricultural context. Following forage evaluation for 2 years and farmers' involvement, the inferences we believe provide a strong basis for practical implementation and promotion of the forages in the areas and by extension in other similar ecologies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

The authors participated in various ways regarding the work reported in the manuscript and they have all agreed the manuscript to be summited to Frontiers special issue on forages. SM designed and established the trials, analyzed data, and wrote the manuscript. RO participated in data collection and engaging the farmers. AJ identified the farmers groups discussed the trials and also engaged the farmers during the trials. DM participated in engaging farmers and observing agronomic measures in the trials and editing the manuscript. AN participated in design of the trial and engaging the partners during the trial as well as writing the manuscript. All authors contributed to the article and approved the submitted version.

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On (Dis)Connections and Transformations: The Role of the Agricultural Innovation System in the Adoption of Improved Forages in Colombia

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Feeding improvement strategies are key in increasing cattle productivity and reducing its environmental footprint. Nevertheless, Colombian tropical cattle systems still feature serious deficiencies in both forage quality and availability. As a result of past and on-going forage Research and Development (R&D) processes, institutions have released 23 grass and legume cultivars of superior characteristics in terms of forage quality, supply, or adaptation to different soil and climate conditions, while providing numerous environmental benefits. However, low levels of adoption are observed: although R&D processes are a necessary condition for adoption, they are still not sufficient to guarantee agricultural technification in Colombia. The ultimate success occurs only when end-users make effective use of a technology–a link constantly interrupted. Agricultural innovation requires complex processes of interaction in which knowledge is shared amongst organizations involved in the Agricultural Innovation System (AIS), namely: suitable links, attitudes, practices, governance structures, and policies. The objective of this study is to identify limitations and opportunities in R&D, adoption, and diffusion of forage technologies in Colombia from an AIS perspective. Particularly, we present a study case pertaining to research institutions only, to (a) map the involved actors and describe their roles and links, and (b) identify the events that marked the evolution of the AIS and the course of forage R&D in its research-related components. We applied a qualitative methodology based on focus group discussions, in-depth interviews, literature review, and historical analysis. Results show that the complex nature of institutions and the interactions between them determine the historical transformation of diffusion of forage technologies. The lack of connection between institutions and the weak intensity of the relationships, prevent the convergence of interests and objectives, leading to vicious cycles that hamper technology adoption. Insufficient synchronization between institutions of different nature (and even between those that share similar objectives) results in efficiency losses due to an unnecessary repetition of activities and processes. We provide recommendations for policy- and decision-makers that will help in both a restructuration of the AIS and a better allocation of funds for R&D, and thus support the development of more effective pathways for forage adoption and scaling.

Keywords: improved forages, research and development, AIS, technology adoption, sustainable intensification

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INTRODUCTION

It is no secret to anyone that the livestock industry is constantly growing and evolving. It is estimated that by 2027, the demand for livestock products will increase by 15.5% worldwide in response to population growth, urbanization and increased incomes in developing countries (OECD/FAO, 2020). It is also well-known that Latin America and the Caribbean at large hold an essential place and role in the livestock sector worldwide, as they contribute more than 25% of the production of beef and 10% of milk (CEPAL, 2017). This activity generates internal and external benefits, guaranteeing to a certain extent food security goals in countries, boosting their economies. This livestock trend in the region is not only historically traceable, but is projected into a promising future. According to the Inter-American Development Bank–BID (2018) and based on world population growth, it is projected that by 2050 meat consumption will increase by 100%, a scenario that would favor Latin American producers given its geographic location and access to human and natural resources. Hence, the supply response to this increase will be located mainly in developing countries (where forage-based systems predominate), according to the availability of resources and the possibilities of increasing productivity (OECD/FAO, 2020). Although historically larger livestock production numbers have been achieved in comparable periods (for example, it tripled between 1980 and 2002 according to Rajalahti et al., 2008), the context has now radically changed. There is a growing scarcity of natural resources (e.g., soil fertility, water and soil availability), as well as political pressure on the incorporation of better environmental practices. This constant political and social pressure seeks to promote actions aimed at reducing the environmental impacts of the livestock sector, being then the main challenge of tropical ranching to increase the efficiency of productive systems, mitigate the environmental impact, and advance in adaptative efforts in the advent of climate change. In addition to this, other impacts and improvements in the livestock industry and its actors become urgent, not only at the primary producer level (in terms of the promotion and implementation of sustainable intensification practices) (Rao et al., 2015), but also in the more equitable and environmentally sustainable value chain structuring processes, as they encourage the elaboration of differentiated products (Charry et al., 2019). Currently, a multiplicity of actors and sectors, political, economic, and academic, are promoting livestock agendas toward sustainability.

In a context of urgent reinvention and growing demand, the livestock industry finds it decisive to implement agricultural innovations, such as improved forages. The deficiencies in the quality of the forages appear as a constant in the tropical territories where cattle activity takes place (Peters et al., 2012). Improving said quality, as well as the availability of food, has been established as one of the key strategies to increase productivity and reduce the environmental footprint (Gerber et al., 2013; Herrero et al., 2013). Thus, and as a result of the Research and Development processes in Latin America (R&D) (some of which we address in this article), 26 cultivars have been released in Colombia, including grasses and legumes that have shown to have better characteristics in terms of quality, forage supply, adaptation to different soil and climate conditions, and various environmental benefits (Peters et al., 2012; Rao et al., 2015; Enciso et al., 2019).

However, and despite the fact that there is little evidence in this regard, low levels of adoption of these forage technologies have been observed (Shelton et al., 2005; White et al., 2013; Labarta et al., 2017). This shows, at least partially, that while research processes are a necessary condition, they are not sufficient to guarantee agricultural innovation. The success of R&D processes occurs when producers make effective use of technology, a link that still falters in the Colombian case. Globally, the impacts on adoption have been evaluated for less than half of the 118 million hectares (Mhas) documented to have improved forages (White et al., 2013). In the Colombian case, the national forage adoption rate is around 62% with respect to the total area in pastures in the lower tropics, being the varieties *B. humidicola* and *B. decumbens* (pastures introduced in the 70s) the most adopted (Labarta et al., 2017). Yet, many of these areas are in some state of degradation (IDEAM UDCA, 2015; Rincón et al., 2018). An adoption of <1% is estimated for the case of hybrids of the Brachiaria genus, as a result of the breeding work carried out by the International Center for Tropical Agriculture (CIAT) in Colombia (Labarta et al., 2017).

The analysis of forage technology adoption processes in Colombia indicate decisive elements in the understanding of the causal relationship between producers and their adoption behavior, but to date there are no explanatory studies that offer a macro perspective to understand the barriers in the access to technology and dissemination mechanisms (see Vera and Seré, 1989; Seré et al., 1993; Rivas and Holmann, 2004; and more recent approaches in White et al., 2013 and Labarta et al., 2017). Available literature has explained, to a certain extent, the factors that limit or promote the adoption of technologies from the perspective of the primary producer, delving into the socio-demographic characteristics of the unity, and the conditions of the enablers, such as access to credit and technical assistance (e.g., Lapar and Ehui, 2004; Jera and Ajayi, 2008; Dill et al., 2015). Some revised studies mainly describe adoption processes in regions of East Africa and Latin America, focusing on the identification of adoption factors mostly from a primary producer's perspective with both quantitative and qualitative approaches. Although still privileging primary producer's perspectives, qualitative studies have done more to document and unveil the experiences and lessons learned related to the adoption of improved forages, taking analysis one step further (e.g., Reiber et al., 2013; Gil et al., 2015; Ashley et al., 2018). Although theoretically and methodologically vital, here we point out that these studies lack deeper perspectives that allow historical decision-making, and thus highlight the complex relationships between agents and institutions that participate in the adoption and diffusion of agricultural technologies. Although it is undeniable that the scientific and research sector plays a fundamental role in the creation of technologies that help to increase productivity, mitigate the effects of climate change, and improve the quality of life of small producers (especially when working in partnering with the public sector and non-governmental organizations), these investments turn out to be insufficient to enable agricultural innovation. This process requires the existence of broader competencies, links, enabling attitudes, practices, governance structures, and policies that facilitate the productive use of the knowledge generated (The World Bank, 2006). This comprises the set of all organizations and people (public and private) involved in the generation, dissemination, adoption, and social and economic use of new agricultural technologies (The World Bank, 2006; Hambly et al., 2012). The network formed in this process, and the conceptual lens of this study, is called the Agricultural Innovation System (AIS).

The AIS approach recognizes that innovation is a dynamic and complex process of interaction between different activities, actors and relationships associated with the creation and transmission of innovation to its productive use (The World Bank, 2006). This approach recognizes the role of actors, markets, institutions, political contexts, and networks in the adoption of new technologies and, therefore, in the evolution of innovation in a system (Rajalahti et al., 2008). Different authors have used the AIS approach as a framework to identify conditions that limit or promote the adoption of technologies in the rural sector (e.g., Spielman et al., 2011; Kebebe, 2018). Among the factors commonly mentioned are: (1) the scarce presence of public policies on innovation and agriculture; (2) problems related to asymmetries in communication; (3) weak links and lack of trust between actors; and (4) norms and cultural attributes of society that impede development and innovation processes, as well as behaviors, practices and attitudes that condition the roles and interactions between actors.

Taking into account the comprehensive nature of the AIS approach, the objective of this study is, through the use of it, to identify limitations and opportunities in the process of development, adoption, and diffusion of forage technologies in Colombia for the case of the actors related to the research/science component. For this we have decided to integrate qualitative approaches when addressing the phenomenon, with the intention of providing a detailed analysis that addresses the nature of inter-actor relationships and the contingencies that determine their transformations. To do so, we rethink the processes of adoption and diffusion of forage technologies through a historical perspective, highlighting the variables and actors that participate in said processes. In addition to highlighting the importance and delving into the investigative component, this article identifies some of the main events that have directed the course of research and dissemination of forage technologies in the country; and maps the actors that are part of the innovation system, describing their roles, links and attitudes, and the way in which they have catapulted or hindered forage innovation processes.

As mentioned before, the network formed in this process is called the Agricultural Innovation System (AIS), a network of actors and institutions that we are just beginning to elucidate. Thus, identifying the limitations and opportunities in the process of development, adoption and diffusion of forage technologies in Colombia implies an understanding of the dynamics that shaped inter-institutional relations, as well as their internal functioning mechanisms. This document is then structured as follows: first, it specifies the methodological tools used for the analysis. Subsequently, it delves into the historical context that has directed the course of research and dissemination of forage technologies in the country, laying the foundations for the analysis. Thirdly, links and levels of influence between the different actors and institutions of the research component are analyzed and mapped. The last two sections expose the bottlenecks and main obstacles that stand in the way of the proper development of the innovation and diffusion processes in general, and provide some ideas on future steps to follow in the matter.

MATERIALS AND METHODS

In order to identify the factors that limit or promote the development, diffusion, and adoption of forage technologies in Colombia, this study used qualitative methodologies, including: literature review, focus groups, and in-depth interviews. The Net-map tool was used to identify actors, their roles and importance in the AIS. The qualitative data generated was analyzed using the following tools: (i) transcription of interviews and focus group meeting; (ii) coding and categorization of key aspects; and (iii) interpretation of the information. The analytical purpose of the instruments used is explained in detail below.

Net Map Tool

Net-map is a participatory mapping research method developed by Schiffer (2007), and has been applied in different agricultural research problems to analyze networks and power dynamics in the promotion of technologies (e.g., Aberman et al., 2015; Ilukor et al., 2015; Daum and Birner, 2017; Lubungue and Birner, 2018). In the present study, the application of the tool was carried out through a focus group session, made up of five participants (active researchers from CIAT's Tropical Forages program), in-depth interviews, and a review of secondary sources. The application of this tool was directed to the research component of the AIS of forage technologies. Thus, the following objectives were proposed for the focus group discussion: (i) identify the actors that are part of the AIS in forage technologies at the national level, and (ii) describe the roles, links, and attitudes of the agents involved in the activities of the AIS.

The Net-map process was divided into two main activities. First, the participants identified the main people, institutions, and organizations that participate in the process of development, dissemination, and adoption of forage technologies in Colombia. Each participant wrote the name of the identified actors on separate cards (one actor per card), also writing down information about the role they play within the process and their level of influence in the AIS. The latter was defined as the actor's ability to influence the specific problem. The measurement of the level of influence was established using a Likert-type scale from 0 (no influence) to 4 (greater degree of influence). At this point, it should be mentioned that the participants in the group session are part of the population under study, and each one has experienced the process from different perspectives. For this reason, different colored cards were assigned to each participant, in order to identify the responses of each one. Next, the cards were collected and grouped according to the different components and distributed on a sheet of paper. During this activity, various questions for discussion and reflection were generated among the participants, related to the absence of actors in some component, and the divergences between roles and influences presented by the participants.

Second, the links, influences, and attitudes of the actors identified in the previous activity were identified. In this section, an open discussion was held among the participants, based on the following questions posed by the facilitator: which of the identified actors have any link to each other? What is the direction of the link (one-way or two-way)? What is the type of exchange (information flow, use of resources, planning, training, etc.)? And what is the strength of this relationship (weak, medium, strong)? According to the response of the participants, arrows were drawn, indicating the existence of a relationship and its characteristics. In the development of this activity, various discussion questions were generated associated with the characteristics of the relationships perceived between the actors, about the attitudes and practices that have restricted and/or promoted the interaction, and about the possible limitations that may have hindered or restricted the linking activities between the different actors. The full program of the focus group session and an implementation guide for the facilitator are presented in the **Supplementary Material**.

In-depth Interviews

Based on the focus group session and the review of secondary sources, some of the different actors that are part of the AIS were identified, which belong to various components. This information was organized in a spreadsheet, grouping the actors according to their membership in each component. Based on this information, the people to interview were selected, according to their experience and relevance within the processes of development, dissemination, and adoption of improved forages. The in-depth interviews (12 in total) were conducted between September 2019 and March 2020. Of these interviews, six focused on relevant actors in agricultural research (CIAT, Agrosavia, CIPAV), five on private sector agents (PAPALOTLA, ALQUERIA, MATSUDA, SEMILLANO, SAENZ FETY) (to understand their relationship with the research component and its actors) and a relevant actor in the field of politics in agriculture in Colombia that offered a general panorama on the matter (ICA). The interviews followed a logical format of open questions, each one lasting ∼1.5 h. For each interview, between 5 and 7 questions were selected from a comprehensive guide that included relevant topics for this research, previously carried out by the authors. This guide contains a general list of questions that are grouped into the following categories: (i) roles, attitudes, and practices, (ii) historical moments, (iii) patterns of interaction between actors, (iv) facilitating environment, and (v) gender inclusion. The selection of the questions was made according to the profile of each actor to be interviewed, prior to the interview. Six of the 12 interviews were conducted remotely, and the remainder in person.

Literature Review

Regarding secondary sources, long-standing studies were integrated on the establishment of livestock in Colombia and the continuous state and private searches to promote through the use of selected pastures) a productive and extensive and continuous livestock sector throughout the Twentieth century (1900–2000). This selection was focused in the existing literature regarding livestock, livestock practices in Colombia and Latin America at large, and improved forages. Our query included reports published by research institutions, peer-reviewed articles and databases. The search included documents published from 1980 to 2020. Conducting in-depth interviews allowed the integration of issues related to the change of research institutions and agendas, while delving into the gradual transformation of social relations that determine the course of research programs and projects. Choosing as informants subjects with a long history in their respective institutions enabled us to obtain a more precise overview of the changes over time of the institutions and professionals linked to the research field in livestock.

CONTEXTUAL AND HISTORICAL FRAMEWORK

Scientific literature conceptualizes improved forages as species that present superior agronomic characteristics compared to native forages and that, in addition, adapt to the agroecological conditions of a given region (Shelton et al., 2005; White et al., 2013; Labarta et al., 2017). These forages are the result of improvement processes, which may include: (i) selection of materials from germplasm banks according to a previous evaluation of visual characteristics, adaptability, forage production, seed, nutritional quality, and animal response (e.g., *Brachiaria*, *Megathyrsus*, *Cenchrus*, *Leucaena*, *Cratylia*, *Arachis*, among others); and (ii) genetic improvement of a material in which desirable characteristics of the parents are combined (e.g., *Brachiaria* hybrid CIAT 36061 cv. Mulato I, *Brachiaria* hybrid CIAT 36087 cv. Mulato II, and *Brachiaria* hybrid CIAT BR 02/1752 cv. Cayman). In general terms, the process of multiplication and diffusion of the seeds/vegetative materials of varieties already formally released, usually follows two routes: formal and informal.

In the formal route, cultivars are developed by a national research institution (e.g., Agrosavia) or private company (e.g., Papalotla) based on a release proposal (breeding by selection or plant breeding). Some materials in this group are: *Brachiaria brizantha* cv. Toledo, *Brachiaria humidicola* cv. Humidicola, *Arachis pintoi* cv. Forage Mani. Under this route, 26 cultivars have been released in Colombia, mainly for low-tropical conditions. In **Table 1,** we present a list of the total improved forages released in Colombia. On the other side, in the informal route, the cultivar is introduced to the country by an individual and/or national seed company which initiates the distribution and/or dissemination. As an example, there are materials in commercial use such as: *Decumbens* grass (*Brachiaria decumbens* CIAT 606), Tanzania 1 grass (*Megathyrsus maximus* CIAT 16031), Maralfalfa grass, Guinea Massai grass (*Megathyrsus*

TABLE 1 | Forage species released in Colombia.

Region		Genus and species	Accession	Variety name	Year of release	Releasing institution	Adoption registration year	Adoption rate (%)	Commercialization
Gramineae	Lower tropics (0–2,000 m elevation)	*Brachiaria brizantha*	CIAT 26646	La libertad	1987	ICA	2016	2.8	No
			CIAT 26110	Toledo	2002	Corpoica	2016	1.24	Yes
			CIAT 26124	Caporal	2021	Corpoica	N.D	ND	No
		Brachiaria humidicola	CIAT 679	Pasto humidicola	1992	Corpoica	2016	22.6	Yes
			CIAT 6133	Llanero	1987	ICA	2016	8.15	Yes
		Brachiaria hibrido	CIAT 36061	Mulato	2003	Papalotla	2016	0.05	Yes
			CIAT 36087	Mulato II	2005	Papalotla	2016	0.03	Yes
			BR02/1752	Cayman	2013	Papalotla	N.D	N.D	Yes
		Sorgo forrajero JJT-18		Sorgo dulce Corpoic JJT-18	2014	Corpoica	N.D	N.D	No
		Andropogon gayanus	CIAT 621	Carimagua 1	1980	ICA	N.D	N.D	No
		Megathyrsus maximus	CIAT 6799	Agrosavia sabanera	2018	Agrosavia	N.D	N.D	No
Fabaceae	Lower tropics (0–2,000 m elevation)	*Arachis pintoi*	17434	Mani forrajero	1992	Corpoica	2016	0.1	Yes
			22160	Centauro	2020	Agrosavia	N.D	N.D	No
		Centrosema acutifolium	5277	Vichada	1987	ICA	N.D	N.D	No
		Cratylia argentea	CIAT 18516+18668	Veranera	2002	Corpoica	N.D	N.D	No
		Desmodium heterocarpon	13651	Maquenque	2002	Corpoica	N.D	N.D	No
		Leucaena leucocephala	21888	Romelia	1992	Cenicafe	N.D	N.D	No
		Stylosanthes capitata	10280	Capica	1983	Corpoica	N.D	N.D	Yes
		Vigna unguiculata		Sinu		Corpoica			
Avena	Higher tropics	ICA Bacatá			1963	ICA	N.D	N.D	No
		ICA Soracá			1965	ICA	N.D	N.D	No
		ICA Gualcalá			1968	ICA	N.D	N.D	No
		ICA Cajicá			1976	ICA	N.D	N.D	Yes
		Avena Obonuco Avenar			2003	Corpoica	N.D	N.D	Yes
		Avena Forrajera Altoandina			2018	Agrosavia	N.D	N.D	No

Own elaboration based on Peters et al. (2011), Labarta et al. (2017), and expert consultation and information provided by seed distributors. ND: no data available. Note: In 1992, ICA was restructured and the research activities passed to the newly created Corpoica; Corpoica is now called AGROSAVIA.

maximus cv. Massai), *Stylosanthes* cv. Campo Grande (Mix between *Stylosanthes capitata* and *Stylosanthes macrocephala*), Pennisetum cv. Cuba 22, and *Pennisetum* cv. Clone 51.

At the national level, we find that there is an adoption level of 34.97% of fodder released under formality channels. Of this percentage, 34.89% corresponded to introduced species of the genus *B. humidicola* and *B. brizantha*; introduced ∼30 years ago (Labarta et al., 2017). In relation to hybrid forages (Mulato I and Mulato II) an adoption level of 0.08% was registered (Labarta et al., 2017), while the varieties released informally such as *B. decumbens, M. maximus* cv. Tanzania, and cv. Mombaza report an adoption percentage of 0.98, 0.29, and 1.61%, respectively (Labarta et al., 2017).

Different studies have carried out, during the last nine decades, documentations of the benefits and costs associated with the adoption of improved forages (see **Table 2**). These studies show the potential of improved forages to improve animal production and contribute to the sustainability of production systems at different scales. In particular, CIAT developed the LivestockPlus concept, demonstrating how the introduction of improved forages in the tropics can lead to sustainable intensification, producing multiple social, economic, and environmental benefits (Rao et al., 2015). These benefits are mainly associated with the increase in the availability and quality of pastures, which results in better indicators of animal development, productivity, and profitability of the livestock activity. In addition, improvements in the quality of feed allows improving the ruminal fermentation process and, therefore, reducing greenhouse gas (GHG) emissions, and achieving greater intensification of the livestock activity (Oliveira et al., 2007; Hristov et al., 2013). It is necessary to clarify that these potential benefits of the use of improved forages depend on the appropriate agroecological and management conditions.

The introduction of technologies to improve the livestock sector has taken place for more than a century (Van Ausdal, 2012). Between 1850 and 1950, the nascent cattle ranchers of Colombia made significant efforts to improve their agricultural practices through the introduction of new breeds and bovine crosses, the improvement of fences and farm care, as well as the introduction of Africanized pastures [e.g., Pará (*Brachiaria mutica*), guinea (*Panicum maximum*)], among others (Rao et al., 1998; Rincón et al., 2010). Since the introduction of pastures of the Brachiaria genus, there has been a rapid and sustained growth of grazing areas in the country: by 1900 there were already two million hectares sown in Pará and Guinea, and by 1958 this number amounted to 10 million, this is, one third of the grazing land of the entire national territory (Van Ausdal, 2012). Said dissemination and adoption processes were spontaneous and massive, they did not follow established guidelines or regulations. They obeyed, rather, to the commercial need to establish a solid industry (especially meat) with an export industry that was never consolidated (Rao et al., 1998; Rincón et al., 2010; Van Ausdal, 2012; Ponce de León-Calero, 2019).

Two historical moments stand out as decisive in regards to R&D processes: the so-called "green revolution" and the advent of neoliberal economic policies in developing Latin American countries (Lynam and Byerlee, 2017). The first moment took place between the 1960s and 1970s, and was marked by an increase in agricultural investment and marked concerns about productivity and quality of life in rural settings, triggered by the need to promote agricultural development in a world increasingly unequal caught up in the political ups and downs of the Cold War (Lynam and Byerlee, 2017; Ponce de León-Calero, 2019). The flourishing and consolidation of programs such as CIAT's Tropical Forages and Agrosavia (Colombian Agricultural Research Corporation former CORPOICA, in Colombia) are also highlighted here, which shows a growing multilateral interest in promoting agricultural innovation processes (Lynam and Byerlee, 2017).

The second moment is framed by the political and economic agendas of Latin American governments (including Colombia) at the beginning of the 1990s, within the framework of neoliberal transformations and economic flexibility (Tirado-Mejia, 1997; Palacios and Stoller, 2006; Van Ausdal, 2012; Ponce de León-Calero, 2019). Previously solid institutions dedicated to research (such as Agrosavia) underwent important restructuring processes due to budget cuts limiting their research possibilities, the continuation and monitoring of ongoing projects and adequate and complete process of technological diffusion. The changes and contingencies experienced by institutions such as Agrosavia show that, as far as agricultural research processes and actors are concerned, continued state funding is necessary. From the interviews carried out with the actors in agricultural research circuits, we were able to establish the causality between state funding and the success or continuity of research programs, as several of the interviewed informants narrated the processes of transformation and historical decline of their scientific agendas because of budget cuts. Untimely budget reductions, as well as the relegation of investigative processes to second place, have undoubtedly been determining factors for efficient dissemination processes, thus affecting the viability of adoption processes. It should be noted that since the 1980s the national research institution Agrosavia has released new forage species, grasses, and legumes, previously evaluated by CIAT. Among these, the cultivars of *Brachiaria dictyoneura* (cv. Pasto Llanero, 1987), *B. brizantha* (cv. La Libertad, 1987), and *B. humidicola* (cv. Humidicola, 1990) stand out. Likewise, the creation in 1979 of the International Tropical Pasture Evaluation Network Foundation (RIEPT) stands out as a fundamental milestone to promote research in the subject and discuss the use of methodologies for evaluating forage technologies (Lynam and Byerlee, 2017). The existence of the RIEPT originated an invaluable database of forages studied and analyzed in detail and allowed the distribution of germplasm among researchers dedicated to the matter, materializing the advances of their research and strengthening institutional relationships between various groups and scientific niches (Lynam and Byerlee, 2017).Below are explained in more detail (i) key processes and their influence on the R&D processes of forage technologies in Colombia and (ii) the agents of the process and their respective interactions.

TABLE 2 | Benefits and costs of improved forages.

Benefits and costs		Effects at different scales			References
Direct benefit	**Impact**	**Farm**	**Regional**	**Global**	
Increment in the availability and nutritional quality of forage	Increment in milk and beef production				Rincón et al., 2010; Rao et al., 2014, 2015; Maass et al., 2015
	Higher number of animal heads per unit area	✓	✓		
	Better productive parameters of animal development (e.g., mortality and birth rate)				
	Social impact: improvement in income, food security and nutrition.				
Reduction of enteric methane emissions (CH_4)	Reduction of GHG emissions per unit of livestock product, given the improvement in feed efficiency. Mitigation and adaptation to climate change				Oliveira et al., 2007; Hristov et al., 2013; Peters et al., 2013; Herrero et al., 2016
Increase in atmospheric nitrogen (N) fixation (legumes)					Shelton and Dalzell, 2007; Reckling et al., 2016
Carbon (CO_2) accumulation in the soil					Oliveira et al., 2007; Soussana et al., 2010; Peters et al., 2013; Rao et al., 2015
Reduction of nitrous oxide (NO_2) emissions, associated with Biological Nitrification Inhibition (BNI)			✓	✓	Subbarao et al., 2009, 2017; Moreta et al., 2014; Karwat et al., 2017; Nuñez et al., 2018
Improvement of soil quality indicators	Improvement of the biological conditions of the soil (increase of biological indices of diversity of micro and macro fauna)				Rousseau et al., 2013; Lavelle et al., 2014; Rao et al., 2015
	Improvement of the physical conditions of the soil (reduction of erosion, compaction, and apparent density)				
Costs					
Establishment of materials (increased use of inputs, labor, equipment)		✓			Carey and Zilberman, 2002; Pannell et al., 2006
New knowledge and skills to maintain the technologies					Thomas and Sumberg, 1995; Lapar and Ehui, 2004
Development of appropriate extension and training packages			✓		Reiber et al., 2013
High perceived risk/uncertainty of technology		✓			Marra et al., 2003

Own elaboration based on the references mentioned.

RESULTS

Mapping of Actors

The information collected shows that the AIS in Colombia for improved forages includes actors from both public and private sectors. **Table 3** presents the list of actors and functions of the AIS for improved forages in Colombia, according to secondary sources, the focus group, and interviews. These actors can be grouped into the following six main components: (i) Politics; (ii) R&D; (iii) Extension, training and information; (iv) Supply of seeds; (v) Financing, and (vi) Primary producer. Each organization can fulfill one or different functions within the system: generation of knowledge, coordination, supervision and control of dissemination processes, bridging, or intermediary institutions, generation of spaces for the articulation of actors, or support structures at the institutional and political level (**Figure 1**).

The component of R&D consists of a total of 11 institutions dedicated to research on tropical forages. It includes national, regional, international and private research institutions. Among national research, Agrosavia, is the main public organization dedicated to research in the sector. It has 13 regional research centers (CIR) spread throughout the country, as well as offices in 10 locations. Of the total number of Agrosavia centers, eight include livestock and forages within their research lines. Mainly, Agrosavia has had a great impact on the development and release of new forage materials through the evaluation and selection of germplasm. At the international level, the Tropical Forages program of the International Center for Tropical Agriculture (CIAT) stands out for its role in the development of plant breeding hybrids, evaluation of materials, and the promotion of concepts of sustainable intensification through improved pastures. Likewise, CIAT has one of the largest collections of forage accessions in its germplasm bank, estimated at 22,694 accessions (from 75 countries). Historically, both CIAT and Agrosavia were identified as vital agents and leaders within the improved forage development processes. At the regional level, public universities have played a fundamental role both in the evaluation of technologies and in their application and promotion, through specific scaling-up projects. Among these,

TABLE 3 | List of actors and functions of the AIS for improved forages in Colombia.

Component/Category actor			Functions															
			F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
Policy	Ministry of Agriculture and Rural Development (MADR)		x	x	x	x	x						x				x	
	Ministry of Environment and Sustainable Development (MADS)			x	x													
	Colombian Roundtable for Sustainable Cattle (MGS-Col)		x		x	x												
	Colombian Agricultural Institute (ICA)			x	x		x											
	Rural Agricultural Planning Unit (UPRA)				x		x											
	Departmental Agriculture Secretariats				x	x	x						x					
Research and development	National research	The Colombian Agricultural Research Corporation (Agrosavia)				x		x	x	x	x							
		Center for Research in Sustainable Systems of Agricultural Production (CIPAV)				x		x	x	x	x							
	International research	International Center for Tropical Agriculture (CIAT), Tropical forages program				x		x	x		x							
		The Tropical Agricultural Research and Higher Education Center (CATIE)				x			x		x			x				
	Regional research	University of Cauca- Research group NUTRIFACA				x			x		x							
		National University of Colombia				x			x		x							
		University of Antioquia- Agricultural Sciences				x			x									

(Continued)

TABLE 3 | Continued

Component/Category actor			**Functions**															
			F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**F16**
		University of Llanos- Research group in Agroforestry				x			x									
		University of Nariño-FISE PROBIOTEC				x			x									
		University of Córdoba- Research group in tropical animal production				x			x									
	Private research	Papalotla							x	x	x							
Extension, training, and information	Colombian Cattle Federation (FEDEGÁN)				x	x					x				x			
	Agricultural extension	Municipal Units for Technical Assistance in Agriculture (UMATAs)											**x**					
	Training and education	National Training Service (SENA)											**x**	**x**				
		Private sector (e.g., Nestlé, Alquería, Alpina) and Outreach initiatives (e.g., Sustainable Colombian Cattle Project)												**x**				
	NGO's	Food and Agriculture Organization of the United Nations –(FAO)									**x**		**x**					
		GANSO									**x**		**x**					
Seed supply	Papalotla Colombia SAS								x	x	x	x			x		x	

(Continued)

TABLE 3 | Continued

Component/Category actor			Functions															
			F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**F16**
	Sáenz Faety, Impulsores Internacionales, Semillas & Semillas, Agrosemillas, among others											x			x			
	Seed producers in Brazil (e.g., EMBRAPA)							x	x			x						
Financing	Financial services	The Colombian Fund for the Financing of the Agricultural Sector (FINAGRO)														**x**		
		Banco Agrario of Colombia														**x**		
		Private banks														**x**		
		Producer associations and cooperatives														**x**		
		Informal credit														**x**		
	R&D financing	Ministry of Agriculture and Rural Development (MADR), Grupo Papalotla, high-income countries and international agencies, donors															**x**	
Primary producer	Producer associations and cooperatives										**x**		**x**	**x**				**x**
	Individual cattle producers										**x**							**x**

F1 Promotion of spaces for articulation, coordination and integration of actors.
F2 Design of regulatory and normative frameworks.
F3 Execution and supervision of public policies.
F4 Design and execution of programs and/or projects.
F5 Coordination, supervision and control within the dissemination processes.
F6 Technology development.
F7 Technology assessment (at the experimental level).
F8 Technology release.
F9 Promotion and demonstration of technology.
F10 Technical advice and information.
F11 Extension and/or agricultural technical assistance.
F12 Training and certification of labor competencies.
F13 Seed multiplication and/or distribution.
F14 Financial services.
F15 Research and development financing.
F16 Demand and use of technology.

Source: Own elaboration.

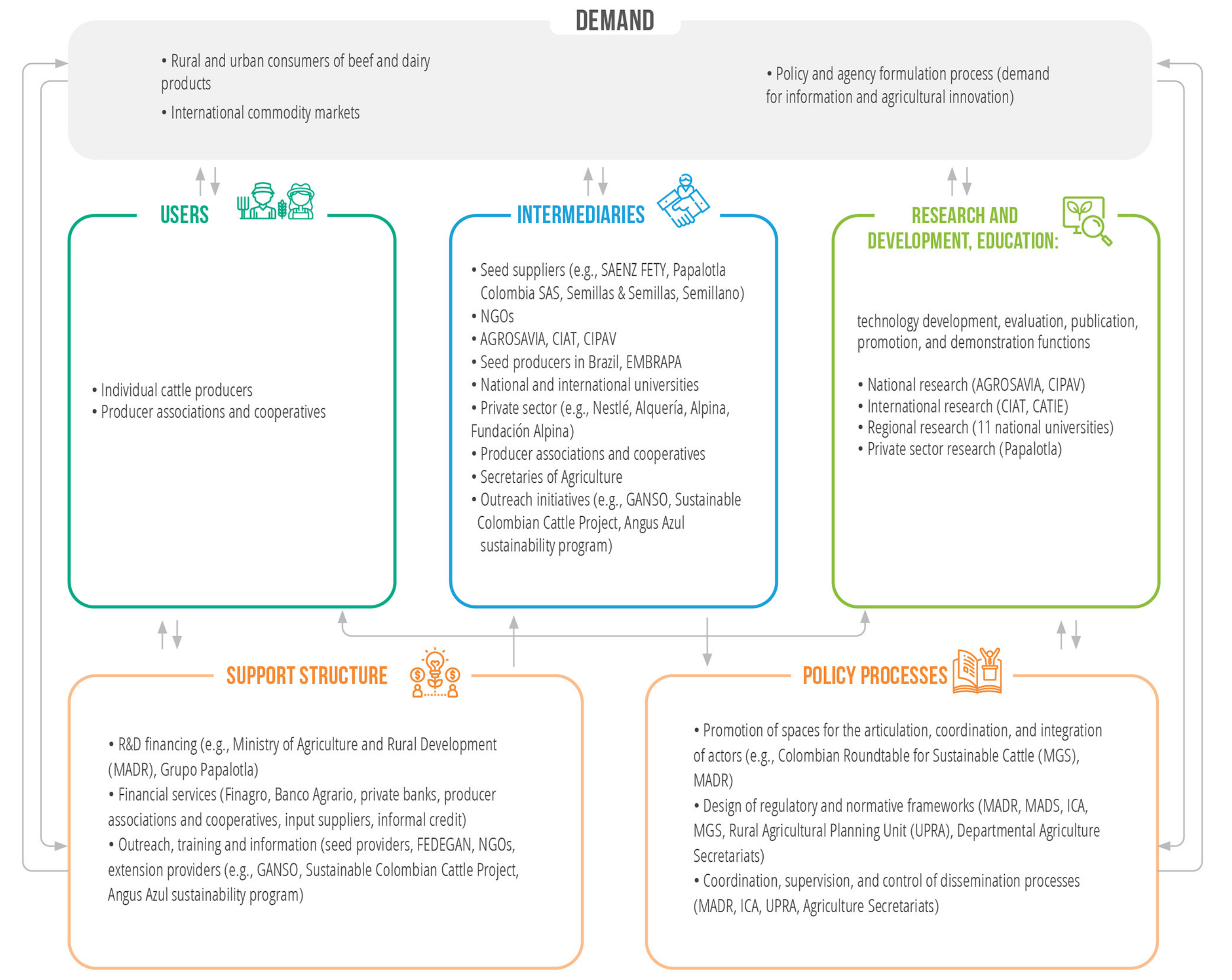

FIGURE 1 | Main agents of AIS for improved forages in Colombia.

the following stand out: The National University of Colombia and the University of Nariño (research conditions of the high tropics).

Bridging organizations or intermediaries, in particular, extension and training services, seed supply, and producers' organizations, facilitate interaction and/or link knowledge generation of R&D agents with users of technologies. Extension services for agricultural production in Colombia go back to the 1950s. At that time, the international trend for the creation of agricultural research institutes and extension services began to grow. From that moment, rural extension services have been through important transformations and organizational arrangements toward a decentralized technical assistance at the territorial level. Currently, the national technical assistance has a framework in the law 1876 of 2017 and the guidelines for the formulation of departmental plans of agricultural extension (PDEA, as per its acronym in Spanish). PDEA are regulated by the Ministry of Agriculture and Rural Development (MADR, as per its acronym in Spanish) in the resolution 407 of 2018. According to these guidelines, there are key stakeholders for delivering extension services such as local units of technical assistance for agricultural production (also known as UMATAs, as per their acronym in Spanish), provincial centers of agrobusiness management (CPGA, as per their acronym in Spanish), the national service for vocational education (SENA, as per its acronym in Spanish), professional associations of the sector, unions, associations, and community-based organizations.

Regarding the national seed supply of improved pastures, it is carried out by commercializing companies that import seeds from Brazil, Mexico, the United States, and Canada (comparative advantages from geographical conditions). **Figure 2** shows the network of importing and exporting companies of improved forage in Colombia. These companies can be divided in two

FIGURE 2 | Network of companies that import and export improved forage seeds in Colombia. Own elaboration based on trade statistics from Legiscomex (2020). Note. The blue nodes refer to the importing companies, and the yellow nodes refer to the exporting companies. The size of each node represents the level of participation regarding to the total in imports and exports, respectively.

groups: importers of introduced varieties and importers of hybrid varieties. The market of introduced varieties has a share of the 98% of all seeds commercialized nationally. This group is comprised of 27 companies. The most relevant are SAENZ FETY, Impulsores Internacionales, and Semillas & Semillas with a market share of 20, 15, and 10, 1%, respectively. These companies commercialize and distribute varieties from Brazil. For low tropics conditions (mainly the species *Brachiaria* and *Panicum*) and for high tropics conditions (mainly varieties such as Ryegrass, Alfalfa, Festucas, Pasto Azul, and clover) sourcing from the United States and Canada. The second group refers to the market of hybrids, still under development with a share of <2% of all commercialized seeds nationally. In this group, from 2017, the company Papalotla Colombia SAS imports and directly distributes through sales advisors and authorized distributors. Direct presence of Papalotla has increased the market of hybrids since 2017. They import hybrid grasses from Semillas Papalotla in Mexico and Brazil. The nationally commercialized seeds are *Brachiaria* hybrids cv. Cayman and Mulato II, with a share of 75% (32 tons in 2018) and 25% (10 tons in 2018), respectively (Rosales and Papalotla, 2019, personal communication).

All stakeholders are influenced by a context of agricultural policy, institutions, and informal general practices that might support or limit innovation processes. Stakeholders here recognize the role of the MADR for its relevance in the formulation, coordination, evaluation of agricultural and rural development policies, sustainable livestock production policy, and financing of programs and/or projects related to the development of forage technologies. Furthermore, stakeholders highlight the role of MADR in the establishment and regulation of the national policy of technical assistance for agricultural production.

Actors and Levels of Influence

Here, a linkage mapping exercise is presented, in which CIAT's relationships with other actors (that CIAT recognizes as key agents in the development and dissemination processes of improved pastures) in Colombia are analyzed. The following results are based on the focus group discussion.

Relationships between R&D institutions mainly occur for collaborative research as part of specific projects. The links are strong between some institutions (e.g., Agrosavia and CIAT and their Forages Network). In most cases, however, we observe weak links that generate duplication of research efforts and competition for resources. There are not many strong links between R&D institutions and intermediary agents such as seed supply companies. CIAT, as exemption, has a strong link with Papalotla regarding the financing, co-development, and exchange of information on forage hybrids. The lack of other possible examples denotes a relational crisis between institutions that still needs to be overcome. Seed companies play a key role in providing technical assistance and training to primary producers, although mainly at the regional level. National universities have a high level of influence regarding the application of technologies (e.g., University of Cauca, University of Antioquia, Amazonia University, and National University of Colombia). However, this is done through specific scaling projects and requiring allies. In the interviews, it was pointed out that the impacts of dissemination processes depend on the collaboration among institutions, and that the competitive nature of funds increases the participation of universities in R&D processes.

Milk and meat trading companies have high potential in terms of technology diffusion due to their direct relationship with producers. Although these companies are key players in accompanying producers, they require a better communication with technical knowledge research and development institutions that effectively bring technological innovations closer to their target populations, harmonizing concepts, and reducing the circulation of confusing information. Currently, there are initiatives and approaches between private companies and research institutions (e.g., Fundación Alpina and CIAT). Associations and/or cooperatives of producers are recognized as having a strong role in the processes of diffusion and scaling of forage technologies. Among the roles they can fulfill are the collaboration with the research component and/or in the selection of pilot farms for the evaluation of technologies, the dissemination of information on technologies, supply of inputs, as well as training and extension among associated producers.

The MADR is identified as an actor with high influence within the processes of development and diffusion of forage technologies. This influence is associated with its role in the construction of a sustainable livestock policy at the national level, the financing of research programs in forage technologies, and the contribution to the Colombian Roundtable for Sustainable Cattle (MGS-Col). In recent years, the MADR and the Ministry of Environment and Sustainable Development (MADS) have increasingly aligned their agendas supporting sustainability more strongly. Thus, the lack of association between most of the innovation actors and the support structures has resulted in the existence of a generally weak innovation system. However, it is important to highlight outreach initiatives to strengthen institutional links and communication between actors that have been taking place in recent years, such as the participation of the main actors of the livestock sector in multi-actor platforms such as the MGS-Col, and approaches of the sector private sector and research institutions. The Rural Agricultural Planning Unit (UPRA) has a growing level of influence on livestock policy given the zoning exercise they conducted for livestock production in the country.

Bottlenecks

The mapping exercise carried out here allows us not only to identify the complexity of the AIS research component in the field of forages, but also provides insights to deepen and contextualize the existence of serious and persistent bottlenecks that affect agricultural innovation in forage matters. Below we describe the limitations that have had a direct impact on the technology adoption and diffusion processes, identified by the actors interviewed during the study.

Extensive Tradition of Livestock

Structural conditions are evident factors in discouraging sustainable intensification and, along with it, the adoption of improved species. For example, for traditional extensive ranching it is much more efficient (cost-effective) to acquire more land for the establishment of the crop than to intensify the use of a certain amount of land through the adoption of technologies. Deforestation as a result of livestock activity, an increasingly critical and urgent topic, also stands as one of the bottlenecks as far as livestock is identified as one of the main culprits behind the invasion of conservation/protected areas for the agricultural exploitation. The low cost of land in pastoral areas, and the still precarious controls over land tenure due to long-standing historical dynamics in which a fragile state predominates, favors land accumulation. This not only encourages sustained land accumulation by illegal actors (who have monopolized or decades large tracts of land, some of which are indeed dedicated to extensive livestock projects) but also encourages small livestock producers to upsurge agricultural areas instead of intensifying their production. In this way, a trend toward the purchase of land or expansion of the agricultural frontier is promoted.

Low Budget for Research

Budget cuts in the 1990s limited Colombian scientists and researchers, both in the formulation and in the follow-up and monitoring of ongoing projects. The paradigm shift in funding brought new consequences: scientists, who were dedicated solely to research work, now have as their main mission a systematic procurement of resources. This led to important distortions in the development of research agendas, fragmented personal and institutional relationships, and weakened sustained advances in the matter (e.g., programs such as the International Network for the Evaluation of Tropical Pastures -RIEPT) were eliminated. Even today there are certain misunderstandings derived from the new role of the scientist/extension worker.

Influence of Public Order Problems on Technology Diffusion Processes

On the one hand, technology transfer processes were affected as a consequence of the various dynamics of the armed conflict between the Colombian state, guerrillas, and paramilitary groups. Concrete examples of this correlation are found in the narratives about kidnappings and threats to research personnel, as well as in the uncertainty in the arrival of seeds to conflict zones. The manifest weakness of the state in disputed territories, as well as the fluctuating (and violent) political order in certain areas of the country has, without a doubt, affected the adequate implementation of extension projects, leading seed supply companies to register large economic losses. On the other hand, the consequence of the illegal drug trafficking market and the scarce state regulation of the seed market led, between the 80s and 90s, to the importation of large quantities of them for money laundering. The existence of a poorly regulated industry facilitated its use as a "facade" between drug traffickers and cartels, which resulted in the importation of large quantities of seed with low quality standards, affecting the domestic market.

Different Objectives Causing a Low Articulation Between National R&D Institutions and Seed Companies

With the help of donors, research entities aim to evaluate and release forage seeds. However, seed production is determined primarily by their own perspective on actual demand and profitability. This disparity causes the processes of diffusion and releasement of seeds to be distorted, and that results in turn in a low impact on the adoption processes: materials are released without commercially available seed, or else, these materials are not suitable for the territories in which they are that are traded informally.

Absence/Weakness in the Social Support of the Research

According to informants, the average duration of projects for the promotion and adoption of forage technologies is 3 years. This period constitutes a limitation because it makes it difficult to adequately measure the impact and scope of the introduction of a new species, and furtherly impossible to obtain accurate data about the adoption of technologies. Scarce times hinder the evaluation of the sustained use of new species, so a complete picture on the adoption of improved pastures at the national level remains a long way off. The foregoing is also a consequence of the disarticulation between different areas and research professionals, as well as between centers and entities in charge of formulating and executing technological innovation projects.

Speculation in the Brazilian Market as a Determinant of the Livestock Landscape in Colombia

The geographical and climatic conditions of the country limit the production of forage seeds, making Colombia dependent on seeds from its Brazilian partner, the main producer in the world. This high level of dependency makes Colombia susceptible to suffering from internal shocks to the economy in Brazil; that is, in the face of a change in the perception or in the projections about the profitability of a certain crop (e.g., sorghum, corn, soybeans) or between forage varieties, companies may prefer to produce seeds of the crop or a certain variety of pasture perceived as more profitable in the short term. These changes not only occur between substitute varieties but also between crops that are not directly related to livestock, which greatly limits the options of the demanding countries. Thus, changes in the Brazilian supply derived from speculative processes lead to an impact on the price level and availability of seeds in Colombia, so that a producer can in turn vary the level of preferences without having been able to evaluate the effectiveness of a previously acquired species.

Cultural Gaps and Personal Relationships

Personal relationships are key in the scaling of technologies (insofar as they allow or hinder the interaction of various agents and entities, the continuation of projects and their follow-up); they prevent or facilitate access to information and resources and at the same time chain inter-institutional relations to the personal sphere. Expedited and transparent interpersonal relationships facilitate scientific praxis, while rivalries, budgetary struggles, and fragile ties hinder the viability of a given project. The interviews carried out shed important light in this regard, where testimonies or narratives such as "our relationship was not good" or "relations between institutions depend on those who work in it" were a constant that allowed us to elucidate the importance of assertive interpersonal relationships for the development, achievement and continuity of research projects and initiatives that, by default, affect the processes of diffusion and adoption of agricultural technologies.

Weakness of Extension Processes in the Promotion of Forage Technologies

The neoliberal reforms of the 1990s (e.g., protectionist and decentralization policies at municipal and departmental levels) also weakened the key components of the national technical assistance system, which led to its progressive exhaustion and disarticulation. The lack of permanent updating in knowledge, methodologies, and technologies is highlighted in the UMATAS (Municipal Units of Agricultural Technical Assistance), and later, in the CPGA (Provincial Centers of Agribusiness Management) and EPSAGROS (Providers of Agricultural Technical Assistance Services). This has generated a knowledge gap between the generation of technologies and demanding users. In addition, the creation of EPSAGRO led to the attraction of resources and to the detriment of the quality of the service provided. To this is added that the service has focused primarily on agricultural issues, leaving aside the components of livestock development. All of the above is reflected in an institutionally weakened extension system where access to information, particularly on livestock technology issues, is seen as an important bottleneck.

Traditionally, Credit Lines Have Not Promoted Investment in Sustainable Intensification Systems

The actors recognize the importance that credit has had for agricultural development in the country, however, they highlight key bottlenecks associated with the low provision of credit in rural areas, information asymmetries that mainly affect small producers, and credit orientation rather toward productivity than sustainability. Despite the fact that the Fund for the Financing of the Agricultural Sector (FINAGRO) has established Special Credit Lines (LEC) for the promotion and renovation of pastures, as well as productive intensification through silvo-pastoral systems (e.g., Colombia Siembra, Livestock Sustainability), a pronounced effect has not been observed in the application of these lines, as the credits for livestock are mainly oriented to the purchase of animals. This has been accentuated as a consequence of credit dynamics such as growth in the substitute portfolio, where resources have been directed toward links in the chain with less risk than toward small producers (e.g., transformation and commercialization). The previous dynamics suggest that the spirit of agricultural credit is being lost, as it works more to attenuate the asymmetries and inequalities between the actors of the Colombian rurality. However, it is important to note that, in recent years, credit institutions have established mechanisms for adoption such as the Rural Capitalization Incentive (ICR), whose objective is to help subsidize up to 40% of the debt of small producers that request credits for the establishment of silvopastoral systems.

The aforementioned issue clearly indicates, in the voice of some of the main agents and historical moments that play a role in the processes of diffusion and adoption of forage technologies, the difficulties that persist and hinder the way of a sustained and successful technification. Despite the many advances obtained in the matter and the valuable and decisive work of research institutions, there is still a long way to go, not only in the transformation of livestock landscapes in Colombia and the efficient implementation of improved pastures, but also in the understanding and study of agricultural innovation systems as historical processes, contingent, subject to change, and deeply affected by inter-actoral relationships. In closing, here are some possible insights on how these R&D processes can be refined.

DISCUSSION

Although mapping the interactions and dialoguing with key agents allowed the identification of the main actors and their interactions in the research and dissemination of forages in Colombia evidence important findings that we explain in detail, trends in academic literature show the changing historicity of R&D of agricultural technologies, its challenges and opportunities and the complex nature of inter-actoral relations and the contexts under which it takes place. This discussion is then framed taking into account these three key elements. We first address the historical context and the main transformations of the AIS. Next, we delve into the conceptualization of inter-actoral relations and their importance within the AIS, and lastly, we discuss the main bottlenecks found to be key in the AIS in Colombia.

Investments in agricultural research have had important changes over time with relevant effects affecting the development of research processes. For example, in the case of the CGIAR, research funding has changed dramatically: it went from being historically constituted in the long-term and directed through central institutions that were in charge of coordinating and managing projects, to being based on short- and medium-term programs, oriented to smaller projects, and of less scope. The mode of financing has also been significantly transformed, moving from unrestricted institutional allocations to concrete projects with concrete deadlines and strict budgets (Beintema and Echeverría, 2020). In turn, the thematic focus of the research has expanded significantly, with much more emphasis on politics, the environment, and biodiversity conservation (Beintema and Echeverría, 2020).

Both research and extension components have been oriented more toward the direct involvement of the producers in the identification of their demands, making rural subjects participants of their own transformation (Ardila, 2010). This has been due to theoretical transformations and methodologies on how to think, intervene, and transform rural livelihoods, a trend that has been growing since the late 1990s known as participatory research (World Bank, 2012). In the case of the CGIAR, the budget (in inflation-adjusted terms) remained fairly flat between 1980 and 2000, even though its mandate was broadened to cover a wide range of research topics. As a result, the continued search for sustained funding for public agricultural research at the global and national levels remains one of the main challenges (Beintema and Echeverría, 2020). The data indicate that, in general, the participation of the private sector in agricultural research in Latin American countries has been increasing over time, and currently it is private companies that supply most of the seeds and animal genetics to farmers in the region (Stads and Beintema, 2009).

Regarding one of our main findings, which is the explicitness that most of the intra-actoral exchanges registered are weak, unidirectional and without feedback loops, several authors refer to the existence of weak links between national agricultural research institutions and rural extension actors in most developing economies (e.g., Anderson, 2004). It is noted that the information used by extension institutions is not necessarily accurate or generated by research institutions, and research priorities do not necessarily align with the needs of extension institutions. Also, on many occasions both types of organization compete for resources (Anderson, 2004). A study carried out by FAO/BID (2016) illustrates this problem. This study evaluated the technical assistance service in South America. For the case of Colombia, 117 surveys were applied to service providers and 38 to producers. According to the results, 38% of the organizations stated that they had weak ties with other organizations, 30% had moderate ties, and 20% had close ties (11% did not respond). The strongest links are with local government agencies and banks and microcredit institutions (31%). In the case of Colombia, a manifested weakness is evident in the relationship between the organizations that provide technical assistance (UMATA, EPSAGRO, NGOs, or unions) and the organizations that can contribute to the provision of the service. For example, the link between research centers and UMATA and EPSAGRO was considered by 50% as weak, and only 43% of the unions consider it strong (FAO/BID, 2016).

Thus, a key to improving rural extension is the articulation between the actors that provide technical assistance services with the research actors, and so is strengthening of their capacities (Garrido-Rubiano et al., 2021). Therefore, one of the greatest challenges is to achieve coordination between the actors (Garrido-Rubiano et al., 2021). Problems related to weak links and lack of trust between actors, as well as asymmetries in communication between them, are recognized as factors commonly mentioned in the literature that uses the AIS approach to examine the problems of adoption of agricultural technologies (e.g., Spielman et al., 2011; Kebebe, 2018).

Although there is a historical presence of national and international institutions promoting research and innovation in agricultural technologies (forages for the example that concerns us here), we find that the assertiveness of interpersonal links has determined immensely the adoption processes. For example, in this case-study, CIAT plays a leading role in the development of new and improved technologies for the country. However, the prominence of institutions has not translated into a higher adoption rate or a more expeditious path toward the goal, insofar as, as mentioned above, personal relationships directly influence inter-institutional ones.

Literature on this subject defines how the domain of intermediaries and/or bridging institutions (e.g., extension services that facilitate the transfer of knowledge and information between domains) is essential in the case of a successful AIS, which for the Colombian context, as mentioned, is in deficit. Thus, new technologies resulting from R&D processes in the agricultural sector have improved the quantity and quality of production and, therefore, have contributed to economic development, agricultural development, and poverty reduction in Latin America (Stads and Beintema, 2009). However, properly designed national agricultural research systems and adequate levels of investment are important prerequisites for agricultural development, food security and poverty reduction in all countries in the region (Stads and Beintema, 2009). Some recent research indicates that problems at the institutional and policy levels explain the low adoption of technology by small producers more than aspects of the producer (e.g., Birner and Resnick, 2010; Schut et al., 2016).

Widely discussed bottlenecks, such as extensive livestock farming, reductions in research budgets, weakness of the extension processes in the promotion of forage technologies, the low impact of agricultural credit lines, scarce articulation between R&D institutions and seed companies, as well as unpredictable speculation in the Brazilian seed market, have largely affected the Colombian context. First, the extensive nature of livestock can be explained here from structural conditions that discourage sustainable intensification, since in many cases, it is more efficient to acquire more land than to intensify. Low land prices, as well as the predominance of a fragile state to control access to it, have played a decisive role. Thus, structural factors that affect extensive livestock are (i) the higher profitability associated with new forage technologies that could lead producers to increase their herd size and hence the pasture area (Kaimowitz and Angelsen, 2008), and (ii) low land prices in many regions (e.g., Orinoquia) that make acquiring new land more efficient than intensifying existing land (White et al., 2001). Likewise, profitable technologies can also provide farmers with the additional capital they need to finance livestock expansion (Kaimowitz and Angelsen, 2008). Thus, if one of the main reasons for planting pastures is to have secure land tenure, the forest conversion to pasture can (and will) continue (Kaimowitz and Angelsen, 2008). This can be favored by price speculation processes, where acquiring more land would increase capital gains (Smith et al., 1997; Van Ausdal, 2012; Gutiérrez-Sanín and Vargas, 2017; Ponce de León-Calero, 2019).

In the research component, budget reductions experienced during the 1990s were decisive. Different reports of the ASTI (Indicators of Agricultural Science and Technology) (Stads and Beintema, 2009; Stads et al., 2016) evaluate trends in R&D in Latin America, pointing out the reduction of resources in all countries of the region in the 1980s and 1990's. These reports highlight direct effects of this reduction in research centers, such as the elimination of several long-standing research programs, and the deterioration of facilities and laboratories. Similarly, changes in the financing model since the 1990s (from long-term to short-term projects) and the constant struggle to obtain resources affected institutions such as the CGIAR, which in turn transformed the way of doing research and research and duration and impact of the projects themselves (Beintema and Echeverría, 2020).

In Colombia, the most relevant reform associated with technical assistance services was related to the State's decentralization processes, through which the National Government delegated the provision of this service to the territories. However, the limited capacity of the municipalities to assume obligations of such magnitude was not considered. Most local governments did not have the required capacities, the necessary administrative procedures, the external financing mechanisms, or the sufficient skills for the investment project management process (such as planning, monitoring, and evaluation) (FAO/BID, 2016). According to the National Agricultural Census (DANE, 2014), only 16.5% of the producers have access to extension services. Thus, among the bottlenecks identified in the technical assistance service in the country are the lack of capacities installed in the regions, the institutional disarticulation among those who generate, disseminate and accumulate knowledge, the low levels of associativity of producers, the loss of confidence of the latter in the effectiveness of the service, and a deficient monitoring and evaluation system (Hurtado et al., 2020).

Another element worth bringing to the discussion is that of credit lines. Although actors recognize the importance of credit for agricultural development in the country, they also highlight key bottlenecks associated with the low provision of credit in rural areas, asymmetries in access to information that mainly affect small producers, and a credit orientation more geared toward productivity than toward sustainability. Different studies have found empirical evidence where access to credit has a positive and significant effect on the adoption of new technologies and practices in the livestock sector (e.g., Lapar and Ehui, 2004; Turinawe et al., 2012). According to DNP calculations (2015), FINAGRO condition credits only reach 38% of the rural producers in Colombia, and credit lines have been directed toward profitability instead of sustainability in the livestock sector. According to FINAGRO's accountability figures (FINAGRO, 2020), credit applications from the livestock sector at the national level have been mainly channeled toward the purchase of animals, machinery, or the payment of the labor force, while credit applications designed to promote sustainable intensification systems, such as pasture renewal or the establishment of silvopastoral systems, have been very limited. This orientation is more pronounced in small and medium producers with participation percentages of 96.5 and 75.75%, respectively. For its part, the investment dedicated to sowing forages does not exceed 2% (FINAGRO, 2020). The advance of the substitute portfolio constitutes a problem in the accentuation of inequalities in the rural sector: despite the fact that the resources for agricultural credit lines have increased over time, the majority of resources have been directed toward other links in the value chain with a lower level of risk. Regarding total credit by type of producer, there has been a decrease in the share of credit granted to small producers, and an increase for large producers. While in 2010 small producers participated with 26% of total credit, for 2019 this percentage was 23% (FINAGRO, 2020). For their part, the large producers in 2010 participated with 28%, while in 2019 this participation increased to 59% (FINAGRO, 2020).

Besides this, a poor coordination between national R&D institutions and seed companies is also profoundly telling. As institutions of diverse nature, both have different goals, and in many opportunities the release of materials is carried out without being able to guarantee the availability of seed at a commercial level. To illustrate, materials such as *Andropogon gayanus* cv. Carimagua 1, *Brachiaria dictyoneura* cv. Llanero, and *Brachiaria brizantha* cv. La Libertad, released by ICA (now AGROSAVIA) in the 1980s, failed despite promotional efforts due to the lack of basic and commercial seed supply (Ferguson, 1993). The low articulation between research institutions and seed companies was a priority issue during the workshops carried out by the International Network for Tropical Pasture Evaluation (RIEPT for its acronym in Spanish) (Ferguson, 1993), which denotes that the research sector identified a poor relationship with seed companies as one of the great obstacles to generating an impact on the adoption of improved forages.

Finally, speculation in the Brazilian market stands as one of the main bottlenecks, applicable to the Colombian case due to its high dependence on market conditions in the neighboring country. According to Legiscomex (2020), of the total imported seed in Colombia, more than 90% comes from Brazil, from where varieties mainly of the *Brachiaria* and *Panicum* species are imported. Forage seed production began in Colombia in the 1970s, a period in which seed production and marketing companies emerged (Ferguson, 1993). At this time, companies such as Semillano Ltda. directly produced seed in the company of farmers and in their own lots for varieties such as *B. decumbens*, *B. dyctionerura*, *Stylosanthes capitata,* and *Arachis pintoi*. Only a small amount of seed was imported from Brazil to meet the demand. However, the forage seed industry in Brazil took an important advantage. This was mainly favored by the environmental conditions that are particularly conducive to seed production, such as the altitudinal level that allows longer periods of light and, therefore, greater flowering and better synchronization (Hopkinson, 1981). These comparative advantages allowed the Brazilian industry to specialize and become one of the most important producers, consumers, and exporters of forage seed worldwide.

CONCLUSIONS

By way of conclusion, we highlight firstly and as a constitutive and conclusive element of this research, the importance of institutional alliances as a cross-cutting element in the adoption of agricultural technologies. We believe that, in addition to the relationships between institutions, it is urgent to promote greater communication and exchange between them, though research, dissemination platforms in which they present results, trends, and research proposals (future and ongoing). The temporary exchange of personnel, as well as guided visits between entities, could play vital roles in strengthening ties, increasing bonds of trust and maintaining this symbiosis over time. We also consider it essential to promote articulation between research and dissemination institutions and distributors of improved seeds, companies, and actors that are part of sustainable livestock strategies (e.g., Sustainable Livestock Table Colombia, zero deforestation agreements) in order to improve the dissemination and opening channels of communication between them, establishing dialogues that facilitate the development of public policies for the sector and contributing to the development of institutional and field capacities. Likewise, and as far as "third parties" are concerned, we maintain that it is of the utmost importance to take advantage of the potential of the milk processing industries to reach the primary producer: the direct link that has been created between these companies and producing communities can be useful for disseminating technologies through training and education programs. Since companies do not have the technical knowledge related to forage management, it is important to promote projects in association with research and extension institutions.

Solid relationships with policy makers, in which the benefits (economic, productive, competitive, and environmental) that the country has from promoting plans and projects that contribute to the implementation of forage technologies in Colombia is also a necessity for the sector. The involvement of public institutions with private actors in the development of technologies should be established in the agendas, not only of universities and research centers, but also between them and government agencies. Said dialogues could be aimed at consolidating strategies that allow the articulation at municipal, departmental, and national levels of each of the local initiatives where the nascent extension system can play an important role. It is well-known in academic literature that producer cooperatives and associations are fundamental actors in technology diffusion processes. Here, we propose to encourage the creation of these institutions in territories where they do not yet exist or in territories where existing ones are located far away from the producers. This can be done during vaccination periods or during technical visits by control bodies (e.g., ICA). It is also useful to point out that, in those consolidated associations, the sharing of experiences and cultural practices in the management of pastures and properties is encouraged. Together with extension agents, knowledge about scientific innovations can be addressed, thus generating fertile and lasting exchanges.

Through the creation of innovation networks (such as the Forages Network between CIAT and Agrosavia), alliances between research institutes, higher training centers, rural extension services, and producer associations can also be fostered in order to advance faster in technology adoption processes. Another possibility for improvement and transformation of the R&D system lies in the promotion of incentives for adoption. The creation of credit instruments for the adoption of technologies and the articulation of agricultural credit lines with extension services, can positively transform the panorama in terms of the adoption of improved forages. This is important not only for forage technologies but also for silvopastoral systems, which tend to be long-term investments as well.

Finally, the strengthening and prioritization of livestock production chains in the Departmental Agricultural Extension Plans (PDEA) in those territories where livestock predominates and there are high levels of deforestation and low adoption of forage technologies is a fundamental and unquestionable axis. The training that is established for this purpose should not only involve the management of pastures and forages; For success to be sustainable over time, we are convinced, extension strategies must include a holistic campaign in which producers are interested in the effective use of support information, social appropriation of knowledge, and problem solving, mainly through open or collaborative innovation, participatory research, and the use of Information and Communication Technologies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Alliance of Bioversity International and CIAT. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NT, KE, MD, and SB: conceptualization, methodology, writing the original draft and review and editing, and resources. NT, KE, and MD: formal analysis. SB: supervision, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Public Policies for the Development of a Sustainable Cattle Sector in Colombia, Argentina, and Costa Rica: A Comparative Analysis (2010–2020)

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Projected food demand increases highlight the importance of Latin America as one of the big global future food suppliers, due to its agricultural potential, in particular regarding cattle farming. Despite the importance of the cattle sector for the region, its negative environmental impacts are numerous and the shift toward sustainability is perceived as slow and uncoordinated. This study aims at identifying successes and difficulties in the implementation of public policies for a sustainable cattle sector in Colombia, Argentina, and Costa Rica. Based on the review of scientific articles, government reports, and publications of international organizations, a qualitative comparative analysis was carried out, documenting the political developments between 2010 and 2020. Our findings suggest that public policies mainly focus on the reduction of greenhouse gas emissions and the implementation of silvo-pastoral systems. Common successes exist among the three countries, e.g., a large number of public policies for promoting sustainable cattle farming or the inclusion of goals to reduce greenhouse gas emissions and implement silvo-pastoral systems, but they also coincide in difficulties, e.g., disconnection between policies and the lack of continuity of development programs. The efforts made with regional and national public policies, in addition to legislative advances, can be seen as initial steps in a long-term process toward sustainable cattle farming, and thus, recommendations are provided for increasing their success at different stages, from the identification of the problem to its evaluation, particularly in the face of financing difficulties, disconnection among policies and initiatives, and participation of citizens and livestock producers.

Keywords: public policies, sustainable intensification, cattle, silvo-pastoral systems, climate change

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INTRODUCTION

Framework of Reference

Latin America and the Caribbean plays an essential role in the global cattle industry since it contributes with more than 25% to the global beef and 10% to the global milk supply (CEPAL, 2017). The cattle sector generates both external and internal benefits, as it supports both the region's and global food security and contributes to the economy of the countries, highlighting the need to increase the efforts to preserve the growth of the sector (Núñez et al., 2015). Cattle production in the region not only goes back a long way, but also appears to be facing a promising future.

The Inter-American Development Bank (BID, 2018) projects a growth in global meat production by 100% until 2050, considering the global population growth—a scenario that would favor Latin American cattle producers due to the region's geographical position, experience, and human and natural resources.

Despite the importance of the sector for the region and its growth potential, the environmental effects of traditional or conventional cattle production systems are multiple and include e.g., impacts on water sources, soil resources, a loss of biodiversity and greenhouse gas emissions (IDB, 2018). The principal greenhouse gases generated by the cattle sector are methane (CH_4), produced in the enteric fermentation process of cattle, carbon dioxide (CO_2), resulting from land-use and land-use changes, and nitrous oxide (N_2O), emitted during manure and slurry management (Rao et al., 2015). The cattle sector contributes significantly to global warming and climate change because of deforestation for feed and forage crops, degradation of pastures and greenhouse gas emissions from cattle production (Abbasi et al., 2015).

This has led to discussions about the transition from a conventional to a sustainable cattle sector. A *sustainable cattle sector* is characterized as economically viable for farmers, respectful of the environment, and socially accepted (Varijakshapanicker et al., 2019). Related to this is the concept of *sustainable intensification*, understood as an approach that uses innovations to strengthen agricultural productivity, while reducing the environmental footprint (e.g., greenhouse gas emissions), promoting ecosystem services (e.g., soil quality improvements, reduced erosion, increased biodiversity) and supporting social development of rural communities (Rao et al., 2015).The challenge is to provide quality food for a growing human population, while managing to reduce the negative environmental impacts caused by food production (Tedeschi et al., 2015). In this regard, sustainable intensification is not reduced to specific practices, but rather involves heterogeneous processes and therefore, its implementation requires adjusting to the different agricultural systems and socioeconomic conditions of the target populations (Xie et al., 2019).

To achieve sustainable intensification, it is necessary to implement a broad set of different actions and innovations, such as the use of environmentally responsible technologies, the implementation of silvo-pastoral systems, or good animal husbandry practices, among others (Departamento Administrativo Nacional de Estadística, 2015). *Silvo-pastoral systems* are defined as the integrated use of grasses, legumes, forage shrubs and trees in livestock production systems. There exist different types of them, which include e.g., trees in pastures (living fences, scattered trees, and forest area), protein banks and shrubs, or the integration of fruit and timber trees. The benefits of silvo-pastoral systems are diverse and range from productivity increases (more forage biomass and higher nutritional quality), animal welfare (e.g., reduced heat stress, better diet), income increases and diversification (e.g., more meat and milk, fruits or timber), to environmental benefits such as better rainwater capture, soil protection and recovery, biodiversity conservation, and greenhouse gas emissions reductions, among others (IICA y Ministerio de Agricultura de la República Dominicana, 2016; Buitrago Guillen et al., 2018). Murgueitio et al. (2014) add that the presence of trees and shrubs contributes to mitigating climate change through mechanisms such as increased carbon deposits in the soil and lower nitrogen losses. They also state that the use of silvo-pastoral systems can increase beef production levels by 12 and 4.5 times, compared to extensive grazing and improved pastures without trees, respectively, while methane emissions per unit beef product are 1.8 times lower.

The implementation of such actions or innovations requires the commitment of different actors along the beef and dairy value chains, service providers (e.g., for credit, extension), and, above all, the support from the public sector. In this regard, this study differentiates between *governmental policies* and *public policies*. *Governmental policies* are all the actions carried out by a government at different levels (e.g., national, departmental, or municipal) in response to social problems, without considering citizen participation. *Public policies*, although part of the previous ones, are more complex processes that involve a strong intervention of the communities and involve four stages: (i) identification of the problem, (ii) design of the policy, (iii) implementation, and (iv) evaluation (Arias and Herrera, 2012). Yalmanov (2021) delves into this differentiation by pointing out that public policies cannot be reduced to a technical function of governments, but rather are complex dynamics influenced by socio-political forces that alter both processes and results. Likewise, it is necessary to consider the existence of individuals and groups that try to shape public policies in search of their particular interests, thus constituting a power struggle (Cochran and Malone, 2014).

To understand how governments support and manage such processes, it is necessary to have an in-depth look at how they have responded to social demands in the past, i.e., through the implementation of policies. This is precisely the objective of this article: to identify successes and difficulties in the implementation of public policies for the development of a sustainable cattle sector in Colombia, Argentina, and Costa Rica between the years 2010–2020. The selection of these countries takes into consideration that they present different social and economic realities, which allows for a comparative analysis. The countries were also selected because of the strong efforts they already made toward the transition to a sustainable cattle sector, evidenced by the existence of e.g., multi-actor platforms for sustainable cattle (in Colombia and Argentina) or the carbon-neutrality objective set by the Costa Rican government. It is worthwhile to answer the question why this study is justified. Primarily, because it adjusts to the reality of a global climate crisis that requires concrete actions, such as public policies, for both adaptation and mitigation. Likewise, the study is justified to the extent that the evaluation of such policies generates knowledge that can allow their reformulation in the medium- and long-term, overcoming the difficulties identified in pursuit of sustainability objectives. The study thus serves as a reference document for various actors, such as national and local governments, cattle producers, and value chain actors, and helps in the design, implementation, and evaluation of existing and future policies.

This article is structured as follows: Section Materials and Methods explains the methodological approach used; Section Results provides first insights into the successes and difficulties of implementing policies in the three countries of analysis, namely Colombia, Argentina and Costa Rica; Section Comparative Analysis and Discussion deals with the comparative analysis of the results among the three countries and a corresponding discussion; in Section Conclusions the conclusions of this study are presented; and Section Recommendations for Public Policy provides useful recommendations for a broad set of stakeholders.

The Latin American Cattle Sector

The Latin American cattle sector currently faces a series of circumstances that determine its development and, consequently, the public policies that govern it. Among these, productivity increases to meet the growing demand for animal-source food, climate change and the search for environmental sustainability stand out (CEPAL, 2015; FAO, 2019).

Regarding cattle production and productivity increases, beef production in the western hemisphere had a recent displacement toward South America, resulting from a reduction in cattle numbers and several years of droughts that affected both the United States and almost all of the Central American countries, mainly El Salvador, Guatemala and Honduras, but also to a lesser extent Nicaragua, Costa Rica and Panama. Between 2000 and 2013, Latin America doubled its beef exports, with exemplary cases such as Uruguay and Paraguay that exported almost two-thirds of what was produced (CEPAL, 2015). However, this contrasts with the situation on Argentina over the same period, whose cattle sector was affected by the 2008 drought and the sale of cattle in 2009, which caused a 44% drop in its beef exports (CEPAL, 2015), although with a notable recovery since 2015 (Cano, 2019). Although the United States have managed to overcome the drought-related crisis and are now again an important competitor at the global level, beef production volumes are almost 70% higher in Latin America. For their part and despite the signing of free trade agreements, the competitiveness of countries in Central America are lagging behind due to a negative perception of their animal health and food safety systems (CEPAL, 2017). Brazil is the beef export leader in the region, contributing 19.3% of the global beef trade (SAGARPA, 2018). The highlighted increases in beef production and exports in Latin American countries has also led to a greater co-responsibility for mitigating climate change. Regarding the adverse effects of cattle production on the environment, the fact that the region generates 30% of the greenhouse gas emissions of the global cattle sector stands out (FAO, 2019). This is further aggravated by the on-going deforestation, which, in addition to contributing to generating greenhouse gas emissions, causes the extinction of hundreds of species of flora and fauna and the destruction of ecosystems. In Central America the situation is particularly worrying since the forest area had a reduction of 40% between 1960 and 2000 (FAO, 2010). Regarding Latin America as a whole, the scenarios vary depending on the climatic, historical, political, and economic conditions of each country. While in Brazil, for example, there still exist large forest areas in the Amazon (which at the same time is one of the global deforestation hotspots), forests have almost disappeared in El Salvador (Sanhueza and Antonissen, 2014).

Apart from the abovementioned implications of the cattle sector on climate change and environmental degradation, climate change itself is also affecting the cattle sector, resulting in a need for climate change adaptation strategies and policies. Changes in the global climate affect the quality of water and animal feed, influence the physiological conditions of cattle, and lead to extreme climatic events (e.g., drought, flooding), among others, all contributing to variations in productivity and a reduction of areas suitable for cattle production. These ambivalent interactions between cattle farming and climate change, in addition to environmental problems caused by other economic sectors, have led the Latin American countries to adhere to environmental commitments, such as the Paris Agreement in 2015. In general terms, the treaty seeks to control the future temperature increases, protect food production systems, and promote sustainable agricultural production systems (FAO, 2019). The Latin American countries have also subscribed to the Sustainable Development Goals (SDG) promoted by the United Nations, which contain 17 goals that aim at guaranteeing prosperity at a global level. All goals set for the 2015–2030 period include components related to the livestock sector, in particular sustainable cities and communities, responsible production and consumption, climate action, and life in terrestrial ecosystems (ONU, 2021).

It should be noted that, beyond the aforementioned factors, the livestock sector in the region is complex and affected by multiple elements. These range from the economic liberalization processes of the 1980s and 1990s that still lead to repercussions, such as job insecurity and the excessive use of natural resources (FAO, 2013; Rojas Villagra et al., 2015), to issues such as political uncertainty, foreign investments, production technologies and animal diseases (CEPAL, 2017).

MATERIALS AND METHODS

To address the proposed objective, we decided to write a review article with a qualitative-descriptive approach. Literature review was used as the main data collection technique. In the analysis we related fragmented knowledge, contrasted different sources, and updated the existing literature, aiming at clarifying the state of the art of public policies that have promoted the development of a sustainable cattle sector. We selected three Latin American countries, namely Colombia, Argentina and Costa Rica and focused on the analysis of policies implemented during the years 2010–2020. This selection corresponds to the efforts made by the countries to develop a sustainable cattle sector: all of them have ratified the Paris Agreement and adopted the Sustainable Development Goals (SDGs), and both Colombia and Argentina have implemented roundtables for sustainable cattle. For its part, Costa Rica has set out the goal of achieving carbon neutrality, which stands out at the Latin American level. It is also noteworthy that, despite the efforts mentioned, in the three countries the agricultural sector is the main cause of GHG emissions (Banco Mundial, 2014), which shows the importance of investigating their public policies to understand how they have faced both this and other environmental problems. Brazil is excluded from the study despite being the largest exporter of beef in the region (SAGARPA, 2018), since it still has excessively high figures of deforestation and GHG emissions (Observatorio do Clima, 2020), which contrasts with the progress made by the three selected countries, where, despite room for improvement, a relatively favorable outlook is observed. This, however, does not state that Brazil does not have laws or public policies oriented toward achieving sustainability of its cattle sector, but rather that the study prioritized slightly more successful experiences that allow it to be a point of reference for other countries.

Data collection was carried out from January to May 2021 and prioritized three types of data sources: (A) Scientific articles, which were especially used for defining concepts and theoretical principles regarding sustainable cattle, particularly but not exclusively in the introduction. (B) Government reports and other official documents, which include publications of national and local governments, ministries, secretaries, congresses, and other public entities of the respective countries. National and local public policies were searched in these documents (including budget figures and intervened areas), and the legislation promoted in each of the contexts addressed. They were used in both the results and analysis sections. (C) Publications by international organizations, such as the Food and Agriculture Organization of the United Nations (FAO), the Economic Commission for Latin America and the Caribbean (CEPAL), and the Inter-American Institute for Cooperation on Agriculture (IICA). Such sources were consulted to contrast the official figures and positions of the countries, specifically in the analysis section. Among the three categories, 115 sources were cited. With the aim of presenting a picture as complete as possible of each of the studied scenarios, the results considered five factors, namely (i) the context, (ii) National Development Plans, (iii) legislative advances, (iv) multi-sector initiatives, and (v) regional policies. At the end of the section corresponding to each country, a table-summary of successes and difficulties in the implementation of public policies is presented (**Tables 2**, **4**, **6**). These arise from the authors' own interpretation, considering the five elements previously exposed, while at the same time allowing the formulation of a set of recommendations for the development of the different stages of the policies (**Table 7**). Regarding the analysis, to evaluate the impact of the public policies described in each of the three scenarios, figures related to deforestation, GHG emissions and conservation of natural areas were consulted.

Hernández et al. (2014) describe that in qualitative studies, the research process is holistic, since it is not reduced to the analysis of the parts, but rather addresses the whole picture. This was especially important for the present study, as it sought to understand how the set of policies have contributed to the transition toward a sustainable cattle sector. Despite the qualitative focus of this research, the importance of quantitative information was not neglected. In turn, it is necessary to point out that, due to the breadth of identified policies and the complexity of exposing them in their entirety, those with the greatest impact in terms of budgets, intervened areas, and importance of the regions they are aimed at for respective national cattle sector of each country were selected.

RESULTS

Colombia

Context

Unliske many other Latin American countries and despite the internal armed conflict that lasted for more than 60 years, Colombia has a relatively stable political and economic system. The first neoliberal reforms were presented in the 1980s and consolidated in 1989 with the Washington Consensus, including elements such as a reduction of the role of the State in social intervention, privatizing public institutions and promoting private ownership and enterprises (Tejedor Estupiñán, 2012). With a more or less rigorous application of these principles, all national governments have since then followed the same guideline, without making abrupt changes. It is within this framework that the various economic sectors have developed, including the agricultural and livestock sectors.

Regarding the cattle sector, its contribution to the national economy is highlighted by generating 1.1 million jobs, which is equivalent to 6% of the national employment (Fedegán, 2021). With ∼35 million hectares, the sector uses most of the available land for agricultural purposes, most of it under extensive cattle ranching systems (Banco Mundial, 2019). In relation to this figure, the Rural Agricultural Planning Unit (UPRA, 2015) has stated that the sector exceeds the maximum amount of land use by 15 million hectares, making it necessary to rethink the rural land use. Colombia is the 17th largest beef producer in the world and contributes with 1.2% of the global beef supply. Exports to countries from the Middle East, Russia, and Vietnam, however, make up only 4% of the overall production volume while the rest is consumed domestically (Venugopal et al., 2021). Despite the occurrence of the COVID-19 pandemic and the fear of its implications on the sector, beef export figures showed a positive development at the end of 2020, with 3,247 tons of beef exported in September, exceeding the figures in the same months of 2019 (1,681 tons) and 2018 (1,899 tons) (Fedegán, 2021).

To this extent, the public policies addressed are located in a scenario where two characteristics stand out: (i) the stability of the political-economic model for more than three decades, and (ii) a cattle sector that, despite its limited international importance, is fundamental at the national level in terms of job creation and food security.

National Development Plans

During the last decade, the different national governments of Colombia have indicated the importance of environmental protection as the basis of their policies. In this regard, the *National Development Plan* 2010–2014 stated that environmental sustainability should be a priority and an essential practice for the wellbeing and equity of future generations (DNP, 2011). For the 2014–2018 period, this premise continued, emphasizing more strongly the importance of protecting natural reserves, regulating land use and preventing socio-environmental conflicts (DNP, 2015). The current *National Development Plan* for the period

FIGURE 1 | Actors involved in the design and execution of public policies in Colombia.

2018–2022 adds to that a long-term project perspective, which allows achieving the SDGs by 2030 (DNP, 2019).

Legislative Advances

Although the legislative framework for the cattle sector is very broad and involves elements such as animal welfare and marketing there are three regulations that stand out in the period of analysis for their influence on the sector in terms of sustainability:

- *Decree 870:* Establishes the framework for payments for ecosystem services, in addition to other incentives for conservation (Presidencia de la República de Colombia, 2017).
- *Law 1876:* Creates the National Agricultural Innovation System (SNIA) with the purpose of improving the productivity and sustainability of the national agricultural sector (Congreso de la República de Colombia, 2017).
- *Law 1931:* Establishes guidelines for the management of climate change in the decisions of public and private entities (Congreso de la República de Colombia, 2018).

Multi-Sector Initiatives

As a further effort to adapt to and mitigate the effects of climate change, multi-sector initiatives have emerged in Colombia, such as the *National Plan for Adaptation to Climate Change* and the *Colombian Strategy for Low Carbon Development* (ECDBC). In the same sense, but focusing entirely on the cattle sector, the *Colombian Roundtable for Sustainable Cattle* (MGS-Col, made up of one national and 12 regional roundtables, was established in 2014 and is an inter-institutional space where the public and private sectors, academy and NGOs converge with the aim of being a benchmark in the design and implementation of sustainable cattle programs and policies, capacity building in rural areas, inter-institutional exchange and link with global initiatives such as the *Global Roundtable for Sustainable Beef* (GRSB) (**Figure 1**). Recently, the MGS-Col presented a technical proposal for the formulation of a national level sustainable cattle policy to the Ministry of Agriculture, which is now under revision. Among the objectives of this proposal is the promotion of the cattle sector from the green growth paradigm and the

TABLE 1 | Overview on the objectives and geographical reach of the Departmental Agricultural Extension Plans (PDEA) in Colombia.

Department	Objectives related to sustainable cattle farming	Source
Antioquia	- Increase productivity, competitiveness, and sustainability in coordination with rural actors	Gobernación de Antioquia, 2020
Boyacá	- Promote the development of productive systems aimed at the conservation and proper management of natural resources	Gobernación de Boyacá, 2020
Casanare	- Improve the competitiveness and sustainability of the sector - Strengthen the contribution to food security and the development of the agricultural producer as an integral human being	Asamblea Departamental de Casanare, 2020
Cauca	- Develop skills in producers to increase the knowledge base and support behavioral change with the aim of improving competitiveness and sustainability	Gobernación del Cauca, 2020
Cesar	- Strengthen the capacities of producers to make decisions about their agricultural production systems, so that they can develop processes that respect the ecosystem	Gobernación del Cesar, 2020
Guainía	- Improve cattle production facilities for associations in the department - Complement and articulate actions through the project "Implementation of a comprehensive and fair rural extension plan" - Raise awareness about cattle regulations	Gobernación del Guainía, 2019
Santander	- Encourage producers to use water resources efficiently and develop soil conservation practices	Secretaría de Agricultura de Santander, 2020
Vichada	- Increase the profitability of the cattle sector through genetic improvement, balanced nutrition and more and better pastures - Reduce the negative environmental impacts of traditional cattle farming through the development of low carbon cattle systems and silvo-pastoral systems	Gobernación del Vichada, 2020

conservation of the environment and natural resources (Mesa de Ganadería Sostenible Colombia, 2019). Likewise, it is pertinent to refer to the zero deforestation value chains initiative for beef and dairy, which is part of the *Zero Deforestation Agreements* contemplated in the *National Development Plan* for 2018–2022. The initiative, understood as a voluntary commitment to collective action among the public and private sectors, commits the involved entities to stopping cattle-farming-related deforestation and, at the same time, developing processes of ecological restoration, such as the reestablishment of a degraded areas, among others (Alianza Colombia TFA, 2021).

Another multi-sector initiative was the *Sustainable Colombian Cattle Project* (GCS), executed from 2010 to 2019 and financed by World Bank, the Global Environment Fund, the Government of the United Kingdom, which aimed at strengthening the Colombian cattle production through the integration of environmentally friendly practices. Among the specific objectives of this project were e.g., the transformation of 35,500 hectares of traditional production systems into silvo-pastoral systems, the preservation of 15 hectares of native forests, the development of payment schemes for ecosystem services, the creation of forage nurseries, and technical assistance for 3,900 cattle farms to support sustainable intensification efforts (Ganadería Colombiana Sostenible, 2018). The *Integral Program for Productive and Environmental Reconversion of the Cattle Sector* (PIRPAG), whose objective is to support the transition of the national cattle sector toward sustainability over a period of 30 years, is another example of multi-actor initiatives in Colombia. It focuses on the modification of traditional cattle landscapes into more productive systems that include environmental commitments, allowing for a reduction and capture of greenhouse gas emissions. The initiative works in several selected regions, such as the humid and dry Caribbean and the foothills of Magdalena Medio and the Orinoco, where pilots have been carried out on integrating live fences, forage hedges and mixed forage banks into the traditional livestock systems (Lozano, 2020; Colombia Sostenible, 2021).

Finally, as one of the *Nationally Appropriate Mitigation Actions* (NAMAs), the Colombian *Sustainable Cattle NAMA* is being developed among a broad group of stakeholders. This future policy will be focused on involving public-private sector participation, addressing the mitigation of climate change through the reduction of greenhouse gas emissions as well as an increase of carbon sequestration (Ministerio de Ambiente y Desarrollo Sostenible, 2019). Its actions will impact 434 municipalities and 3.6 million hectares (Banco Mundial et al., 2021).

Sub-national Regional Level Public Policies

Parallel to the above-mentioned initiatives, there also exist various public policies implemented at the regional level. Among these, the *Departmental Agricultural Extension Plans* (PDEA) stand out, which, although they are still in the initial phase of design and implementation, are macro level policies that define the provision of agricultural extension at departmental level (**Table 1**). For the transition toward sustainable cattle systems, credit is needed. The Colombian government launched a credit line program for silvo-pastoral systems in 2020, which is being implemented at a regional level (mainly in 82 municipalities) and seeks to promote sustainable practices, such as the conservation of biodiversity and the protection of water and soil resources, in the different cattle regions of the country. The credits are directed to the purchase and planting of tree species and the implementation of living fences, among others, and is the first initiative in this regard (Ministerio de Agricultura y Desarrollo Rural, 2020).

National Level Public Policies

In 2019, the *2018–2022 Agricultural and Rural Development Policy: a field for equity* was launched. This policy aims at promoting agricultural competitiveness and productive transformation based on three pillars: (i) rural development, (ii) productivity + profitability = competitiveness, and (iii) modern and technical institutions. The sustainability component is in the second pillar and has the objective of positioning the country as leading actor at international level, boosting employment, diversifying the productive offer, and encouraging environmentally responsible production practices. To achieve this, farm planning, the establishment of silvo-pastoral systems, the division of pasture areas and the use of aqueducts that prevent contamination of water sources by animals, are the most prominent approaches. The *National Conversion Strategy* focuses on three main objectives: (i) technology (access and implementation), (ii) agricultural extension (in correspondence with Law 1876 of 2017), and (iii) financial instruments. In addition to these objectives, the policy aims at establishing a pilot cattle farm in each of the country's cattle regions (Antioquia, Boyacá, Caquetá, Cauca, Humid Caribbean (Córdoba and Sucre), Coffee Triangle and northern Valle del Cauca, Guajira, Magdalena Medio, Nariño, Orinoquía (plains), Orinoquía (flooded savannas), and Tolima-Huila), and at strengthening the 12 regional roundtables. Once the initial network has been completed, progress will be made so that in 2022, the implementation of silvo-pastoral systems on 75,000 ha in 25,000 properties will be achieved (Ministerio de Agricultura y Desarrollo Rural, 2019).

Successes and Difficulties in the Implementation of Public Policies

From the reading and analysis of the aforementioned policies, it is possible to identify a set of successes and difficulties in their implementation (**Table 2**). Successes and difficulties arise both from the political and economic context, as well as from the content of the policies and the relationship between them. The existence of macro policies stands out, such as the *National Development Plans* and legislative advances, but also do regional programs with specific objectives, facilitating short-term implementation, monitoring and evaluation. One of the main successes is the promotion of silvo-pastoral systems, which is strengthened by the creation of a specific credit line. Financial resources are precisely one of the main problems since some policies show a lack of clarity on how to finance their objectives.

TABLE 2 | Successes and difficulties in the implementation of public policies in Colombia.

Successes	Stability of the political-economic system for more than three decades
	Continuity of the promotion of a sustainable cattle sector in the National Development Plans
	Existence of a national level Roundtable for Sustainable Cattle
	Strong progress in the formulation of a national level public policy on sustainable cattle
	Promotion of silvo-pastoral systems through on regional and national policies
	Existence of a credit line for the establishment of silvo-pastoral systems
Difficulties	The national public policy framework is still very young and at its early stages
	The Departmental Agricultural Extension Plans are still very young and at their early stages, and they also do not exist for all departments with relevance to the cattle sector
	Insufficient coordination between national and regional level public policies
	Lack of clear budgets to carry out some of the policies, particularly from the National Development Plan and the Departmental Agricultural Extension Plans
	Different levels of acceptance of public policies by producers, many of whom relate sustainable cattle farming with the need for economic investment

Argentina

Context

In recent decades, the political, economic and social narratives for development pathways of Argentina have been determined by two clearly differentiable and opposed development models: the first, established between 1990 and 2002, gave a fundamental role to foreign investment and was characterized by an outward-oriented economy; the second, between 2003 and 2015, appealed to a state with greater regulation of markets, internal savings, food production with social inclusion and strengthening of commercial ties at the regional level (Taraborrelli, 2017). Since 2015, Argentina has had two governments with opposing views, with clear tendencies toward the former development models: between 2015 and 2019, liberal policies adjusted to the requirements of the International Monetary Fund were prioritized (Morresi and Vicente, 2019), while in 2019, the path that started in 2003 was resumed (Scaletta, 2020).

Regarding the cattle sector, a reactivation was sought in 2015 with the lifting of various obstacles, such as export controls and interventions in the internal market (Patrouilleau et al., 2017). According to recent figures, the Argentinian cattle herd counts approximately 53 million heads (Secretaría de Gobierno de Agroindustria de la Nación, 2019). Despite the increase in beef sales on the international market, particularly to China, and the decline in domestic demand for beef due to high inflation and lower wages, domestic consumption continues to be the basis of the sector. Argentina has recovered its privileged position in the global beef market, occupying the fifth place in production and fourth in exports (Cano, 2019). The COVID-19 pandemic, although having effects on the sector, did not slow the growth rate significantly and by October 2020, 730,000 tons of beef had been exported (Villamil, 2020). An additional aspect to highlight is the expansion of the agricultural frontier, particularly resulting from plantations of monocultures such as soy. Their growth has displaced livestock, pushing it to less productive lands and into forests (Pincén et al., 2010).

In summary, a context is revealed in which the changes in the political model and economic instability are elements of great influence on the cattle sector, despite the preservation of growth and export levels making Argentina one of the global leaders for beef.

National Development and Land-Use Management Plans

The *Participative Federal Agri-food and Agroindustry Strategic Plan* for 2010–2016 presented some of the challenges the Argentinian cattle sector was facing, such as a water deficit and drought in 2008, which obliged cattle farmers to sell their animals earlier than planned (lack of feed and water) and led to lower calf birth rates in 2009, among others. To counteract these problems, the plan proposed that by 2015 all national policies should integrate the principles of sustainable development and thus reverse the loss of natural resources (Ministerio de Agricultura Ganadería y Pesca, 2010). For its part, the *Territorial Strategic Plan* (PET), launched in 2011, recognized the cattle sector as cause of desertification, particularly through pasture overgrazing. Although the plan did not delve into the cattle sector, it proposed that all citizens need to achieve environmental sustainability and included the promotion of a sustainable productive development in the guidelines for territorial and land-use planning in rural areas (Ministerio de Planificación Federal Inversión Pública y Servicios, 2011). The *National Policy and Strategy for Territorial Development and Planning*, launched in 2016, defined the achievement of an environmentally sustainable society as the main objective, for which it proposed a series of strategies, such as improving knowledge about natural resources and including the environmental dimension as a transversal axis in public territorial policies and actions at the federal, provincial and local levels (Ministerio de Planificación Federal Inversión Pública y Servicios, 2016).

Legislative Advances

Although there are several laws that directly and indirectly influence the Argentinian cattle sector and its sustainable development, three stand out in this regard:

- *Law 26.331, Minimum Budgets for Environmental Protection of Native Forests:* promotes the sustainable management of forest reserves, in addition to creating the National Fund for the Enrichment and Conservation of Native Forests (Congreso de la Nación Argentina, 2007).
- *Law 27.066. Regime for the Promotion of Cattle Production in Arid and Semi-arid Zones:* aims at increasing the supply of bovine livestock (by-)products, which follow the principle of environmental balance (Congreso de la Nación Argentina, 2014).
- *Law 27.520. Law of Minimum Budgets for Adaptation to and Mitigation of the Global Climate Change:* establishes strategies that allow guaranteeing human and environmental development. Article 24, in particular, refers to practices to mitigate climate change linked to the agricultural and livestock sector (Congreso de la Nación Argentina, 2019).

Multi-Sector Initiatives

One of the principal multi-sector initiatives is the *Argentine Sustainable Beef Board* (MACS), an association of public and private entities, NGOs, academia, and other organizations (e.g., input and service providers), with the aim of promoting sustainability policies for the cattle sector (**Figure 2**). It currently has more than 40 members committed to the development of specific goals, such as proposing innovations in inputs and services, anticipating the response to market trends, and promoting the improvement of the beef value chain (MACS, 2021). Another multi-sector initiative is *Carne del Pastizal*, which has the objective to stimulate cattle production based on practices that respect biodiversity, in addition to generating positive impacts in economic and social terms. One of its main achievements was the export of certified grass-fed beef to Europe (INTA, 2014). At this point, it is worth mentioning that there exists no policy for the cattle sector yet that properly responds to the *NAMA concept*, although (see subchapters below) there are various actions aimed at reducing the sector's greenhouse gas emissions according to the NDCs defined at the COP21 in Paris in 2015 (Centro Agronómico Tropical de Investigación y Enseñanza, 2019).

Sub-national Regional Level Public Policies

Regarding the traditional cattle provinces in Argentina, it is necessary to refer to four important policies (**Table 3**). In these, the importance of protecting grasslands, good animal husbandry practices, and sustainable grazing stand out. Likewise, the policies consider the quality of life of ranchers and productivity, jointly exposing a sustainability project in which economic benefits and environmental guarantees are integrated.

National Level Public Policies

With the aim of finding solutions to make forests profitable for their owners and, at the same time, provide goods and services to the society, the *National Management Plan for Forests with Integrated Cattle* (MBGI) was launched in 2015, responding to Law 26.331 of 2007 and promoting the design and monitoring of forests with integrated cattle, as well as the implementation of silvo-pastoral systems (Presidencia de la Nación Argentina, 2018). As pointed out by Borrás et al. (2017), the plan is an agreement that seeks to articulate national, provincial, productive and conservation public policies. The *National Program on Natural Resources, Environmental Management and Eco-regions* (PNNAT), developed in 2015, aims at contributing to the protection of the environment in the agricultural sector through a progressive improvement of sustainability in rural areas and production systems. Regarding cattle, two projects of the plan focus on the measurement of greenhouse gas emissions and wastewater treatment, through which it is intended to contribute to both prevention and environmental remediation based on methodological tools for diagnosis and evaluation, technology development, among other measures (INTA, 2017). As one of the most important public policy instruments at the national level, *Rural Change II, Innovation, and Investment* (CRII) stands out. The program emerged in 1993 but was relaunched in 2013 with the objective to support the association of small and medium-sized enterprises, agri-food, and agroindustry to strengthen the sector. For smallholder cattle producers, which are the main group of beneficiaries of the initiative, an improvement plan instrument was developed that contains an environmental sustainability component, in which aspects

FIGURE 2 | Actors involved in the design and execution of public policies in Argentina.

TABLE 3 | Overview on the provincial cattle plans of Argentina.

Province	Objectives related to sustainable cattle farming	Source
Buenos Aires, Corrientes, Entre Ríos, and Santa Fe	Pastures and Savannas of the Southern Cone of South America: initiatives for their conservation in Argentina (2010): - To promote sustainable cattle ranching in grasslands by integrating environmental conservation practices into agricultural production.	Miñarro and Marino, 2013
Santa Fé	Santafesino Cattle Plan (2018): - Launched with the purpose of generating suitable conditions for the growth of cattle production and the adoption of good animal husbandry practices. - Overarching purpose of guaranteeing the quality of life of the Santa Fé citizens and the sustainability of the sector.	Ministerio de Producción Ciencia y Tecnología, 2018
Entre Ríos	Enterriano Cattle Plan (2020): - Beef differentiation and certification. - Implementation of good animal husbandry practices.	Ministerio de Producción Turismo y Desarrollo Económico, 2020; Secretaría de Agricultura y Ganadería, 2020
Neuquén	Plan Ganadero Bovino Provincial (2021): - Implementation of technologies in the management of grasslands and water. - Development and dissemination of sustainable grazing techniques. - Improvement of the meadow productivity. - Strengthening the adaptability to environmental changes.	Gobernación de Neuquén, 2021

TABLE 4 | Successes and difficulties in the implementation of public policies in Argentina.

Successes	National Development Plan with an environmental sustainability component
	Existence of laws that promote forest protection, environmental balance, and climate change mitigation strategies
	Development of multi-sector initiatives that promote sustainable beef production
	Existence of the Argentine Sustainable Beef Board
	National public policies articulated with the provinces
	Regional policies that promote the adoption of silvo-pastoral systems and good animal husbandry practices
Difficulties	Environmental conditions that affected and still affect the cattle sector
	High political instability that has led to changes in the development model
	Increased inflation and unstable exchange rate
	Decrease in wages and reduction in national beef consumption
	Unequal implementation of the MGBI in the provinces

such as the use of agrochemicals and good water management are included (Ministerio de Agricultura Ganadería y Pesca, 2013).

Successes and Difficulties in the Implementation of Public Policies

As highlighted in **Table 4**, the aforementioned policies present both individually and collectively a diversity of successes. The presence of a sustainable beef board, as well as the inclusion of an environmental component in the *National Development Plan* and legislative advances, configure a context for the development of the cattle sector in accordance with international treaties. The policies implemented at the regional level are contributing significantly to achieving sustainability of the sector, since they set specific objectives and focus on results. Nevertheless, there also exist some difficulties, which mainly respond to conjunctural factors, such as inflation, unemployment, and the reduction of consumption, which are largely dependent on the national government in power and can vary positively or negatively in the medium- and long-term, making it difficult to determine how they will affect the cattle sector. Faced with this uncertain panorama, the international treaties signed, and the legislation developed to date become more relevant, guaranteeing that the sustainability of the sector can be preserved.

Costa Rica

Context

Costa Rica has shown continuous economic progress over the last 25 years because of opening up to foreign investments and trade liberalization. The balance between political stability and sustained growth is reflected in human development indicators and one of the lowest poverty rates in Latin America (Banco Mundial, 2021). Costa Rica's economy has focused on the export of goods and services and is characterized by low inflation and stable exchange rates, as well as an internationally competitive export sector. However, there are lags in infrastructure, which affect the different productive sectors and, particularly, green economy efforts (Gobierno de Costa Rica, 2019).

The Costa Rican cattle sector is present all over the country, with major concentration in the regions Huetar Norte (34%), Chorotega (22%) and Central (15%), while Brunca (12 %), the Caribbean (9%), and the Central Pacific (8%) regions are less important (Ministerio de Agricultura y Ganadería, 2019). The cattle sector generates annual profits of close to US$ 1.5 billion and involves 500,000 people, highlighting its social and economic importance (Ministerio de Agricultura y Ganadería, 2017). According to the National Institute of Statistics and Censuses (INEC, 2020), the country's cattle herd counts with ∼1,600,000 animals, out of which 15.4% correspond to dairy cattle, 62.7% to beef cattle, 21.7% to dual-purpose cattle and 0.2% to cattle used for farm work. In terms of beef exports, China and the United States are the most important buyers. In 2019, China imported 14,014 tons of beef with a value of US$ 56.72 million, representing 57% of Costa Rica's beef export volume (Barquero, 2020). The United States bought 23% of the beef export volume in the same year for a value of US$ 26.5 million (Procomer, 2020). It is important to mention that the livestock sector remains stable despite the crisis generated by the COVID-19 pandemic, which is due to factors such as the productive system, local consumption, and the use of national productive inputs (Garza, 2020).

Consequently, the sector operates in a stable political and economic situation, which has allowed its development and the opening of important international markets. However, Costa Rica has not yet established itself as a fundamental actor on the global beef market, generating contributions mainly at the national level in terms of employment and food security.

National Development Plans

The 2011–2014 *National Development Plan* sets out environmental protection as one of its main objectives. It suggests the incorporation of fundamental elements of sustainable development into the national policies and the reversion of natural resource degradation, while promoting an economy with minimum levels of greenhouse gas emissions in search of carbon neutrality by 2021 (Ministerio de Planificación Nacional y Política Económica, 2010). This last goal is reiterated in the 2015–2018 *National Development Plan*, as well as the need for climate change mitigation and adaptation actions of the agricultural sector (Ministerio de Planificación Nacional y Política Económica, 2014). For the period of 2019–2022, these precepts are continued by proposing specific measures, such as interventions on cattle farms applying the *NAMA model* and the strengthening of the capacities of micro-producers through silvo-pastoral system and agroforestry models (Ministerio de Planificación Nacional y Política Económica, 2019).

FIGURE 3 | Actors involved in the design and execution of public policies in Costa Rica.

Legislative Advances

The regulations regarding environmental sustainability are very broad in Costa Rica, but there exist two important decrees with direct effects on the cattle sector[1]:

- *Executive Decree 37.017*: authorizes the use of cattle slurry to improve the chemical, physical and microbiological characteristics of the soil (Presidencia de la República de Costa Rica, 2012).
- *Executive Decree 39,482:* declares the *National Strategy for Low Carbon Cattle* (ENGBC) 2015-2034 as of public interest, taking into consideration the objective of becoming a carbon-neutral country (Presidencia de la República de Costa Rica, 2012).

[1]Although there exist other regulations that stimulate sustainable cattle farming in the country, they are not cited because they were launched prior to 2010. The present study covers developments between 2010 and 2020. Law 7837 of 1998 (Law for the Creation of the Cattle Corporation), Law 8408 of 2004 (Program for the Promotion of Sustainable Agricultural Production), among others, stand out.

Multi-Sector Initiatives

In connection with the previously described policies, the Costa Rican *Cattle NAMA* stands out as an example for multi-sector initiatives (**Figure 3**). The strategy was developed in 2013 and aims at transitioning the cattle sector toward productive efficiency, adaptation to climate change and greenhouse gas emission reductions. The mitigation potential of the NAMA is understood from the promoted practices, being mainly increased forest cover, rational grazing, living fences and improvement of pastures and fertilization (Ministerio de Agricultura y Ganadería, 2019). In turn, the NAMA is expected to improve the quality of life and income of ranchers, while raising consumer awareness of the need to reduce GHG emissions from the cattle sector (UNFCCC, 2014).

Sub-national Regional Level Public Policies

In 2015, the design of *Regional Livestock Development Plans* started, which respond to local problems, but conform to the national purpose of carbon neutral cattle production. These plans

TABLE 5 | Regional level development objectives for a sustainable Costa Rican cattle sector.

Region	Objectives	Source
Central Oriental	- Development of sustainable cattle practices for the conservation of natural resources	Ministerio de Agricultura y Ganadería, 2019
Central Sur	- Implementation of technical assistance programs - Promotion of climate change mitigation and adaptation practices on cattle farms	Ministerio de Agricultura y Ganadería, 2019
Central Occidental	- Consolidation of the payments for ecosystem services program for individuals or entities that promote silvo-pastoral systems	Dirección Regional Central Occidental, 2015
Huetar Norte	- Promotion of ecosystem service programs - Establishment of silvo-pastoral systems and agroforestry for adaptation to climate change - Enabling the access to environmentally friendly technologies at primary producer level	Ministerio de Agricultura y Ganadería, 2019
Brunca	- Promotion of actions to adapt production processes to climate change	Comité Sectorial Regional Agropecuario, 2015

are being carried out in Central Oriental, Central Sur, Central Occidental, Brunca and Huetar Norte, while Chorotega, as well as the Central Pacific and the Caribbean regions show delays (**Table 5**).

National Level Public Policies

In addition to the aforementioned initiatives, Costa Rica started the *National Strategy for Low Carbon Cattle 2015–2034*, which, among others, proposes the promotion of cattle production in areas with less exposure to climate vulnerability, an increase the establishment of silvo-pastoral systems, and a set of low-carbon technologies, which e.g., includes living fences, improved pastures, forage banks, rational grazing and the moderate use of slurry (Ministerio de Agricultura y Ganadería, 2019). These purposes continue with the *National Decarbonization Plan 2018–2050*, whose ninth axis[2] exposes the importance of consolidating the eco-competitive cattle production model based on productive efficiency and the reduction of greenhouse gas emissions (Gobierno de Costa Rica, 2018). The *Costa Rican Policy for the Agri-Food Sector and Rural Development 2010–2021* incorporates climate change and agri-environmental management as one of its four pillars. It also refers to the need of promoting sustainable production systems through an ecosystem approach, for which payment schemes for ecosystem services were adopted as an instrument (Ministerio de Agricultura y Ganadería, 2011). The *Policy for the Agricultural Sector and the Development of Rural Territories 2015–2018* emphasizes on some mitigation strategies, such as economic incentives for producers that contribute to the reduction of greenhouse gas emissions or the promotion of silvo-pastoral systems (Secretaría Ejecutiva de Planificación Sectorial Agropecuaria, 2015).

[2]The 10 axes of the National Decarbonization Plan 2018–2015 are: (1) Development of an efficient and renewable mobility system. (2) Conversion of the vehicle fleet to zero emissions. (3) Boosting cargo transportation with zero emissions. (4) Consolidation of a national electricity system of renewable energy. (5) Promotion of buildings with low emissions. (6) Modernization of the industry. (7) Promotion of a waste management system. (8) Development of efficient agri-food systems. (9) Promotion of an eco-competitive livestock model. (10). Promotion of a territorial management model that allows the protection of biodiversity.

TABLE 6 | Successes and difficulties in the implementation of public policies in Costa Rica.

Successes	Political stability for several decades
	Low inflation and stable exchange rates
	National Development Plans include sustainable production components
	Diversity of national and regional public policies framed in the objective of carbon neutrality
	Regional Livestock Development Plans with focus on environmental sustainability
	Promotion of silvo-pastoral systems through both national and regional policies
	Existence of payment schemes for ecosystem services and agroforestry programs
Difficulties	Absence of a sustainable cattle roundtable or any similar initiative
	Postponement of the carbon neutrality objective

Successes and Difficulties in the Implementation of Public Policies

As shown in **Table 6**, the political and economic stability of Costa Rica, in addition to the commitments acquired through the Paris Agreement and the 2015–2030 SDGs, has allowed continuity to a set of governmental initiatives focused on sustainable production models. The commitment to achieve carbon neutrality is also reiterative, which is promoted at both the national and regional levels. However, the absence of a sustainable cattle roundtable or any similar initiative stands out as an important bottleneck, despite its potential to contribute to the articulation of public policies, information exchange and validation, and the promotion of new practices and technologies.

COMPARATIVE ANALYSIS AND DISCUSSION

From the elements raised, it is possible to identify relationships between the studied countries Colombia, Argentina, and Costa Rica. To this extent, macro and micro aspects are highlighted that allow understanding the public policies developed, while evaluating their impacts through e.g., figures on deforestation or greenhouse gas emissions, among other indicators, taking into consideration an international scenario from which environmental sustainability strategies are formulated and results from the individual governments are demanded.

Explanatory Factors of Public Policies

As has been outlined, public policies involve a set of stages that go from the identification of the problem to the evaluation of the implemented actions. Macro-level factors intervene in this process, such as the political will of the state institutions, understood as an ideological commitment to respond to the demands of citizens (Goldfrank, 2006), the articulation among the involved actors or the continuity and linkage of the programs. At the same time, micro-level factors related to the perception of the unions, associations and producers about sustainability strategies become relevant. To understand the policies outlined in this document, it is necessary to delve into both aspects.

At the macro-level, the *National Development Plans* and legislative advances of the three countries show a willingness of state institutions to promote a sustainable cattle sector. This circumstance is expressed in their *National Development Plans* and legislative advances. The strategies proposed by all are quite similar, focusing on the need to reverse the loss of natural resources, reduce greenhouse gas emissions, stop deforestation, and promote the use of silvo-pastoral systems. Although these documents usually contain general lines regarding the problems, often without being expressed in tangible indicators or results, it is necessary to recognize that they have also been the starting point for large-scale initiatives. In relation to this, the carbon-neutrality objective proposed by Costa Rica stands out, a commitment that has made the country an international benchmark for sustainability. The political will of the three countries is also expressed by the existence of national and regional multi-sector initiatives. In this regard, the capacity for articulation among the actors stands out, linking public, private, academic, and various other entities to achieve a common goal—the sustainability of the cattle sector and value chains. This aspect is fundamental since it responds to the very concept of public policy where decisions are not made by a top-down decision but are the result of collaborative efforts. It should be noted that the *Roundtable for Sustainable Cattle* in Colombia and the *Argentine Sustainable Beef Board* have had a preponderant role in the processes, since they are considered as important pools of national and international actors with different institutional backgrounds. Both institutions support the sharing of feedback and experiences made by their members with sustainable cattle practices. In the case of Colombia, its main contribution has been the creation of a base document for the formulation of a National Public Policy on Sustainable Cattle, which is currently under review by the Ministry of Agriculture and would not have been developed without the initiative of the Roundtable.

Regarding the continuity and association of the programs, disparate circumstances are evident. In the case of Costa Rica, the carbon-neutrality objective has been preserved by the different governments and *National Development Plans*, as well as in the multi-sector and regional initiatives, such as the *Cattle NAMA*. In Argentina, although not as well as defined as in Costa Rica, national policies have managed to articulate with the provinces, i.e., regarding the adoption of silvo-pastoral systems. The situation in Colombia, however, has not been so favorable, since for many years, there was no public policy that coordinated local sustainability efforts, and thus, they rather developed independently and in a disorderly manner.

These macro-level factors, which are related to the actions of governments and institutions, converge with the way in which producers perceive the public policies that seek to integrate them. In relation to silvo-pastoral systems, Braun et al. (2016) describe their numerous advantages, but also warn of their disadvantages and, consequently, occurring preventions of producers toward the implementation of related policies. Some of these difficulties refer to the lack of familiarity with the new strategies, in addition to the need for higher initial investments and a certain level of complexity compared to traditional cattle farming systems. The Ministry of Agriculture and Livestock of Costa Rica (Ministerio de Agricultura y Ganadería, 2019) reaffirms these arguments, adding that new technologies including silvo-pastoral systems, face a conservative attitude by the producers, which is due to risk aversion, minimal interest in on-farm investments, and a lack of available information. In the studied scenarios and countries, micro-factors are present to a stronger or lesser extent, with the common denominator of difficulties in financing and training for change, which leads producers to perpetuate their traditional practices. The continuation of the public policies developed in Costa Rica and Argentina, however, suggests a gradual overcoming of these barriers, while they are still more present in Colombia.

The convergence of macro- and micro-level factors has made the implementation of public policies a complex process in different regards, which highlights the importance of strengthening collaborative actions among state institutions, private sector, and other organizations, since this helps overcoming the fears producers have regarding sustainability-related policies.

From International Requirements to National Results

There exist various high-level environmental commitments that involve Colombia, Argentina, and Costa Rica, such as the Paris Agreement and the SDGs 2015–2030. These agreements are mechanisms of the international community to put pressure on national governments to regulate their production systems, beyond political or economic interests. This is how the adhesion of the countries to these initiatives, although voluntary, is not precisely due to a genuine interest, but to an imperative to which it is necessary to respond. To understand how the analyzed countries have acted in the face of such international demands by developing and adjusting their public policies and, at the same time, analyze their impact, it is important to consider some figures. In this regard, reference is made to factors such as forest cover, deforestation, and GHG emissions, which offers an overview of the current situation in terms of sustainability advances.[3]

[3]It should be noted that comparisons between countries are not exact due to the availability of data, which may vary over time or by the way in which they are disaggregated.

Argentina currently counts with 53,654,545 hectares of native forest (Ministerio de Ambiente y Desarrollo Sostenible de Colombia, 2021). As indicated by the Dirección Nacional de Bosques (2021), however, the loss of forest land for 2020 was 333,222 hectares, a rather worrying figure, and 27.8% of this deforestation corresponds to agriculture and livestock sector (only surpassed by fires, with 57.3%). According to the latest National Inventory of Greenhouse Gases, the country's total emissions for 2016 were 364 million tons of CO_2, of which 21.6% correspond to the livestock sector (Secretaría de Ambiente y Desarrollo Sustentable, 2019).

In Colombia, the achievements in terms of environmental sustainability are mixed. For the 2018–2022 period, the national government intends to implement 150,000 hectares of silvo-pastoral systems, agroforestry systems, productive reconversion, and fish farming (DNP, 2019), a low figure when compared to other countries. It has also set the goal of planting 180 million trees by the end of the period, an initiative to which the departmental governments have adhered (Ministerio de Ambiente y Desarrollo Sostenible de Argentina, 2021). Beyond these objectives, which will have to be evaluated in due course, recent figures are worrying: By 2020, according to official figures, the country generated approximately 298 million tons of CO_2 across all economic sectors (Gobierno de Colombia, 2021). Likewise, deforestation affected 2.8 million and 159,000 hectares of forest land from 2000 to 2019 and in 2020, respectively (CONPES, 2020). Although the causes of this phenomenon are multiple, including the exploitation of timber, the construction of roads, illicit crops, among others, extensive cattle farming has a share of this responsibility, and as Kaimowitz (2019) points out, largely explains the destruction of ecosystems both in Colombia and in the rest of Latin America. The author also states that cattle farming is a placeholder for guaranteeing land possession, which is much more lucrative than the production of beef or milk. This scenario is worrisome, since if sustainability initiatives in many cases have little effects on real cattle farmers, much less will they have effects if cattle farming is not the main activity. Another factor that needs to be taken into consideration is the Peace Agreement signed between the Colombian State and the Revolutionary Armed Forces of Colombia (FARC) in 2016, with the aim of ending the internal armed conflict that lasted for over 60 years. Contrary to what might be expected, the Peace Agreement intensified the already existing environmental problems, including deforestation, since the State has not taken control of the territories abandoned by the guerrilla, and reconfigured the relationships between the actors who dispute the land (e.g., landowners, peasants, illegal armed groups) (Armenteras, 2019).

Costa Rica exhibits both a stable political system and significant progress in terms of sustainability: Between 2011 and 2016, CO_2 emissions were $\sim$7 million tons per year (Gobierno de Costa Rica, 2020) and in 2018 11.7 million tons (RAND Corporation, 2020), very low figures compared to Colombia and Argentina. For its part, it should be noted that deforestation continues to be a major problem, mainly linked to the cattle sector, an activity that occupies a large part of the affected areas (MINAE et al., 2018). Between 2000 and 2016, however, the country's forest cover has increased permanently, going from 46.53 to 54.56% in this period (OCDE, 2020). There are also notable advances related to the payment of ecosystem services, which between 2010 and 2020 supported the protection of 585,945 hectares of forest land (FONAFIFO, 2021).

Consequently, the elements exposed for the three countries configure a mixture of successes, difficulties, and contrasts. In the first place, it should be noted that external demands must be understood in positive terms, since they allow the development of strategies that would not be carried out spontaneously. In other words, the importance of international organizations and treaties is recognized in a role of oversight of national governments so that they respond, through public policies and legislative advances, to the demands and problems of their citizens. Likewise, it is important to recognize that international organizations not only exercise a controlling role over national governments, but also promote financing mechanisms for the benefit of developing countries. Deforestation and GHG emissions continue to be a common problem in the three countries, although with more worrying figures in Colombia and Argentina. This highlights that those public policies that are more closely coordinated with each other and implemented over a long-term period are reflected in more encouraging processes and impacts, such as in Costa Rica.

CONCLUSIONS

The sustainable development of the cattle sector is an unquestionable need. International demands, in addition to the role of different actors, deny any possibility of continuing with traditional production practices. This scenario commits the national governments to take forceful actions, which are not always reflected in the same ways, since each country has particularities that determine the processes and, therefore, the results. Colombia, Argentina, and Costa Rica demonstrate such contrasts, and understanding their public policies implies going beyond the figures, taking into consideration their social and economic conditions.

To this extent and although the three countries express a political will to promote sustainable cattle practices, they are at different stages. This does not mean, however, that the realities are completely opposite to each other. On the contrary, the general perception is relatively similar insofar as they are all in a process of evolution and still have many objectives to achieve in the framework of the commitments made at the COP21 in Paris in 2015 and with the SDGs 2015–2030. Even though the results achieved so far are not fully satisfactory, the implemented policies should not be abandoned, but rather persist and be expressed in tangible effects. It is necessary to strengthen both the articulation between the initiatives and their actors, while overcoming the fears producers to adhere to the transition process toward sustainability.

It is important to point out that the public policies analyzed in this document have positive impacts in at least two senses. In the first place, their contributions to the environment

TABLE 7 | Recommendations for the design and implementation of public policies.

Stage	Recommendations
(1) Financing	- Promote taxes for the responsible entities/individuals of GHG emissions or deforestation - Strengthen payment systems for ecosystem services - Involve the private sector in public initiatives and projects
(2) Identification of the problem	- Update the figures on deforestation, GHG emissions, and other environmental indicators to identify the most affected territories - Develop spaces for dialogue with communities and producers to hear their opinions - Evaluate previous public policies to identify successes and difficulties and thus determine aspects for continuance or reformulation
(3) Design	- Articulate local public policies with regional and national policies - Socialize the policies with the different actors (producers, communities, and others) - Prioritize sustainability goals over private interests
(4) Implementation	- Involve communities in projects - Strengthen technical training plans for producers - Support producers in the development of infrastructure
(5) Evaluation	- Create digital platforms where citizens know budgets, objectives, and other characteristics of the initiatives - Periodically evaluate the set objectives (promoted by the governments, but with citizen participation) - Publish final reports comparing objectives and results

stand out, fostering the protection of natural resources for present and future generations. These include, for example, the implementation of a significant number of silvo-pastoral and agroforestry systems in Colombia, the conservation of forests in Argentina, or the advances in carbon neutrality in Costa Rica. Second, they lead to the benefits for the cattle sector, making it essential that producers understand that they favor themselves when implementing the strategies. This is because environmentally responsible measures prevent problems such as climate change and land degradation, phenomena with direct impacts on cattle production. In the short term, the attitude of certain international markets reluctant to buy beef and dairy products from deforestation areas stands out: sustainable practices can capture new buyers and contribute to the economic profitability of the sector at a time when socially responsible consumption is gaining strength worldwide, meaning that consumer choices are being made increasingly by considering environmental and social repercussions products and services might involve (Izquierdo et al., 2018).

Finally, it is emphasized that although the policies achieved so far provide valuable contributions, it is necessary to assume them as a first stage in a long-term process. As such, it is critical to support their continuity and increase their scalability, to achieve the goal of a wider adoption of sustainable production alternatives, such as silvo-pastoral systems. This process implies the contribution of all actors, from international organizations to public entities, cattle producers, unions and associations, the private sector, academia, and society.

RECOMMENDATIONS FOR PUBLIC POLICY

We recommend that for all the evaluated countries, public policies should be developed that contain clear objectives and budgets, facilitating their development, application, and evaluation. The national extension systems and technical assistance programs need to be strengthened to provide the involved actors (i.e., cattle producers) with required information and knowledge and stimulate the transition toward sustainable cattle farming. For Argentina, we recommend the state institutions to increase their efforts regarding deforestation policies, mainly for the Gran Chaco region. Colombia should formulate more ambitious objectives in terms of the implementation of silvo-pastoral systems, and in Costa Rica actions of national and international institutions should be articulated with the objective of establishing a multi-sector platform for sustainable cattle (like the *Roundtable for Sustainable Cattle* in Colombia or the *Argentine Sustainable Beef Board*). Such platforms stimulate sharing the different experiences made within the sector and thus help in both their achievement and in coordinating common objectives at the national level. Likewise, we recommend that in Colombia, the advances made with the Colombian Roundtable for Sustainable Cattle should continue, since they allow for the consolidation of efforts and, in the future, the monitoring of the National Public Policy of Sustainable Cattle. For all countries, we recommend the consolidation of using technological innovations that contribute to the monitoring of deforestation. Finally, communication channels should be established between the studied (and other Latin American) countries that support knowledge exchange, mutual learning and the sharing of successes and difficulties in the implementation of public policies related to the sustainable intensification of the cattle sector. **Table 7** proposes more specific recommendations for the three countries, considering the difficulties identified (**Tables 2**, **4**, **6**) in our study. Common problems are highlighted, such as the lack of economic resources to develop public policies and enforce laws, for which some financing options are proposed. Likewise, the importance of promoting citizen participation in each of the stages of the policies is highlighted, achieving not only that the objectives are consistent with the needs of the territories and communities, but also that the processes carry out an adequate management of public resources. It should be noted that, while the differences between the three countries are recognized, such recommendations fit all of them, whether in the national context or in local settings. In turn, due to the economic, cultural, and political similarities in Latin America, the points made are relevant at the regional level.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LL, MD, and SB: conceptualization, methodology, writing the original draft and review and editing, and resources. LL and MD: formal analysis. SB and MD: supervision. SB: funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

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A Transcriptomic Analysis of Stylo [*Stylosanthes guianensis* (Aubl.) Sw.] Provides Novel Insights Into the Basis of Salinity Tolerance

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Tropical areas have a large distribution of saline soils and tidal flats with a high salinity level. Salinity stress is a key factor limiting the widespread use of tropical forage such as *Stylosanthes guianensis* (Aubl.) Sw. This study was designed to screen the salinity tolerance of 84 *S. guianensis* accessions; In a greenhouse experiment, plants were subjected to Hoagland solution or Hoagland solution with 200 mM NaCl for up to 15 days. Salinity tolerant accession CIAT11365 and salinity sensitive accession FM05-2 were obtained based on withered leaf rate (WLR). Further verification of salinity tolerance in CIAT11365 and FM05-2 with different salinity gradients showed that salinity stress increased WLR and decreased relative chlorophyll content (SPAD), maximum photochemical efficiency of photosystem II (Fv/Fm), and photosynthetic rate (Pn) in FM05-2, but CIAT11365 exhibited lower WLR and higher SPAD, Fv/Fm, and Pn. Leaf RNA-Seq revealed that Ca^{2+} signal transduction and Na^+ transport ability, salinity tolerance-related transcription factors and antioxidant ability, an increase of auxin, and inhibition of cytokinin may play key roles in CIAT11365 response to salinity stress. The results of this study may contribute to our understanding of the molecular mechanism underlying the responses of *S. guianensis* to salinity stress and also provide important clues for further study and in-depth characterization of salinity resistance breeding candidate genes in *S. guianensis*.

Keywords: *Stylosanthes guianensis* (Aubl.) Sw., salinity tolerance, differentially expressed genes, transporter, hormones

INTRODUCTION

Soil salinity is a major limiting factor in agriculture in terms of yield and productivity (Munns and Tester, 2008). Most forage species are salt sensitive; the effect of NaCl on forage is caused by both the reduction of water availability caused by high Na^+ concentration and the toxic effect of Na^+ and Cl^- on plants. Elucidating salinity-tolerant mechanisms, mining salinity-tolerant genes, and improving crop salinity tolerance are good strategies to deal with increased saline soil (Deinlein et al., 2014). Research on the salt-tolerant mechanism of plant includes forage, improvement, and utilization of saline soil, which have become the focus of recent studies (Abiala et al., 2018; Zelm et al., 2020; Zhao et al., 2020).

Stylo (*Stylosanthes* spp.) is an important forage legume that is grown in tropical and subtropical areas, mainly used for pasture and green manure. The Chinese Academy of Tropical Agricultural Sciences (CATAS) introduced more than 500 Stylo accessions to China from the International Center for Tropical Agriculture (CIAT) or other countries since the 1960's. Stylo has become a very important tropical forage legume in tropical areas of China. Till now, CATAS has cultivated 15 nationally approved Stylo varieties (Huang et al., 2017). *Stylosanthes guianensis* (Aubl.) Sw. is one of the most important species in *Stylosanthes* spp., a subshrub with height ranging from 0.6 to 1.2 m and a stem diameter of 0.3–0.8 cm, which grows well in tropical and subtropical climates, and is adapted to acid and drought soils. *S. guianensis* also has the earliest origin, the most branches, the richest genetic diversity, and the widest distribution in *Stylosanthes* spp. Almost half of stylo accessions in the CATAS seed bank are *S. guianensis* (249 accessions) (Jiang et al., 2017).

Coastal saline soils and tidal flats are distributed in vast areas in tropical regions (Ivushkin et al., 2019), and have an adverse effect on tropical forage yield and quality. Screening of salinity-tolerant tropical forage such as Stylo is a sustainable and economical viable option of improving and utilizing such coastal saline soils. Previous studies have shown the high variation of salinity tolerance in 67 accessions from 23 species of *Stylosanthes* spp., *S. erecta* P.Beauv. CIAT11900, and *S. hippocompoides* Mohlenbr. Fine stem, *S. hamata* (L.) Taub. CIAT1010, *S. fruticosa* (Retz.) Alston CIAT11052, *S. debilis* M.B. Ferreira & Sousa Costa CIAT11927, and *S. hamata* Verano have the relatively best salinity tolerance with 200 mM NaCl for 15 days. Only 10 *S. guianensis* accessions were evaluated for their salinity tolerance and most of them had intermediate- or above-level performance (Liu et al., 2017). Considering the high biomass advantage and the high diversity of *S. guianensis*, it is essential to evaluate the salinity tolerance of *S. guianensis* in a wider range of accessions and explore the molecular mechanism of response to salinity stress.

The objectives of this study were to (1) Examine the salinity tolerance of 84 accessions of *S. guianensis*; (2) Clarify the performance of salinity-tolerant and salinity-sensitive *S. guianensis* with different salinity concentrations; and (3) Explore the salinity-tolerant mechanisms and differentially expressed genes (DEGs) by transcriptomic analysis.

MATERIALS AND METHODS

Experiment 1: Screening of Salinity Tolerance in 84 *S. guianensis* Accessions

This study was carried out in the greenhouses of CATAS, Danzhou, Hainan, China. A total of 84 *S. guianensis* accessions were screened for their salinity tolerance (**Supplementary Material 1**). About 6- to 8-cm-long stems of each *S. guianensis* accessions were taken from the field gene bank of CATAS, wrapped with sponge, and planted on a foam board. The foam board was floated in a plastic box (110 × 90 × 20 cm) filled with 40 L Hoagland solution. Pumps supply with oxygen to each plastic box. The equivalent of water lost through evaporation and transpiration was supplied into the plastic box every day, and solutions were changed every week. Salinity treatment was conducted with 200 mM NaCl after 2 months' cultivation when the height of seedlings reached 20–25 cm; NaCl concentration was gradually increased to 200 mM by adding 50 mM NaCl per 12 h to avoid sudden death. Withered leaf rate (WLR) was measured at 15 days of salinity treatment; and a few yellow leaves were removed before salt treatment to avoid impact of WLR. WLR (%) = number of leaves with withered symptoms more than 50%/total number of leaves × 100 (Liu et al., 2017).

A split plot design was used with salt stress treatments as the main plots and the accessions as the subplots. Each treatment had 3 replicates. The 84 *S. guianensis* accessions grown in the plastic box were randomly placed.

Experiment 2: Physiological Responses of 2 Accessions of *S. guianensis* to Different Salinity Levels and Transcriptomic Analysis

Two *S. guianensis* accessions, CIAT11365 (salinity tolerant, ST) and FM05-2 (salinity sensitive, SS), were selected based on experiment 1. Seeds of both accessions were sowed into plastic pots with 20 cm diameter and 24 cm height, filled with sand. Plants were maintained in the greenhouse for 2 months and then treated with 100, 200, 300, and 400 mM NaCl. Both accessions were irrigated daily with 400 ml of Hoagland solution or salt solution for 15 days, and the redundant solution at the bottom of the pot was drained to avoid salinity accumulation. Each treatment had 4 replicates. The exposure of plants to increasing salt concentration allowed a gradual acclimation to salinity conditions to avoid sudden death at high salt concentration. Leaf samples were collected at 5 days with 200 mM NaCl for transcriptomic analysis.

WLR, relative chlorophyll content (SPAD), maximum photochemical efficiency of photosystem II (Fv/Fm), and photosynthetic rate (Pn) were estimated in this experiment. SPAD value was measured on upper-middle leaves with a SPAD meter (TYS-B, Zhejiang, China); Fv/Fm was estimated with a chlorophyll fluorometer (PAM-2500, Heinz Walz GmbH, Effeltrich, Germany) after leaves were dark-adapted for 15 min, and Pn was measured using a portable photosynthesis system (Li-6400 XT, LICOR, Inc, Lincoln, NE, USA).

Transcriptomic Analysis

Total RNA and mRNA Isolation

Total RNA was extracted using Trizol reagent (Invitrogen, CA, USA) purified using the RNeasy Plant Mini kit (Qiagen) according to the manufacturer's protocol. RNA purity was checked using the kaiaoK5500®spectrophotometer (Kaiao, Beijing, China); RNA integrity and concentration were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer

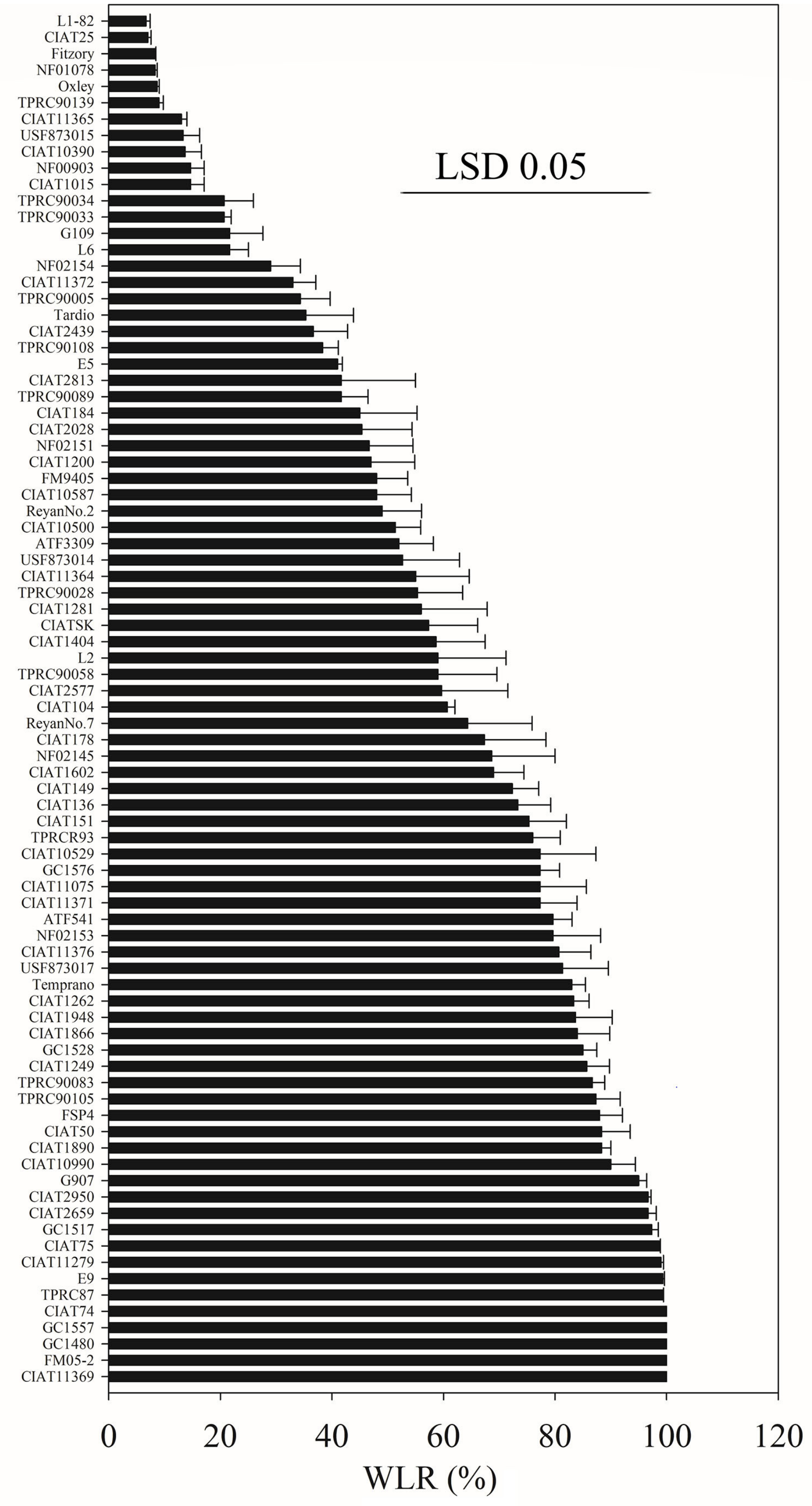

FIGURE 1 | The range in WLR vertical bars of 84 *S. guianensis* accessions treated with 200 mM NaCl for 15 days. The LSD 0.05 was 45.39. Values are means ($n = 3$).

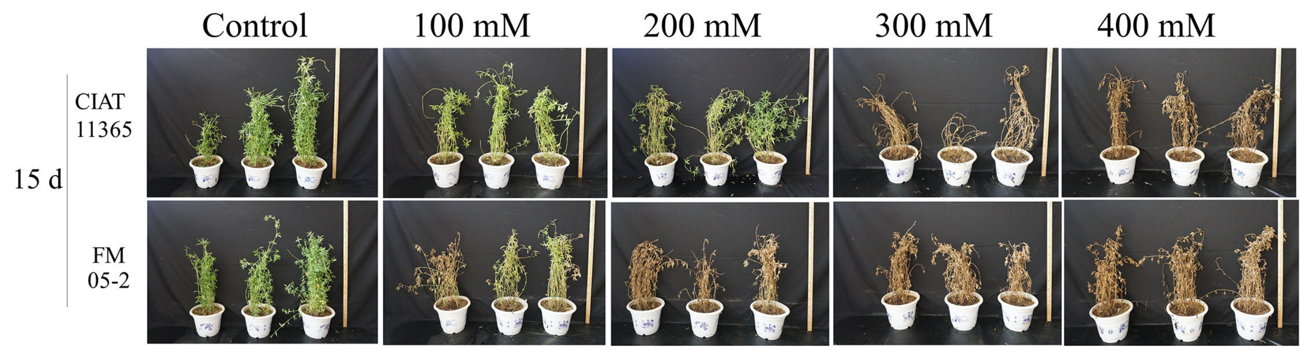

FIGURE 2 | Phenotype of two *S. guianensis* accessions CIAT11365 (salinity tolerant, ST) and FM05-2 (salinity sensitive, SS) under different NaCl concentrations (100–400 mM) at 15 days.

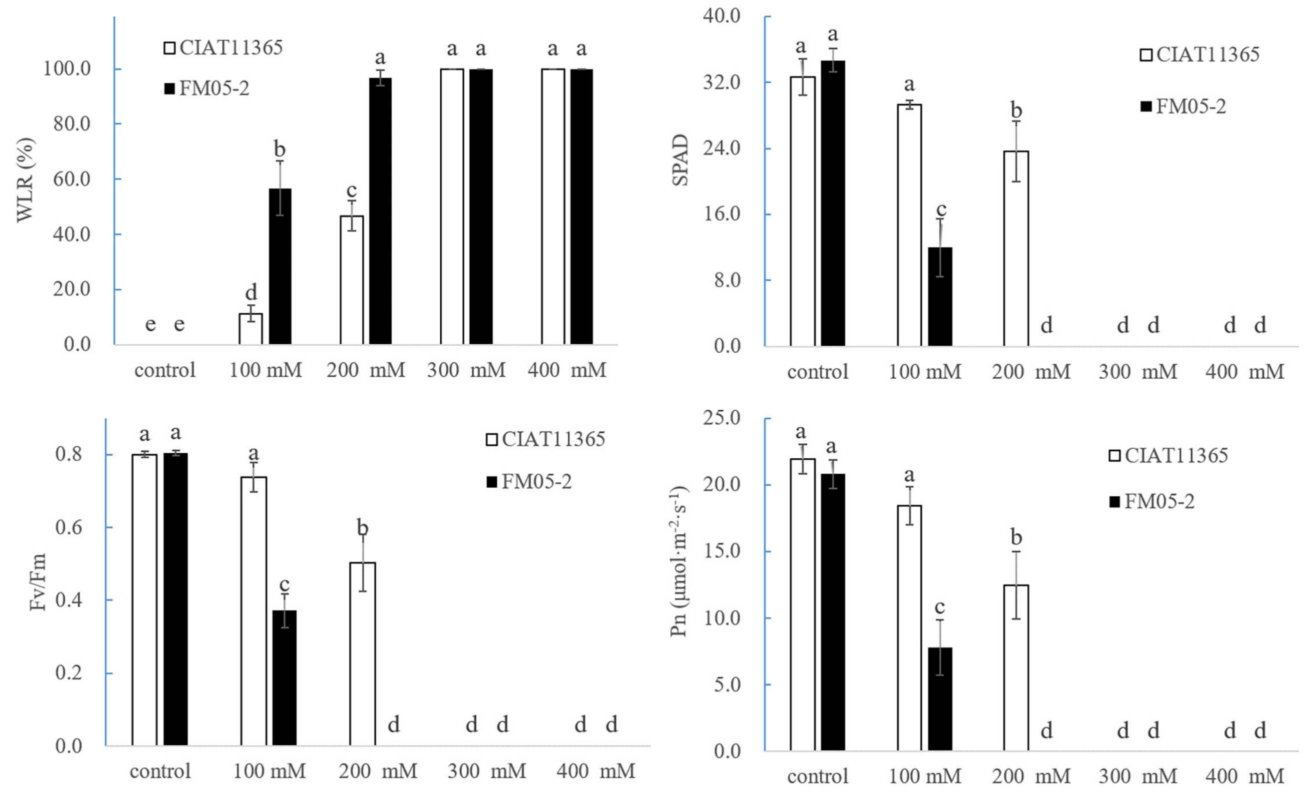

FIGURE 3 | Effect of different salinity concentrations to WLR, SPAD, Fv/Fm, and Pn in ST and SS. ST, CIAT11365; SS, FM05-2.

2100 system (Agilent Technologies, CA, USA) and agarose gel electrophoresis.

Transcriptome Sample Preparation and Transcriptome Sequencing

A total amount of 2 μg of RNA per leaf sample was used for the RNA sample preparations, sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (#E7530L, NEB, USA) following the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First-Strand Synthesis Reaction Buffer (5X). First-strand cDNA was synthesized using random hexamer primer and RNase H. Second-strand cDNA synthesis was subsequently performed using buffer, dNTPs, DNA polymerase I, and RNase H. The library fragments were purified with QiaQuick PCR kits and eluted with EB buffer, and then terminal repair, A-tailing, and the added adapter were implemented. The aimed

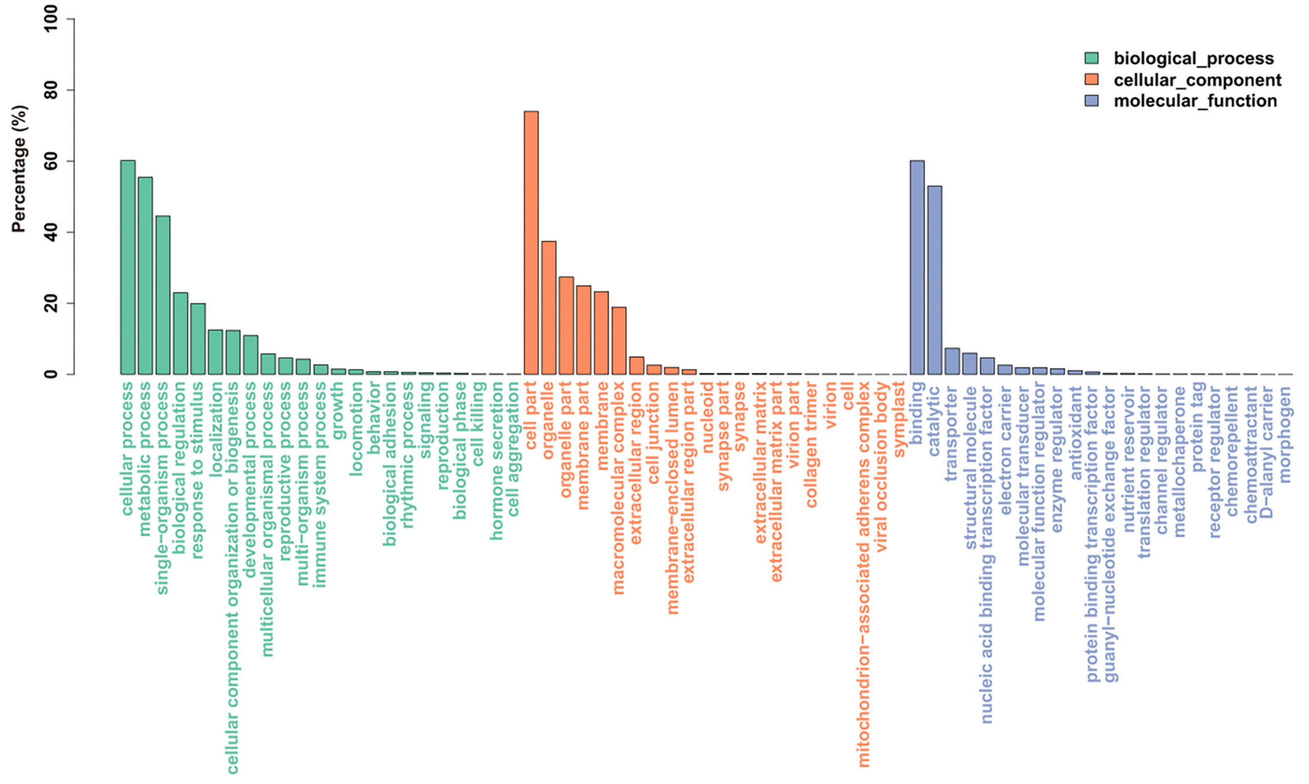

FIGURE 4 | Histogram of gene ontology (GO) classification. The results are summarized in three main categories: biological process, cellular component, and molecular function.

products were retrieved, PCR was performed to complete the library. Preliminary quantification of RNA concentration of library was obtained using Qubit® RNA Assay Kit in Qubit® 3.0 then diluted to 1 ng/μl. Insert size was assessed using the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA), and qualified insert size was accurately quantified using the StepOnePlus^{TM} Real-Time PCR System (Library valid concentration >10 nM). The clustering of the index-coded samples was performed on a cBot cluster generation system using HiSeq PE Cluster Kit v4-cBot-HS (Illumina) according to the manufacturer's instructions. After cluster generation, the libraries were sequenced on an Illumina platform and 150-bp paired-end reads were generated.

Preprocessing and de novo Assembly

De novo assembly was employed to construct transcripts from these RNA-Seq reads because of the absence of reference genomic sequences. Trinity software was used for *de novo* assembly of the Illumina reads. For a quality control before subsequent analysis, raw data were processed with Perl scripts. The raw reads were processed by removing reads containing adapter, the adaptor-polluted reads, the low-quality reads, and reads with number of N bases accounting for more than 5%. The obtained Clean Data after filtering will be subjected to statistics analyses on its quality, including Q30, data quantity and quality, base content statistics, etc.

The software Trinity was used for *de novo* assembly, which was developed at the Broad Institute and the Hebrew University of Jerusalem. Trinity represents a novel method for the efficient and robust *de novo* reconstruction of transcriptomes from RNA-seq data. Trinity partitions the sequence data into many *de Bruijn* graphs, each representing the transcriptional complexity at a given gene or locus. Each graph was processed independently to extract the full-length splicing isoforms and to tease apart transcripts derived from paralogous genes.

Unigene Annotation and Classification

Trinotate was used for performing the functional annotation of unigenes and ORFs. Trinotate is a comprehensive annotation suite designed for automatic functional annotation of transcriptomes, particularly for *de novo* assembled transcriptomes, from model to non-model organisms. Trinotate makes use of a number of different well-referenced methods for functional annotation including homology search to known sequence data (BLAST+/SwissProt), protein domain identification (HMMER/PFAM), protein signal peptide and transmembrane domain prediction (singalP/tmHMM), and comparison to current annotation databases (EMBL Uniprot eggNOG/GO Pathways databases).

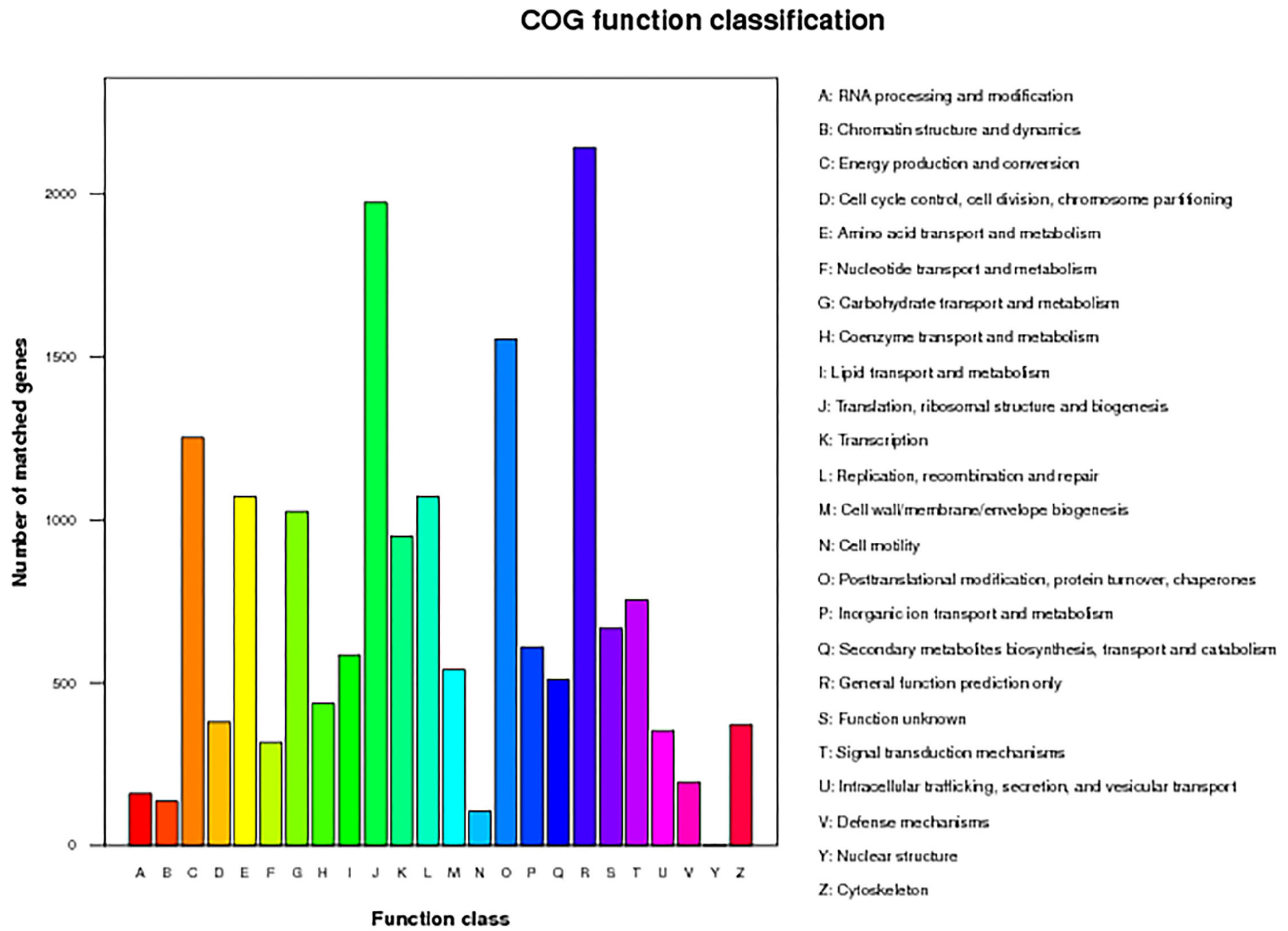

FIGURE 5 | The euKaryotic Ortholog Groups (KOG) annotation of putative proteins. All 14,313 putative proteins showing significant homology to those in the KOG database were functionally classified into 26 molecular families.

Quantification of Gene Expression Levels and Differential Expression Analysis

Read count for each gene in each sample was counted by HTSeq v0.6.0, and RPKM (Reads Per Kilobase Millon Mapped Reads) was then calculated to estimate the expression level of genes in each sample (Guo et al., 2013). DEGseq v1.18.0 was used for differential gene expression analysis between two samples with non-biological replicates. Under the assumption that the number of reads deriving from a gene (or transcript isoform) follows a binomial distribution, DEGseq is proposed based on MA plot and widely used for differential gene expression analysis. The *p*-value could be assigned to each gene and adjusted by the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with $q \leq 0.05$ and $|\text{log2\_ratio}| \geq 1$ are identified as DEGs. DESeq (v1.16) was used for differential gene expression analysis between two samples with biological replicates using a model based on the negative binomial distribution. The *p*-value could be assigned to each gene and adjusted by the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with $q \leq 0.05$ and $|\text{log2\_ratio}| \geq 1$ are identified as DEGs.

The GO (Gene Ontology, http://geneontology.org/) enrichment of DEGs was implemented by the hypergeometric test, in which *p*-value is calculated and adjusted as *q*-value, and data background is genes in the whole genome. GO terms with $q < 0.05$ were considered to be significantly enriched. GO enrichment analysis could exhibit the biological functions of the DEGs. KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.kegg.jp/) is a database resource containing a collection of manually drawn pathway maps representing our knowledge on the molecular interaction and reaction networks. The KEGG enrichment of DEGs was implemented by the hypergeometric test, in which *p*-value was adjusted by multiple comparisons as *q*-value. KEGG terms with $q < 0.05$ were considered to be significantly enriched.

Quantitative Real-Time PCR Analysis

The expression of selected genes was validated by quantitative real-time PCR (qRT-PCR); the same RNA samples as the RNA-seq library construction were used. First-strand cDNA fragments were synthesized using the cDNA synthesis kit (Fermentas, Burlington, Ontario, Canada). Gene primers were designed using

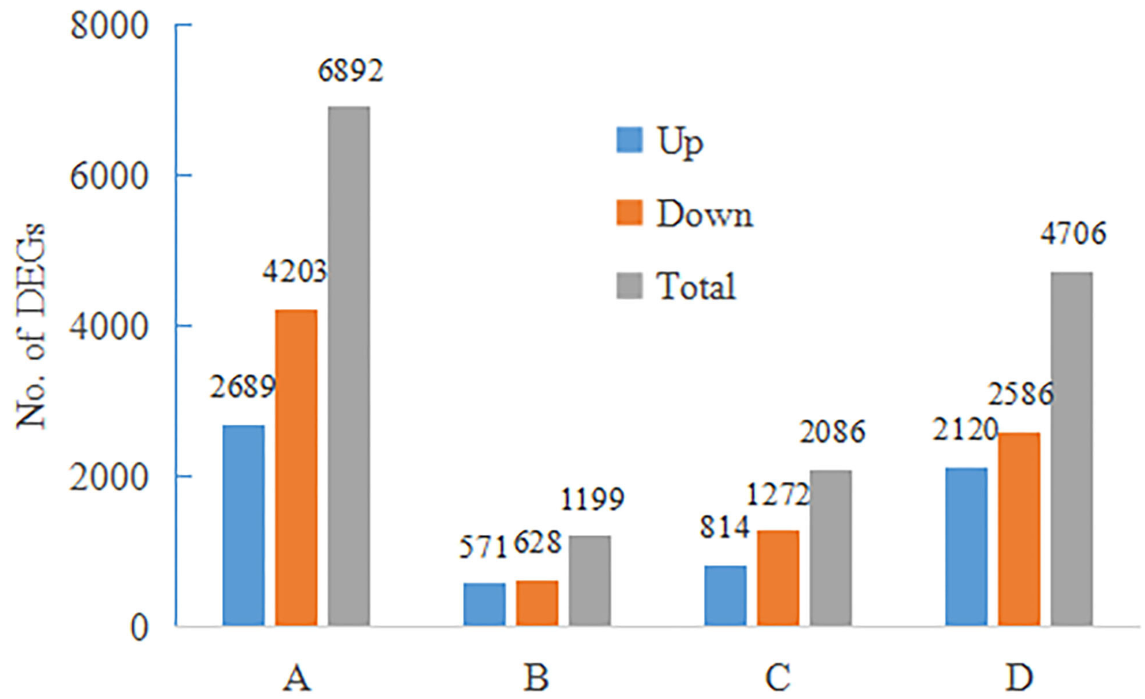

FIGURE 6 | Differentially expressed genes (DEGs) were identified between 4 comparisons, including A (control ST_control SS), B (salt ST_control ST), C (salt SS_control SS), and D (salt ST_salt SS).

Primer 5 software. Ef1A gene was used as reference gene. Each sample had three biological replicates and each biological replicate had three technical replicates; 20 µl of reaction includes 5 µl of cDNA, 10 µl of 2 × SYBR® Premix Ex Taq™ (Tli RNaseH Plus), and 0.5 µl of the forward and reverse primers. The synthesis reaction lasted 39 cycles at 95°C for 10 S and 60°C for 34 S.

Data Analysis

All data were subjected to analysis of variance (SAS 8.1; SAS Institute Inc., Cary, NC). Differences among the mean values were assessed by the least significant difference (LSD) test at $p = 0.05$.

RESULTS

Screening of Salinity Tolerance in 84 *S. guianensis* Accessions

WLR showed that *S. guianensis* accessions had large variation in salinity tolerance (**Figure 1**). Schofield, L1-82, CIAT25, NF01078, Fitzory, Oxley, TPRC90139, ReyanNo.5, CIAT11365, CIAT10594, USF873015, and CIAT10390 had relatively lower WLR (<14%) at 15 days of 200 mM NaCl stress, and these accessions were considered to be salinity tolerant. In contrast, CIAT74, GC1557, GC1480, FM05-2, CIAT11369, E9, TPRC87, CIAT11279, CIAT75, GC1517, CIAT2950, and CIAT2659 were considered to be salt-sensitive accessions with a relatively higher WLR (>96%) at 15 days of 200 mM NaCl stress.

Physiological Responses of 2 Accessions of *S. guianensis* to Different Salinity Levels

Based on WLR (**Figure 1**), two accessions, CIAT11365 (salinity tolerant, ST) and FM 05-2 (salinity sensitive, SS), were selected for physiological responses at different salinity levels, and the result showed that ST can survive at 15 days of 200 mM treatment (WLR = 46.67%) whereas SS almost have no green leaves left (WLR = 100%) (**Figures 2**, **3**). WLR, SPAD, Fv/Fm, and Pn showed that ST and SS had a decline trend under 100–400 mM NaCl treatment, but ST had a significant higher value of WLR, SPAD, Fv/Fm, and Pn at 15 days of 100–200 mM NaCl treatment than SS (**Figure 3**).

Transcriptome Sequencing and Assembly

An overview of the RNA-Seq reads is presented in **Supplementary Material 2**. A total of 151,356 contigs were obtained from the clean reads with a mean length of 1,118.6 bp and length ranging from 201 to 16,419 bp (**Supplementary Material 3**). Among the 151,356 contigs, 74,515 unigenes were obtained with an average length of 879.4 bp. The length of a unigene ranged from 201 bp to 16,419 bp; N50 was 1,617 bp and N90 was 320 bp. RNA-seq data from this article can be found in the NCBI SRA database under the BioProject ID: PRJNA771864.

Gene Annotation

The unigenes were annotated by searching against the seven public databases (**Supplementary Material 4**). A total of 38,426 unigenes (51.57%) were matched in the NR database, 30,953

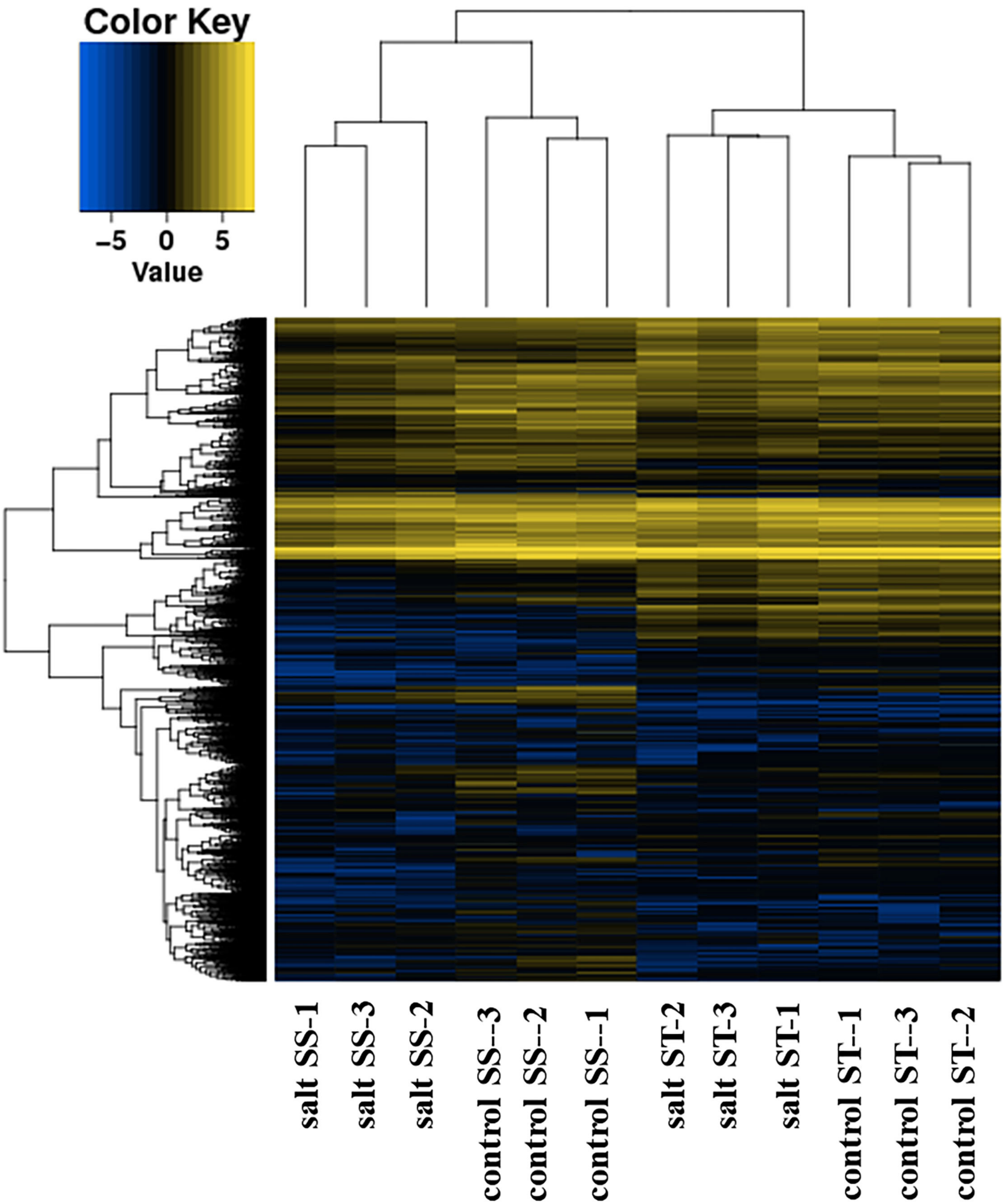

FIGURE 7 | Hierarchical clustering of the differentially expressed genes.

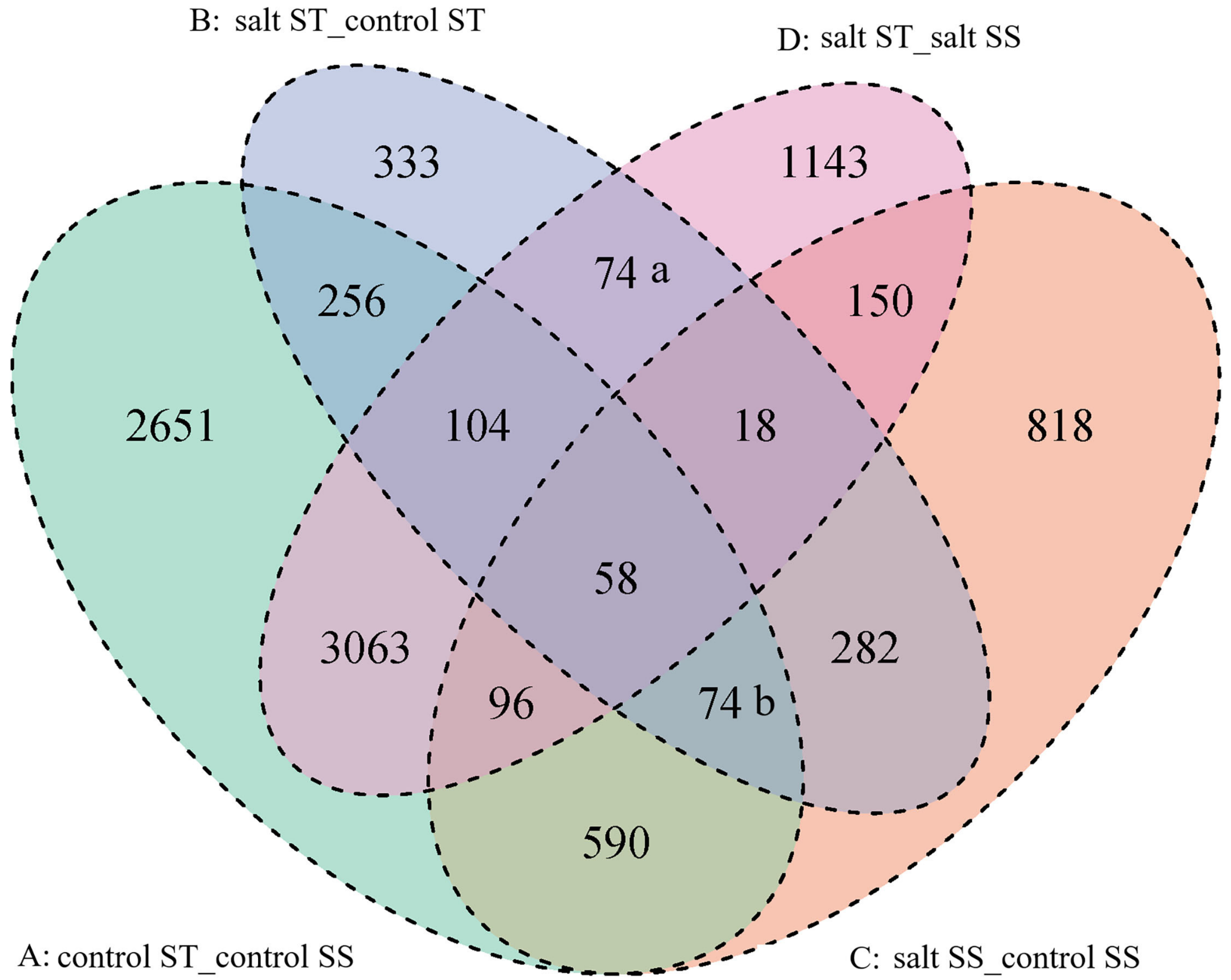

FIGURE 8 | Venn diagram of differentially expressed genes. The sum of the numbers in each large circle represents the total number of differentially expressed genes between comparison, and the overlap part of the circles represents common differentially expressed genes between comparisons. ST: salinity-tolerant accession CIAT11365, SS: salinity-sensitive accession FM 05-2.

(41.54%) in the BLASTX database, 30,420 (40.82%) in the Prot database, 29,963 (40.21%) in the GO database, and 23,209 (31.15%) in the PFAM database. A total of 50,529 unigenes (67.81%) were successfully annotated in NR, BLASTX, Prot, GO, PFAM, BLASTP, NT, eggnog, KO, TmHMM, or SignalP databases.

Gene Ontology Classification

For GO analysis, there were 29,963 unigenes divided into three ontologies (**Figure 4**). "Cellular process"-, "metabolic process"-, and "single-organism process"-related genes were mainly included in the biological process category; "cell part"-, "organelle"-, and "organelle part"-related genes were mainly included in the cellular component category; for the molecular function category, "binding," "catalytic," and "transporter" were the main genes. There were 14,313 unigenes assigned to KOG classification divided into 26 function classes (**Figure 5**). The top 4 classes were "General functional prediction only" (2,143), "Translation, ribosomal structure, and biogenesis" (1,973), "Posttranslational modification, protein turnover, chaperones" (1,555), and "Energy production and conversion" (1,252), respectively.

Differential Expression Genes Analysis Under Salinity Treatments

DEGs ($p_{\text{adj}} < q \leq 0.05$ and log2FoldChange|log2_ratio| ≥ 1) were identified between 4 comparisons, including A (control ST_control SS), B (salt ST_control ST), C (salt SS_ control SS), and D (salt ST_salt SS). The number of DEGs detected in A, B, C, and D were 6,892, 1,199, 2,080, and 4,706, respectively (**Figure 6**). DEGs are clustered by hierarchical clustering using up and down gene regulation and gene enrichment analysis (**Figure 7**). The blue color represents low gene expression quantity, and the yellow represent high gene expression quantity. **Figure 6** showed that more DEGs were detected in comparison A and D than in B and C, suggesting that there are more DEGs in different

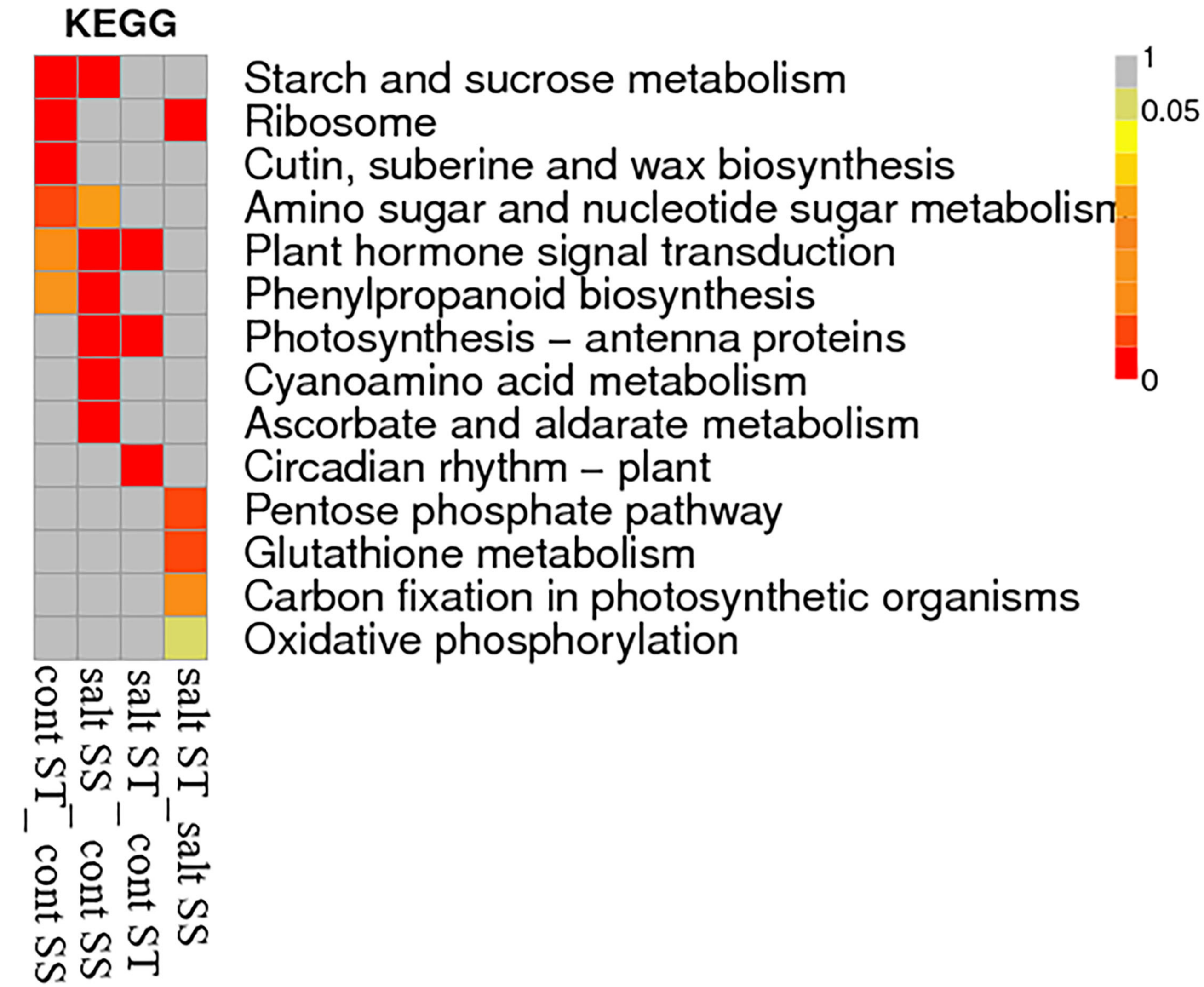

FIGURE 9 | Heatmap of KEGG pathway enrichment analysis for DEGs of four comparisons, namely, control ST_control SS, salt ST_control ST, salt SS_ control SS, and salt ST_salt SS.

accessions than in the same accession, and more up-expressed genes in salt and control of ST than in salt and control of SS (**Figure 7**).

Venn diagram analysis revealed the unigenes were overlapping between the four comparisons (**Figure 8**). There were (a) 74 DEGs in two hybrid combinations of B and D, 18 DEGs in two hybrid combinations of B, C, and D, and 282 DEGs in two hybrid combinations of B and C; and (b) 74 DEGs in two hybrid combinations of A, B, and C, and 58 DEGs in four hybrid combinations of A, B, C, and D. The DEGs were either up- or downregulated; those five groups may contain the most important DEGs that contributed to the salinity tolerance of ST.

Heatmap of KEGG pathway enrichment analysis for DEGs showed that circadian rhythm-plant was a special pathway in comparison to salt ST_control ST; pentose phosphate pathway, glutathione metabolism, carbon fixation in photosynthetic organisms, and oxidative phosphorylation were special pathways in comparison to salt ST_salt SS; and plant hormone signal transduction was a common pathway in comparison to control ST_control SS, salt SS_ control SS, and salt ST_control ST but not enriched in salt ST_control ST (**Figure 9**).

Validate the DEGs by Real-Time RT-PCR Analysis

To validate the data from RNA-sequencing, 41 DEGs mainly including salt response genes from 74 (a), 282, 18, 74 (b), and 58 groups of Venn diagram were selected for real-time RT-PCR analysis in ST and SS accessions in response to salt stress (**Figure 8**). The primers of selected genes are listed in **Supplementary Material 5**. The qRT-PCR results showed a strong correlation with the RNA-seq-generated data (**Table 1**). Among the 41 DEGs, 14 had a significant difference between salt ST and salt SS according to the RT-PCR result (Table 4 in **Supplementary Material 6**), 12 DEGs in salt ST had a significant increase compared to salt SS,

TABLE 1 | Comparisons of RNA-Seq and RT-PCR in 41 DEGs of ST and SS.

Gene id	Venn group (Figure 8)	GO_biological_process			RNA-Seq result of ST			RT-PCR result of ST			RNA-Seq result of SS			RT-PCR result of SS	RT-PCR result
			Expression of control	Expression of salt	Significant	Up/down	Significant	Up/down	Expression of control	Expression of salt	Significant	Up/down	Significant	Up/down	Significance of salt ST vs. salt SS
c38854_g1	74a, 18, 58, 282, 74b	PREDICTED: cytokinin dehydrogenase 6-like [Glycine max]	2136.2	756.5	Yes	Down	*	Down	1968.6	924.0	Yes	Down	**	Down	Significant high
c25614_g1	74a, 18, 58, 282, 74b	PREDICTED: cation/H(+) antiporter 15-like [Cicer arietinum]	1030.7	73.8	Yes	Down	*	Down	1072.8	31.6	Yes	Down	***	Down	Significant high
c34374_g1	74a, 18, 58, 282, 74b	Nodulin MtN21/EamA-like transporter family protein [Medicago truncatula]	56.2	123.3	Yes	Up	**	Up	57.3	122.2	Yes	Down	ns		Significant high
c41938_g5	74a, 18, 58, 282, 74b	Peroxidase 2 [Sesbania rostrata]	43.0	107.0	Yes	Up	**	Up	48.1	102.0	Yes	Up	ns		Significant high
c31061_g1	74a, 18, 58, 282, 74b	PREDICTED: transmembrane protein 45A-like [Cicer arietinum]	81.1	20.2	Yes	Down	ns		81.9	19.4	Yes	Down	*	Down	ns
c25356_g1	74a, 18, 58, 282, 74b	Oligopeptide transporter OPT family protein [Medicago truncatula]	274.9	98.4	Yes	Down	*	Down	271.6	101.6	Yes	Down	ns		ns
c34262_g1	74a, 18, 282, 74b	Annexin [Arachis hypogaea]; response to salt stress	2600.6	5916.6	Yes	Up	ns		1903.3	####	Yes	Up	ns		Significant high
c36834_g1	18, 58, 282, 74b	FAD binding domain; |response to oxidative stress	229.4	98.3	Yes	Down	ns		240.5	87.1	Yes	Down	ns		ns
c39804_g2	74b, 18, 282	PREDICTED: cysteine-rich receptor-like protein kinase 10-like isoform X2 [Glycine max]; Salt stress response/antifungal	174.2	48.2	Yes	Down	ns		206.2	16.2	Yes	Down	**	Down	ns
c18276_g1	74b,18, 282	Putative aquaporin PIP-type 7a [Glycine soja]; response to stress	318.7	122.1	Yes	Down	*	Down	351.9	88.9	Yes	Down	**	Down	ns
c34502_g2	74a, 282	Vacuolar amino acid transporter 1 [Glycine soja]	138.2	58.5	Yes	Down	*	Down	149.0	47.6	Yes	Down	***	Down	Significant high
c41881_g1	74a	Cysteine-rich receptor-kinase-like protein [Medicago truncatula] [Medicago truncatula]	84.5	31.9	Yes	Down	**	Down			No	Down	ns		ns

(Continued)

TABLE 1 | Continued

Gene id	Venn group (Figure 8)	GO_biological_process			RNA-Seq result of ST			RT-PCR result of ST			RNA-Seq result of SS			RT-PCR result of SS	RT-PCR result
			Exp ression of control	Exp ression of salt	Signi ficant	Up/ down	Signi ficant	Up/ down	Exp ression of control	Exp ression of salt	Signi ficant	Up/ down	Signi ficant	Up/ down	Signi ficance of salt ST vs. salt SS
c40459_g1	74a	Auxin-induced protein [Vigna radiata]	2020.2	790.7	Yes	Down	*	Down			No	Down	ns		Significant high
c32634_g1	74a	NAC-like transcription factor [Arachis hypogaea] stress related	21.9	253.0	Yes	Up	**	Up			No	Down	ns		Significant high
c33712_g1	74a	Disease resistance protein (CC-NBS-LRR class) family protein [Medicago truncatula]	77.9	25.0	Yes	Down	ns				No	Down	**	Down	Significant low
c33369_g1	74a	Redoxin [Tilletiaria anomala UBC 951]	12.1	102.9	Yes	Down	ns				No	Down	ns		Significant low
c61867_g1	74a	PREDICTED: cation/calcium exchanger 1-like [Glycine max]	357.0	97.9	Yes	Down	ns				No	Down	**	Down	ns
c57911_g1	74a	PREDICTED: LOW QUALITY PROTEIN: myb-related protein Zm1 [Glycine max] check downstream genes in Arabidopsis	15.6	2.5	Yes	Down	ns				No	Down	**	Down	ns
c51380_g1	74a	Triose-phosphate transporter family protein [Medicago truncatula]	94.0	42.7	Yes	Down	*	Down			No	Down	ns		ns
c41881_g3	74a	PREDICTED: cysteine-rich receptor-like protein kinase 25-like [Glycine max]	11.2	2.4	Yes	Down	*	Down			No	Down	*	Down	Significant high
c34633_g2	74a	Vacuolar cation/proton exchanger [Medicago truncatula]	75.8	36.0	Yes	Down	ns				No	Down	*	Down	ns
c34566_g1	74a	Vacuolar cation/proton exchanger[Medicago truncatula]	1029.7	230.1	Yes	Down	ns				No	Down	*	Down	ns
c32315_g1	74a	PREDICTED: potassium channel SKOR-like [Glycine max]	105.6	31.7	Yes	Down	*	Down			No	Down	ns		ns
c31504_g2	74a	Plant-pathogen	30.4	7.7	Yes	Down	ns				No	Down	**	Down	ns
c27440_g1	74a	GRA–TF	306.7	134.7	Yes	Down	*	Down			NO	DOWN	**	Down	ns

(Continued)

TABLE 1 | Continued

Gene id	Venn group (Figure 8)	GO_biological_process	RNA-Seq result of ST				RT-PCR result of ST		RNA-Seq result of SS				RT-PCR result of SS		RT-PCR result
			Exp ression of control	Exp ression of salt	Signi ficant	Up/ down	Signi ficant	Up/ down	Exp ression of control	Exp ression of salt	Signi ficant	Up/ down	Signi ficant	Up/ down	Signi ficance of salt ST vs. salt SS
c27130_g2	74a	PREDICTED: NAC domain-containing protein 73-like [Cicer arietinum]	52.6	16.7	Yes	Down	ns				NO	DOWN	*	Down	ns
c23342_g1	74a	RING-H2	18.4	6.2	Yes	Down	**	Down			No	Down	**	Down	ns
c10949_g1	74a	K(+)/H(+) antiporter [Medicago truncatula]	171.0	61.5	Yes	Down	**	Down			No	Down	*	Down	ns
c42060_g8	74a	Plant-pathogen	13.7	89.0	Yes	Up	ns				No	Down	*	Down	ns
c41837_g4	74a	plant-pathogen	6.6	31.1	Yes	Up	ns				No	Down	*	Down	ns
c25043_g1	74a	PREDICTED: putative oxidoreductase TDA3 [Gossypium raimondii]	36.4	74.4	Yes	Up	*	up			No	Down	ns		ns
c20795_g1	74a	PREDICTED: probable glutathione S-transferase parA [Cicer arietinum]	13.1	56.2	Yes	Up	ns		2.4	66.9	Yes	Up	**	Up	ns
c34262_g2	282	Calcium ion transmembrane transport; response to cold; heat; oxidative stress; salt	50.6	191.8	Yes	Up	**	Up	55.8	186.6	Yes	Up	ns		Significant high
c32133_g1	282	Uncharacterized protein LOC100305594 [Glycine max]; Universal stress protein family	388.0	921.0	Yes	Up	*	Up	428.4	880.6	Yes	Up	ns		Significant high
c36998_g2	282	PREDICTED: cysteine-rich receptor-like protein kinase 10-like [Glycine max]; Salt stress response/antifungal	250.6	39.9	Yes	Down	*	Down	230.7	59.9	Yes	Down	***	Down	ns
c27912_g1	282	Medicago sativa aquaporin-like transmembrane channel protein (pAFI 8-1) mRNA, complete cds	812.8	297.8	Yes	Down	ns		909.8	200.8	Yes	Down	**	Down	ns
c47236_g1	282	PREDICTED: Glycine max translocator protein homolog (LOC100785785), mRNA; response to salt stress; transport	11.8	140.1	Yes	Up	*	Up	15.4	136.5	Yes	Up	*	Up	ns

(Continued)

TABLE 1 | Continued

Gene id	Venn group (Figure 8)	GO_biological_process			RNA-Seq result of ST			RT-PCR result of ST			RNA-Seq result of SS			RT-PCR result of SS	RT-PCR result
			Exp ression of control	Exp ression of salt	Signi ficant	Up/ down	Signi ficant	Up/ down	Exp ression of control	Exp ression of salt	Signi ficant	Up/ down	Signi ficant	Up/ down	Signi ficance of salt ST vs. salt SS
c41642_g5	282	Sophora davidii dehydrin (DHN) mRNA, complete cds; response to stress	694.6	7787.3	Yes	Up	**	Up	331.9	####	Yes	Up	**	Up	ns
c40061_g6	282	Phaseolus vulgaris clone BE5D1976 In2-1 protein mRNA, complete cds; response to stress	3795.1	7784.4	Yes	Up	**	up	2870.6	####	Yes	Up	ns		ns
c38817_g1	282	Glutathione S-transferase [Medicago truncatula]; response to salt stress	2800.5	8781.9	Yes	Up	*	Up	2153.6	####	Yes	Up	*	Up	ns
c35733_g1	282	Hypothetical protein PHAVU_006G159300g [Phaseolus vulgaris]; response to stress	73.5	184.8	Yes	Up	**	Up	64.8	193.5	Yes	Up	ns		ns

and 2 DEGs in salt ST had a significant decrease compared to salt SS. The functions of the 12 increased DEGs are mainly ion transporter (c25614_g1, c34374_g1, c34262_g1, c34502_g2, and c34262_g2), plant hormone (c38854_g1 and c40459_g1), antioxidant enzyme (c41938_g5), transcription factor (c32634_g1), aquaporin (c18276_g1), and other functions (c41881_g1 and c32133_g1). The functions of the 2 decreased DEGs are mainly redoxin (c33369_g1) and disease resistance protein (c33712_g1).

DISCUSSION

High Variation of Salinity Tolerance in *S. guianensis* Accessions

High variation of salinity tolerance in 84 *S. guianensis* accessions was observed according to WLR, ranging between relative salinity tolerant (ST) with 13.0% WLR at 15 days of 200 mM NaCl stress and relatively salinity sensitive (SS) with 100% WLR. The high variation of salinity tolerance may come from the high genetic diversity of *S. guianensis* (Tang et al., 2009; Jiang et al., 2017). Based on our previous study, WLR is a good physiological parameter for the screening of salinity-tolerant *Stylosanthes* spp. (Liu et al., 2017). This study showed that WLR is also a good parameter for *S. guianensis* accessions. WLR can reflect the salinity stress symptoms from the whole plant level. Other physiological parameters such as chlorophyll content or SPAD, Fv/Fm, Pn, EL and RWC are conventional and reliable.

Salinity Tolerance of *S. guianensis* Accessions Ranged From 100 to 200 mM NaCl

The phenotype of two *S. guianensis* accessions ST and SS under different NaCl concentrations (100–400 mM) for 15 days confirmed that the screening result from WLR is reliable. SPAD, Fv/Fm, and Pn were further proof of different salinity tolerance between ST and SS, as these physiological parameters were consistant with WLR. Previous studies showed that the salinity-tolerant ability of *Stylosanthes* spp. is between 0.9% and 1.2% NaCl (Wu et al., 2013; Dong et al., 2017). In this study, phenotype and physiological parameters showed that salinity-tolerant *S. guianensis* ST can endure 100–200 mM NaCl, which confirms the findings of former studies, so that ST could be applied in moderate saline soil of tropical areas.

The Expression Pattern of Genes Involved in Signaling and Transporters

Ca^{2+} is one of the very important intracellular second messenger molecules involved in many signal transduction pathways in plants (Seifikalhor et al., 2019). The latest research showed that glycosyl inositol phosphorylceramide (GIPC) sphingolipids in the plasma membrane act as Na^{+} receptors for sensing

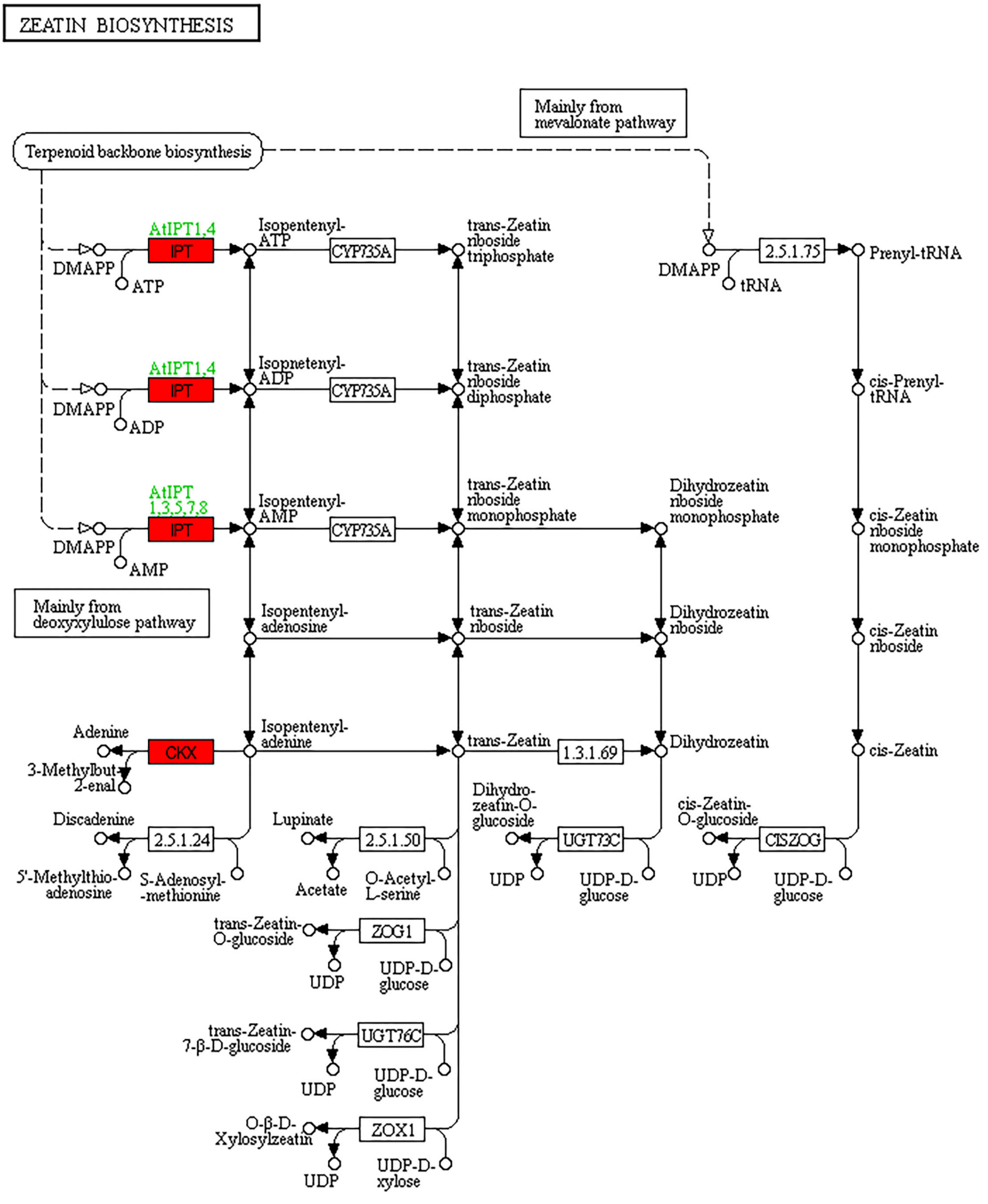

FIGURE 10 | Differentially expressed genes in zeatin biosynthesis KEGG pathway in comparisons of salt ST_salt SS; red denotes upregulated genes.

Na^{+} in the apoplastic and then gate Ca^{2+} influx channels in plants (Jiang et al., 2019). Increased concentrations of the Ca^{2+} activate the classical salt overly sensitive (SOS) signaling pathway (SOS1, SOS2, and SOS3) (Zhang et al., 2021). The activity of the SOS1 exchanger is regulated through protein phosphorylation by the SOS2/SOS3 kinase complex; SOS2 is a Ser-Thr protein kinase belonging to the SNF1-related kinase (SnRK) family and SOS3 is a myristoylated Ca^{2+} sensor (Manishankar et al., 2018). Annexins are calcium-dependent lipid-binding proteins spread through the fungi, plants, animals, archaea, and prokaryotes, which exhibit the conserved core domains in their protein structure (Yadav et al., 2018). Annexins are Ca^{2+} and phospholipid binding proteins, facilitate Ca^{2+} conductance across the plasma membrane, and sense the Ca^{2+} changes in the cell (Saad and Ben Romdhane, 2020). Ca^{2+} transmembrane transport (c34262_g2) and annexin (c34262_g1) showed a significant increase in salt ST compared to salt SS, indicating that higher Ca^{2+} accumulation in ST cytoplasm may lead to activate SOS pathway or other salinity tolerance pathways in ST, which contribute to the high salinity tolerance of ST.

Cysteine-rich receptor-like kinases (CRKs) are one kind of upstream signaling molecules and act as sensing stress signals and responses to various abiotic stresses in plant (Zhang et al., 2018). About 37–170 members of the CRK family in monocots and dicots were found, but their physiological roles and functions on a biochemical and cellular level remain largely uncharacterized. A previous study found that the extracellular domains of typical CRKs contain two unknown function 26 (DUF26) configuration of conserved cysteines C-X8-C-X2-C, the DUF26 domain has antifungal activity and plays a crucial role in salt stress resistance (Zhang et al., 2009). Cysteine receptor-like protein kinase 25 (c41881_g3) showed a significant increase in salt ST compared to salt SS, indicating that CRK may contribute to the salinity tolerance of ST.

Maintaining a dynamic balance of ions under salinity stress is an important strategy for plants, salinity-tolerant plants maintain the ion balance by excreting Na^{+} out of the cell or compartmentalizing Na^{+} into the vacuole to avoid salinity damages (Zhao et al., 2020). Plasma membrane and vacuolar membrane transporters or ion channels such as Na^{+}/H^{+} antiporters (NHX), Ca^{2+}/H^{+} antiporter (CAX), high-affinity K^{+} transporter (HKT), Ca^{2+}-activated vacuolar channel (TPK1/VK), and slow anion channel-associated 1 (SLAC1) play a leading role in mediating the excretion or deposit of Na^{+} in plants (Pantoja, 2021). In this study, cation/H^{+} antiporter (c25614_g1), nodulin MtN21/EamA-like transporter family protein (c34374_g1), and vacuolar amino acid transporter (c34502_g2) were significant increased in salt ST compared to salt SS, indicating that salinity stress upregulated many ion transporters, which led to a better ion homeostasis in ST.

The Expression Pattern of Genes Involved in Plant Hormone

Response to salinity stress requires the integration and coordination of multiple hormones such as abscisic acid (ABA), jasmonic acid (JA), gibberellic acid (GA), ethylene (ET), salicylic acid (SA), cytokinin (CKs), and auxin (Ryu and Cho, 2015). Auxin plays a major role in regulating plant growth and development. Some studies report that high salt stress is linked with greatly remodeled root architecture by altering auxin accumulation and its redistribution (Petersson et al., 2009; Wang et al., 2009). In this study, auxin-induced protein (c40459_g1) had a significant increase in salt ST compared to salt SS, indicating that auxin may increase in ST than in SS and contribute to salinity tolerance of ST. CKs are involved in many physiological and biochemical processes in plants, including cell division, reproductive capacity, leaf senescence, and adaptation to abiotic stresses; however, CKs play negative roles in plant salt tolerance (Yu et al., 2020). Cytokinin dehydrogenase (CKXs) is the key enzyme involved in CK metabolism and can effectively reduce the CK concentration in plants, an increase of CKXs has been shown to cause sensitivity to salt stress in Arabidopsis (Nishiyama et al., 2011). RT-PCR showed that CKXs (c38854_g1) had a significant increase in salt ST, and that in consequence zeatin biosynthesis KEGG pathway showed that CKX was significantly increased as well in salt ST compared to salt SS (**Figure 10**). A reduction of cytokinin biosynthesis in the root system and the subsequent reduction of the cytokinin supply in the shoot could alter the gene expression network and could elicit appropriate responses to ameliorate salinity stress (Tran et al., 2010; Nishiyama et al., 2011).

The Expression Pattern of Genes Involved in Transcription Factor and Antioxidant Enzyme

NAC transcription factors (TFs) belong to a unique class of transcription factors in plants, which play important roles in multiple biological processes including salinity tolerance (Dudhate et al., 2021). A recent study found that NAC TFs could cause the accumulation of proline and glycine betaine to alleviate or avoid the negative effects of ROS in soybean (Li et al., 2021). RT-PCR showed that NAC-like transcription factor (c32634_g1) and peroxidase (c41938_g5) had a significant increase in salt ST compared to salt SS, suggesting that high antioxidant ability may play an essential role in salinity tolerance of salt ST. Interestingly, overexpression of the annexin gene TdANN12 in transgenic tobacco improves stress tolerance through ROS removal (Saad and Ben Romdhane, 2020).

CONCLUSIONS

There was high variation of salinity tolerance in *S. guianensis* accessions, CIAT11365 was a relatively salinity-tolerant accession, which can survive between 100 and 200 mM NaCl. Transcriptomic analysis showed that an increase of Ca^{2+} signal transduction and Na^{+} transport ability, salinity tolerance-related transcription factors and antioxidant ability, as well as an increase of auxin, and inhibition of cytokinin may contribute to the salinity tolerance of CIAT11365. In consequence, CIAT 11365 could be utilized in moderate saline soil of tropical areas.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: NCBI [accession: PRJNA771864].

AUTHOR CONTRIBUTIONS

YL and GL conceived the study as well as participated in its design and coordination. YL carried out all salinity treatment experiments. YL, DK, and WW analyzed the data. YL, HY, SD, MA, BX, and WW wrote the manuscript and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.725656/full#supplementary-material

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