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Effect of cover crops mixtures on weed suppression capacity in a dry sub-humid environment of Argentina

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Cover crops (CC) are increasingly used worldwide within integrated weed management scenarios. They are usually established between two commercial crops and are not harvested, grazed, or incorporated into the soil, but remain on the surface during their growth cycle. The aim of this work was to determine the performance of different CC mixtures and their effects on weed suppression in the south-central region of Buenos Aires province, Argentina. Field experiments were carried out in 2019 and 2020 at the CEI Barrow (MDA-INTA, Tres Arroyos), where both binary and ternary CC mixtures were evaluated. Binary mixtures consisted of winter cereals (*Avena sativa*, *Secale cereale*) and legumes (*Vicia villosa*, *Vicia sativa*) while ternary mixtures were obtained by combining binary mixtures with canola (*Brassica napus*). Weed emergence counting was performed on a 14- day basis to determine weed total density. Vegetation cover of the CC mixtures as well as biomass production from crops and weeds were estimated. The specific composition of the mixtures showed a greater influence on vegetation cover than on biomass production, which would depend mainly on the prevailing environmental conditions. The type of vetch used, the addition of canola, as well as, the proportion of cereals in the mixture determined the CC effect on weeds. CC were consistently more effective in suppressing weed biomass than seedling emergence density. However, weed seedling suppression by CC was similar to or even greater than the chemical-based control (p<0.0001). The average biomass of weeds in all CC (pooled data) was highly reduced when compared to the weedy control (12 vs 259 g m^{-2}, p<0.001), and similar responses were obtained when compared to the chemical fallow (8 g m^{-2}). The mixture *S. cereal+ V. villosa+* canola stood out for presenting the highest values of productivity and vegetation cover and high weed suppression. These

results provide support for the choice of CC mixtures in a dry sub-humid area of Argentina, aimed at maximizing interference with weeds and also to demonstrate their benefits in short- and long-term management. Therefore, CC implementation within crop sequences should be considered as a complementary tool contributing to the development of more sustainable management strategies.

1 Introduction

Given the need to comprehensively address the problem of weeds in agricultural systems, cover crops (CC) are a useful tool that could be included within integrated weed management (IWM) program. CC are usually established between two commercial crops (Reeves, 1994) and non-harvested, grazed, or incorporated into the soil, but remain on the surface during their growth cycle to improve soil fertility and enhance yields (Ruffo and Parsons, 2004; Scavo et al., 2022). There is a growing interest by farmers and researchers in the adoption of diverse CC mixtures (Groff, 2008; Wortman et al., 2013) considering their ability to offer multiple ecosystem services within cropping systems (Brainard et al., 2011). CC diversification has potential to improve weed management (MacLaren et al., 2019), resulting in a key mechanism to facilitate the transition to the "Agroecological Crop Protection" approach, which promotes the reduction of pest impacts through crop management practices compatible with healthy agricultural and food systems, agroecological principles, and the "one health" concept (Deguine et al., 2023). However, their potential suppressive effect on weeds depends both on the number of species as well as their combination within the mixture (Finney and Kaye, 2017; Suter et al., 2017; Baraibar et al., 2018). So far the use of diverse CC mixtures is relatively incipient and the experimental results are both scarce and inconsistent (Davis et al., 2016; Holmes et al., 2017). Therefore, it is necessary to develop empirical evidence to understand how CC interferes with weeds (Florence et al., 2019). Binary mixtures (BM) (i.e., those formed by two botanical families mainly legumes and grasses) are commonly used as CC due to their high resource efficiency compared to other species or functional groups combinations (Dhima et al., 2007; Hayden et al., 2014). Their suppressive effect on weeds was reported by Akemo et al. (2000) and Hayden et al. (2012). Ternary mixtures (TM) (i.e., formed by three botanical families) may confer additional benefits associated with each component, where the most common include grasses, legumes, and cruciferous. The latter has been less explored concerning weed control (Haramoto and Gallandt, 2004; Björkman et al., 2015; Lorin et al., 2015). Holmes et al. (2017) determined that the exclusion of cruciferous

in TM generated an increase in weed biomass, given mainly by their high productivity under the prevailing conditions of interspecific competition. Conversely, Mesbah et al. (2019) observed no differences in weed biomass between both types of mixtures. Therefore, benefits offered by multi-species conjugation versus BM are often considered inconsistent or eventually site-specific regarding weed suppression (Schonbeck et al., 2017).

CC suppress weeds by competition (Ngouajio and Mennan, 2005; Holmes et al., 2017), selective allelopathic activity (Weston, 1996), and physical interference (den Hollander et al., 2007). The allelopathic effect can be species-specific (Norsworthy et al., 2007) and have been reported mainly in grasses such as rye and oats (Kato-Noguchi et al., 1994; Schulz et al., 2013). Therefore, a combination of allelopathic CC might be more effective for a wide range of weeds (Creamer and Stinner, 1997; Wortman et al., 2013). Moreover, since the suppressive effect exerted by CC involves a combination of mechanisms, their suppressive effect on weed would likely depend on the CC specific composition, as well as on the site-specific environmental conditions, cultural practices, and the weed community present (Liebman and Dyck, 1993; Hayden et al., 2012; Baraibar et al., 2018). Therefore, species mixtures are expected to exhibit diverse and complementary suppression mechanisms (physical and chemical) (Baraibar et al., 2017; Schappert et al., 2019), which would increase the suppression capacity (Brainard et al., 2011; Schipanski et al., 2014; Finney et al., 2016).

CC biomass production is often used as an indicator of the capacity to suppress weeds (Brennan and Smith, 2005; Wayman et al., 2015) due to competition for resources (Finney et al., 2016). Also, successful and highly productive CC need a fast initial growth to reach the highest leaf area index to maximize solar radiation interception (Elhakeem et al., 2021). This is why the vegetation cover is used as a parameter to evaluate weed suppression as it correlates negatively with the dry weight of the weeds (Kruidhof et al., 2008; Uchino et al., 2011). The information about the quantity of biomass produced by a species mixture, specifically about to the contribution of each species to the total biomass is scarce (Davis et al., 2016), since many species have been evaluated in monocultures (Holmes et al., 2017). This is why the development of mixtures that favor beneficial interactions for the control of

spontaneous vegetation is of great relevance for the design and reproduction of sustainable cropping systems (Brooker et al., 2021). It is also unclear whether the high CC productivity of grasses and legumes can be further improved by including additional functional groups based on eco-physiological traits that show another pattern of productivity in time and space, such as cruciferous (Cong et al., 2018). In addition, studies on species interactions can be useful for a better understanding of the process and consequently improve biomass production (Wendling et al., 2017). In CC mixtures it would be expected that the diversity in the form of growth would allow to creation of a more complete canopy cover to restrict the availability of light for weeds: grasses and cruciferous grow upright, while legumes make them prostrate or extended (MacLaren et al., 2019). The spatial and temporal complementarity of biomass production between CC components can be a useful tool to increase efficiency in the capture of resources to the detriment of weeds (Döring et al., 2012; Finn et al., 2013). Grasses and cruciferous tend to suppress effectively through rapid growth and high biomass production (Brennan and Smith, 2005; Brainard et al., 2011; Hayden et al., 2012; Dorn et al., 2015; Finney et al., 2016), while legumes grow more slowly and are less competitive (Hayden et al., 2012; Lawson et al., 2015).

In Argentina, the lines of research related to the effects of CC on the physicochemical properties of the soil focused mainly in the availability of nitrogen and/or water (Capurro et al., 2012; Restovich et al., 2012; Cazorla et al., 2012; Vanzolini et al., 2013). Whereas, its effect as part of the IWM has been addressed more recently and to a lesser extent, addressing weed communities present at a specific time of the cycle or in the residues mainly during the onset of commercial crops (Baigorria et al., 2013; Miranda et al., 2014; Acciaresi et al., 2016; Kahl et al., 2016; Lobos et al., 2019). Few approaches characterize weed emergence dynamics throughout the CC cycle to evaluate the suppressive effect in different stages (Buratovich and Acciaresi, 2019). It is essential to determine the dynamics of weeding through the diversity of species and their abundance for a better understanding of the processes that regulate crop-weed interactions, thus facilitating the incorporation of IWM-based practices (Buratovich and Acciaresi, 2017). Likewise, the latest ReTTA (ReTTA Relevamiento de Tecnología Aplicada, 2021) determined that the use of CC in Argentina quintupled in the last 5 years and, that this greater implementation was mainly based on the search for a solution against weeds that are difficult to control.

Based on the previous statements, it is necessary to develop further studies to understand how CC influences weed suppression. Novel information is required to support the choice of the best CC species for weed management and to increase knowledge of the behavior of mixtures under variable environmental conditions.

The objective of this contribution was to study the performance of different CC mixtures (productivity and vegetation cover) on weed suppression during two successive periods in the south-central region of Buenos Aires, Argentina. The hypothesis were that (1) the specific composition of the CC mixtures affects the productivity and vegetation cover; (2) binary and ternary mixtures studied as CC interfere with both seedling emergence dynamics and growth of autumn-winter-spring (A-W-S) weeds; (3) the level of weed suppression by CC mixtures is influenced by the level of biomass production and plant cover generated.

2 Materials and methods

2.1 Experimental design

Field experiments were carried out during 2019 and 2020 at the Chacra Experimental Integrada (CEI) Barrow (Tres Arroyos, Buenos Aires, Argentina; 38° 20" S; 60° 13" W).

CC binary mixtures (BM) consisted of winter cereals (*Avena sativa* or *Secale cereale*) with legumes (*Vicia villosa* or *Vicia sativa*), while ternary mixtures (TM) combined BM with canola (*Brassica napus*) (Table 1). Sowing rates were determined based on previous works (see Hayden et al., 2014; Baraibar et al., 2017; Finney and Kaye, 2017; Holmes et al., 2017), along with the contributions and recommendations of seed suppliers, producers, and researchers of the region. Since early sowing is pointed out by other authors (Baigorria et al., 2011; Baraibar et al., 2018) as the most recommended practice to maximize biomass production, the CC were seeded on March 21th (2019) and March 16th (2020). Before planting, all legumes were treated with *Rhizobium leguminosarum biovar viciae* at a dose of 200 cm^3 50 kg^{-1}. Two control treatments were used as reference: weedy plots (W, without control) and chemical fallow (CF, with non-selective herbicide). In the latter, two glyphosate LS 60% (1.8 L ha^{-1}) applications were performed at different times depending on the composition of the weed community and the relative abundance of each species.

In the entire trial area for the two study years, the predecessor crop was wheat (*Triticum aestivum*), which was harvested in December to simulate a sequence of crops typical of the region under study. In 2019, crop sowing was carried out under conventional tillage using a disc harrow and a field cultivator. In 2020 a non-tillage system was applied and fallow consisted of an application of glyphosate LS 60% (1.8 L ha^{-1}) days before the sowing. The sowing depth was calibrated to 1-2 cm, as it is a recommended value for both large and small seeds (Murrell et al., 2017). The distance between furrows was 20 cm and the planting density was variable depending on the type of mixtures (Table 1). A completely randomized block design with four replicates was used. The experimental units (EU) consisted of 3 m wide by 6 m long plots (18 m^{-2}). The evaluated treatments consisted of eight CC mixtures and two controls. Conforming a total of 40 with a 720 m^{-2} net experimental area (Figure 1).

According to Soil Taxonomy (USDA, 1975), soil belongs to a "Tres Arroyos" series, with original material based on loess sediments and classified as Paleudol petrocalcic. These soils are characterized by having a horizon profile: Ap/A (0-22cm) and BA (22-32 cm) loam-clay-sandy with subangular block structure, Btn (32-75 cm) with a clayey texture and coarse prism structure. At 75 cm is the petrocalcic horizon (INTA, 2014). Chemical soil analyses at a depth of 0-20 cm, for both years at the study site before sowing, indicated adequate conditions for the correct development of the crops: acidic pH, medium to high values of organic matter and phosphorus, although with a low nitrate content (Table 2).

TABLE 1 Seeding rate of each species used for mixtures of binary or ternary cover.

Botanical family	Latin name	Common name	Cultivar	Seeding rate	
				kg ha^{-1}	pl m^{-2}
Poaceae	*Avena sativa*	Oats	Sureña	30	105
	Secale cereale	Rye	Ricardo INTA	20	64
Fabaceae	*Vicia villosa*	Hairy vetch	Ascasubi INTA	20	49
	Vicia sativa	Common vetch	Hilario INTA	40	52
Brassicaceae	*Brassica napus*	Canola	Hyola 830 CC	3	71
2-species: **binary mixtures**	Oats+ Hairy vetch (**OHV**)				
	Oats+ Common vetch (**OCV**)				
	Rye+ Hairy vetch (**RHV**)				
	Rye+ Common vetch (**RCV**)				
3-species: **ternary mixtures**	Oats+ Hairy vetch+ Canola (**OHVC**)				
	Oats+ Common vetch+ Canola (**OCVC**)				
	Rye+ Hairy vetch+ Canola (**RHVC**)				
	Rye+ Common vetch+ Canola (**RCVC**)				

2.2 Meteorological data

The study area is characterized by a dry sub-humid water regime with an average annual total rainfall of 757.8 millimeters (1938-2014 series), being spring and part of autumn the most rainy seasons, while winter the driest. The average annual temperature is 14.9°C, with the warmest month being January and the coldest July (Borda, 2016). Weather data in the study were recorded at the meteorological station of CEI Barrow located in the same experimental site (38° 20" S; 60° 13" W). In 2020, the total
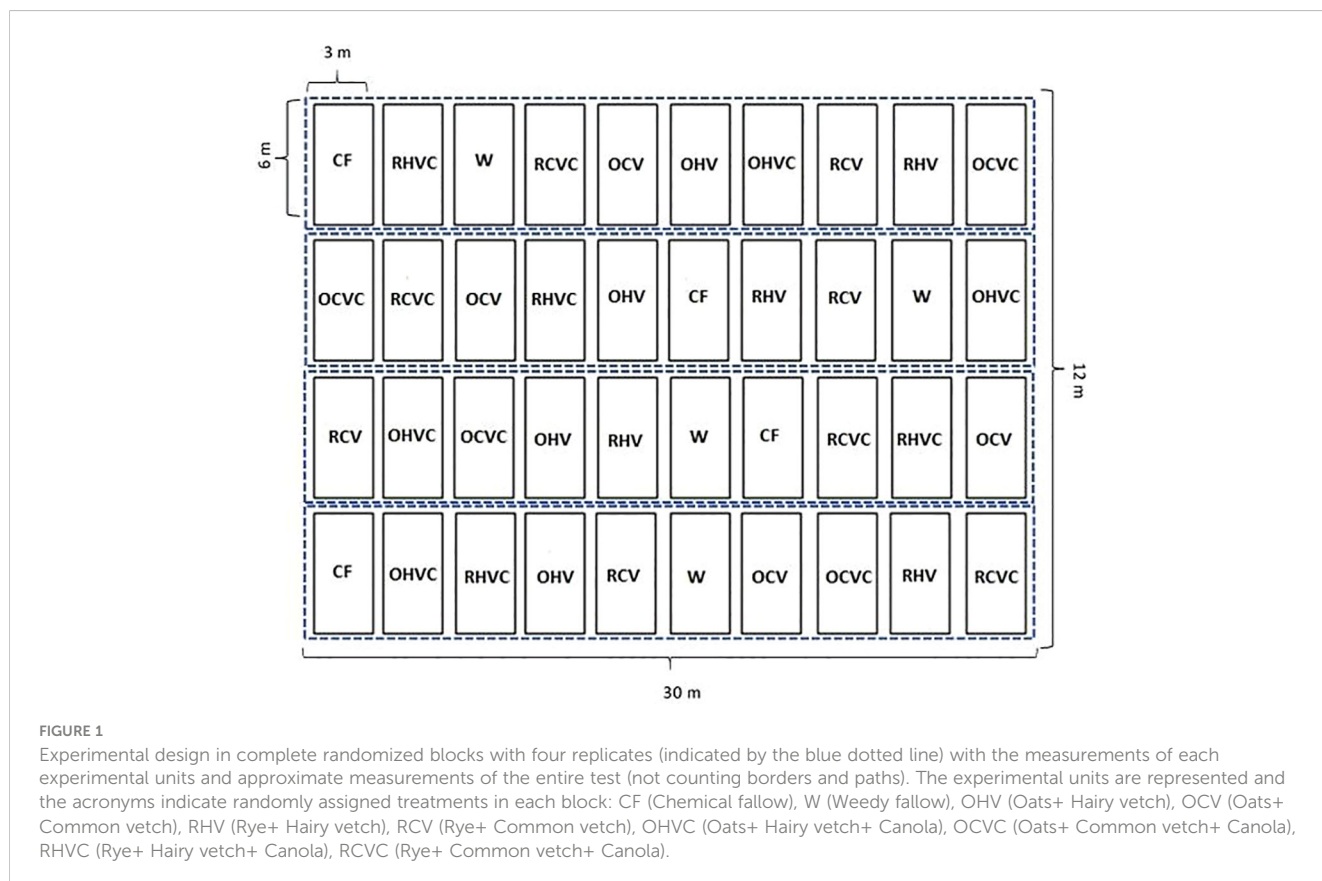
FIGURE 1
Experimental design in complete randomized blocks with four replicates (indicated by the blue dotted line) with the measurements of each experimental units and approximate measurements of the entire test (not counting borders and paths). The experimental units are represented and the acronyms indicate randomly assigned treatments in each block: CF (Chemical fallow), W (Weedy fallow), OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola).

TABLE 2 Results of the chemical analysis of soil (pH, organic matter, nitrates and phosphorus) for the year 2019 and 2020.

Parameter/ Year	2019	2020
pH	6.26	6.4
Organic matter (%)	4.07	3.64
Nitrate (ppm)	7.4	9.6
Phosphorus (ppm)	20.46	25.13

rainfall recorded during the CC cycle was 54% higher than in 2019, the latter showing a deficit of 109 mm compared to historical values. Likewise, the latter showed a longer period of minimum temperatures below the historical value (March, July, and September) and earlier occurrence of frosts in the cycle, compared to the year 2020 (Table 3).

2.3 Data collection

To evaluate the first hypothesis, in the middle of October, vegetation cover percentages (% VC) of CC mixtures were estimated by taking three digital photographs (0.25 m^2 each) per EU. The total number of photographs taken was 120, further processed with the CobCal v 2.1 software. Biomass of both CC mixtures and weeds was determined by harvesting the aerial vegetation during mid-spring present in 0.5 m^2, resulting from the sum of the biomass contained in 2 quadrats of 0.25 m^2 randomly distributed in each EU. Aerial dry biomass values of the different components of the mixtures were obtained after oven drying at 65°C for a week.

To test the second hypothesis, both biomass and density of weed individuals were evaluated. In both years, weekly destructive seedling counts of autumn-winter-spring (A-W-S) species were performed in randomly distributed 0.25 m^{-2} frames (n=4). Total density (pl m^{-2}) was determined as the sum of seedlings that emerged throughout the CC cycle. In order to characterize the

weeds surveyed, the abundance (%) and average frequency of each weed species were assessed. The total number of frames evaluated was 40, one for each EU. Data were represented for those species with abundance or frequency ≥10%.

2.4 Statistical analysis

An analysis of variance (ANOVA) was performed to evaluate the effect of the CC treatments on each study variable. Data were transformed to improve homoscedasticity if necessary. Fisher's least significant difference test (p≤ 0.05) was used for mean comparison. Statistical analysis was performed using Infostat® software (Di Rienzo et al., 2014).

To evaluate the third hypothesis, a linear regression analysis was performed between each variable measured on the CC (vegetation cover and dry biomass) and the variables of the weed (dry biomass and density).

For all the studied parameters, scatter plots of the observed vs predicted residues were analyzed to assess compliance with the model's assumptions (normality, homoscedasticity, and independence). Residual plots indicated that the variances were normally distributed and homogenous. At this point, the total emergence density (pl m^{-2}) and the emergence 85 days after planting (DAP) in 2020 of A-W-S and dicotyledons weed species data, were transformed into a log(x) and square root (x+1) to comply with homoscedasticity of variance and normality of data.

3 Results

3.1 Biomass production and vegetation cover of CC mixtures

The average biomass production of the CC was 39% higher in 2020 compared to 2019 (9360 vs. 5678 kg ha^{-1}) (Figure 2). In 2019, aboveground biomass was 54% higher in the TM formed by oats or

TABLE 3 Record of average maximum and minimum temperature (T°), days with frost and precipitation (mm) during the CC cycle for the years 2019, 2020 and the historical average of the area (1939-2019 series).

Month	Maximum T°			Minimum T°			Days with frost			Precipitation (mm)		
	2019	2020	Historical	2019	2020	Historical	2019	2020	Historical	2019	2020	Historical
May	24.4	29.6	24.9	10.9	14.7	11.3	0	0	0.1	51.4	81.2	82
April	23.8	21.0	20.6	8.4	8.5	7.7	1	0	1.3	27.2	109.5	67
May	17.7	16.9	16.4	5.5	7.9	5.2	6	2	4.2	61.2	45.2	54
June	15.2	14.1	12.9	5.3	3.8	2.7	4	5	8.5	50.5	153	42
July	13.9	12.0	12.5	1.3	2.3	2.1	15	13	10.1	12.4	62.9	41
August	16.5	16.4	14.5	2.4	3.0	2.6	14	13	8.9	11.1	20.6	42
September	18.2	18.1	17	3.5	3.4	4.2	7	10	5.9	42.2	37.1	53
October	19.0	20.5	19.8	6.1	6.2	6.7	1	5	2.2	88.8	83.8	71
Total										234	505	452
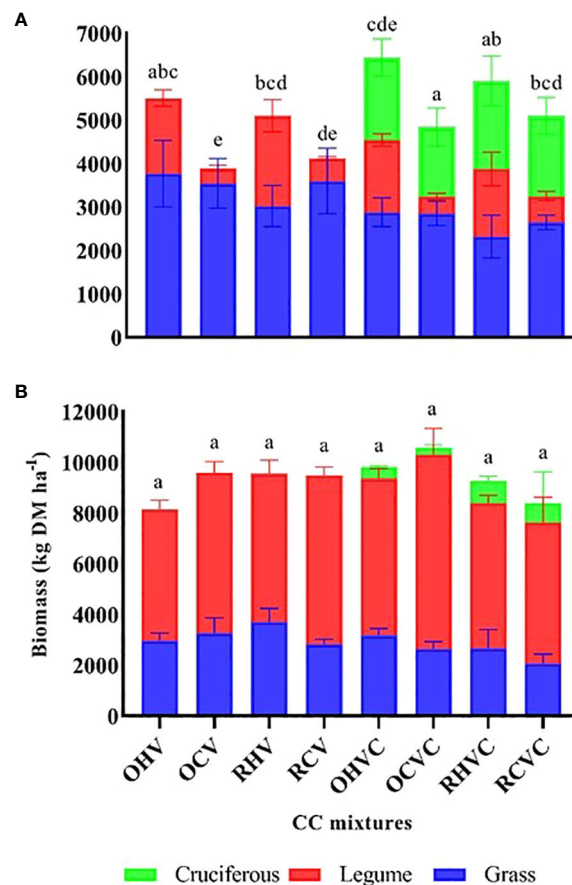
FIGURE 2
Production of total aerial biomass (kg DM ha^{-1}) of the different CC mixtures and each component: grass (in blue), legume (in red) and cruciferous (in green), for the years 2019 **(A)** and 2020 **(B)** in different treatments: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola). The bars represent average values and different letters indicate significant differences among treatments in Fisher's LSD test (p<0.05).

rye + hairy vetch compared to rye or oats + common vetch (p=0.0006) (Figure 2A). Likewise, hairy vetch showed higher productivity compared to common vetch in all mixtures (p=0.0114). In 2020, no differences were observed in biomass production between CC treatments (Figure 2B). However, it is important to note that rye or oats + hairy vetch were among the mixtures with the highest biomass production in both years, with average values of 6168 and 9551 kg ha^{-1}.

Regarding the composition of the mixtures, a negative relationship (p<0.0001) was found between the percentage of legume and grass (r= -0.71) of the mixture (Figures 3A, C). The contribution of each component to the total biomass varied between years. In 2019, the proportion of grasses was higher, but mixtures with hairy vetch were balanced, while in 2020 legumes were dominant. In 2019, the incorporation of canola in the BM negatively affected the biomass of both grasses and legumes (Figures 2A, B), although in the joint balance, the contributions

of aerial biomass of canola exceeded this depression. A negative relationship (p=0.005) was established between the % of grass and the % of canola (r=-0.48) in the mixtures (Figure 3B). In contrast, canola contributed very little biomass to the TM in 2020, which could be due to establishment failures caused by hare damage that reduced the plant stand at the beginning of the cycle.

Regarding VC, in 2019 the TM based on rye + hairy vetch presented the highest values (p<0.0001), followed by the BM oats or rye + hairy vetch and the TM oats + common or hairy vetch. While the BM made up of hairy vetch presented the lowest VC percentage with higher values for the rye-based mixture (Figure 4A). Hairy vetch presented higher (p<0.0001) VC than common vetch in all the mixtures evaluated and the addition of canola (p=0.0001) increased the VC of the BM. In 2020, the TM formed by rye + common or hairy vetch presented higher VC (p=0.0006) compared to all the evaluated mixtures, except for the BM rye + common vetch which did not present significant differences (Figure 4B). An effect of grass
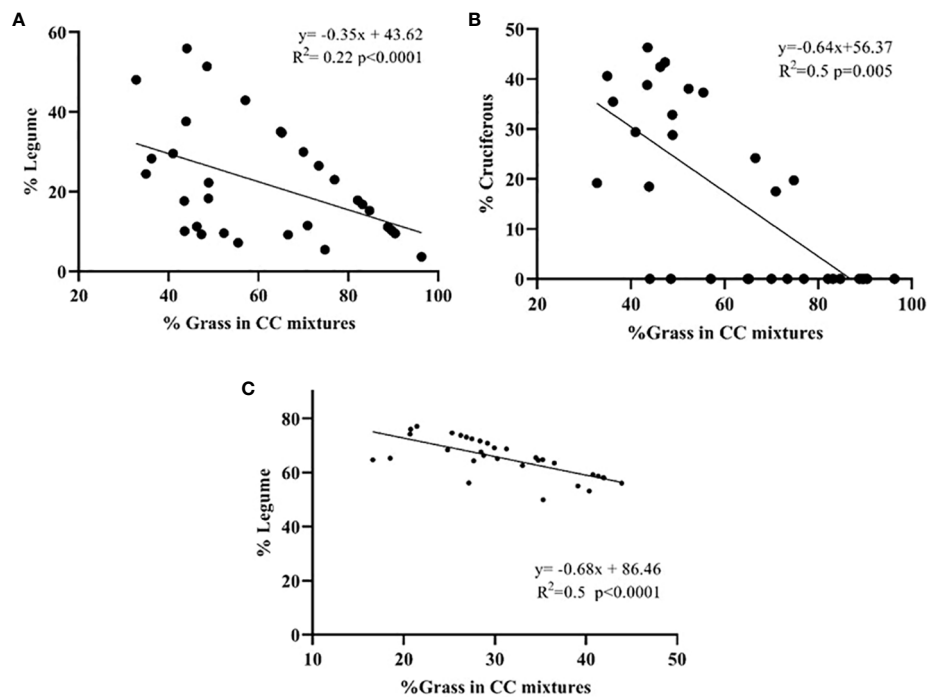
FIGURE 3
Linear relationships between the percentage (%) of grass in the CC mixtures and the resulting legume and cruciferous percentage for the years 2019 **(A, B)** and 2020 **(C)**. The points indicate the biomass production of the groups of botanical families for the different CC evaluated.

was also observed (p=0.0001) and, as in the first year of study, of the canola aggregate in SM (p=0.01). At this point, rye generated higher VC than oats and TM had higher VC than BM (Figure 4B).

The average VC was higher in 2020 than in 2019, highlighting that, in the second year, all mixtures achieved VC > 90%. While, in 2019, the maximum value of VC reached 63%, thus suggesting that the low rainfall regime was a limiting environmental factor.

For both years negative relationships were found between VC and the % of grasses in the CC (r=-0.44 and -0.36). VC decreased (p=0.01 and p= 0.04) when the proportion of grasses in the mixtures increased (Figures 5A, B). This variable also showed a positive correlation with the % of legumes (r=0.45) and Canola (r=0.62), for the years 2019 (p=0.01) and 2020 (p=0.009) respectively. This would determine that the proportion of legumes and cruciferous plants was important (p=0.01 and 0.009) to increase VC (Figures 5C, D).

3.2 Characterization of the weed species surveyed

The A-W-S weed surveyed during 2019 and 2020 consisted predominantly of annual dicotyledonous species. Under conventional tillage (2019), *Anagallis arvensis* presented the highest relative abundance and average frequency of occurrence in all CC and sampling dates, followed by *Conyza sumatrensis* and *Polygonum aviculare* (Table 4). Under no-tillage (2020), *P. aviculare*

showed the highest relative abundance followed by *C. sumatrensis*. In turn, these had the highest average frequency of occurrence while *A. arvensis, G. spicata, Cirsium vulgare* and *Lolium* spp. showed the lowest values.

P. aviculare and *C. sumatrensis* were among the most abundant weeds regardless of the period considered. The variation found in the relative abundance of weed species surveyed between study years could be due to the differences between tillage systems and/or the contrasting rainfall regime between years.

3.3 Weed emergence density

In 2019, no significant differences were observed between CC and the control treatments in A-W-S weed emergence density. Also, no significant differences between the different types of CC mixtures (Figure 6A). In part, this could be due to the limiting water conditions prevailing this year (Table 3).In contrast, in 2020, all the CC mixtures suppressed weed emergence by 88-98% (p<0.0001) compared to chemical fallow and weedy plots, respectively (Figure 6B). Also, it is important to note that in both years, the CC generated an early suppression of weed emergence. As showed in Figure 7A, in 2019, at the beginning of the cycle (53 DAP), a greater emergence of A-W-S weeds (p=0.009) was observed in weedy plots compared to CC (polled data) (48 vs 17 pl m^{-2}). In addition, no significant differences were observed between most CC mixtures and the chemical fallow, with the exception of BM oat +
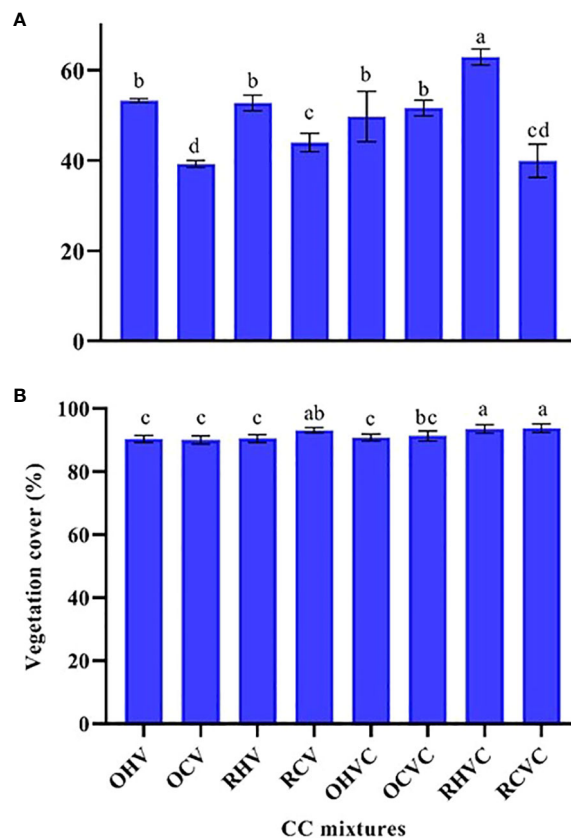
FIGURE 4
Vegetation cover (%) of the different CC mixtures: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), for the years 2019 **(A)** and 2020 **(B)**. The bars represent mean values and the same letters indicate non-significant differences among treatments determined by Fisher's LSD test (p<0.05).

hairy vetch which presented the greatest emergence (p=0.01), comparable to weedy plots (Figure 7A). In 2020 (85 DAP), both controls showed a greater emergence of A-W-S weeds (p=0.0007), which were mainly dicotyledonous species, compared to the CC (polled data) (30 vs 9 pl m^{-2}) and the BM rye + hairy vetch was the mixture with the lowest emergence (p=0.04) of weeds (Figure 7B). No significant relationship was observed between CC biomass production (or vegetation cover) and weed emergence density (p=0.6).

3.4 Weed biomass

CC mixtures reduced weed biomass by 94.5 and 98% compared to the weedy plots for 2019 and 2020, respectively (Figure 8). These values were comparable to those obtained under chemical fallow and, in general, all mixtures showed low biomass levels except oats-hairy vetch and rye-common vetch BM in 2019. In terms of differences between CC mixtures, for the first year, the oats-hairy vetch BM presented higher biomass (p<0.0001) compared to the rye-common vetch TM, the BM, and TM based on rye-hairy vetch and the TM made up of oats-common vetch. Likewise, BM showed higher biomass (p=0.0468) compared to TM (Figure 8A). In 2020,

the TM based on rye-hairy vetch and the BM of the same species with common vetch showed a higher biomass of spontaneous plants (p<0.0001) compared to the oats- common vetch BM (Figure 8B). Rye had a higher biomass (p=0.0325) compared to oats (Figure 8B). No significant relationship was observed between CC biomass production (or vegetation cover) and weed biomass (p= 0.67 and 0.15).

4 Discussion

Results obtained in this contribution suggest that the composition of the CC mixtures would have a greater influence on total biomass production in years with a limited rainfall regime. Under such environmental conditions, the addition of canola to the CC mixture showed a tendency to increase productivity, while *V. villosa* showed greater stability compared to *V. sativa*. This could be explained by the differential behavior of both types of vetch, since *V. villosa* is more tolerant to prolonged periods of water deficit and low temperatures (Renzi, 2013; Renzi et al., 2019). For both years of study, mainly the type of vetch used, the addition of canola in BM, and the proportion of grasses affected the vegetation cover. This reveals the strong competitive capacity of grass species which must
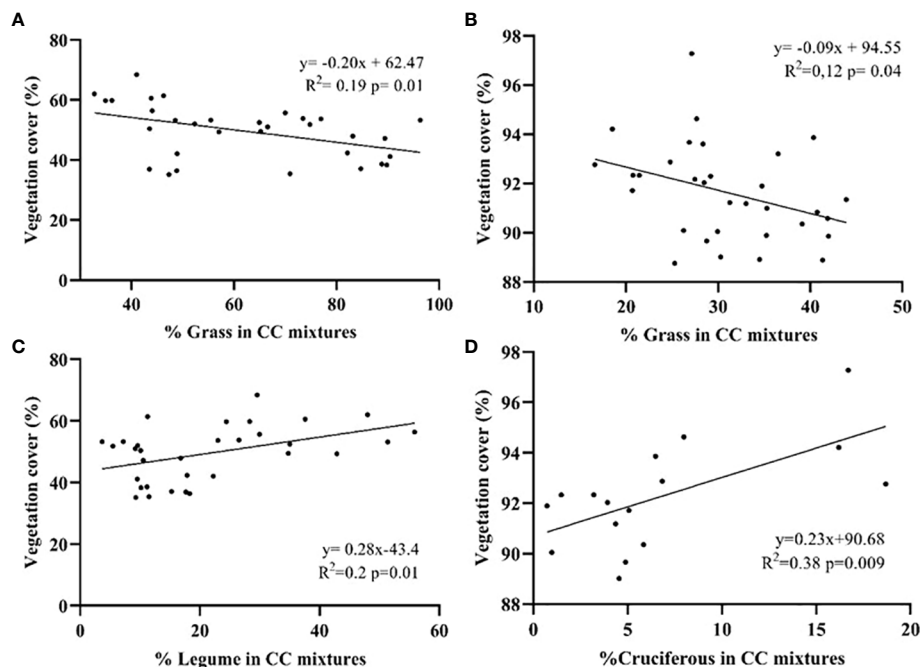
FIGURE 5
Linear relationships between vegetation cover (%) at the end of the cycle based on the % of grass for the years 2019 **(A)** and 2020 **(B)**, legume and cruciferous in the mixtures for the years 2019 **(C)** and 2020 **(D)**. The points indicate the vegetation cover generated for the % of grass, legume, or cruciferous in the different CC evaluated.
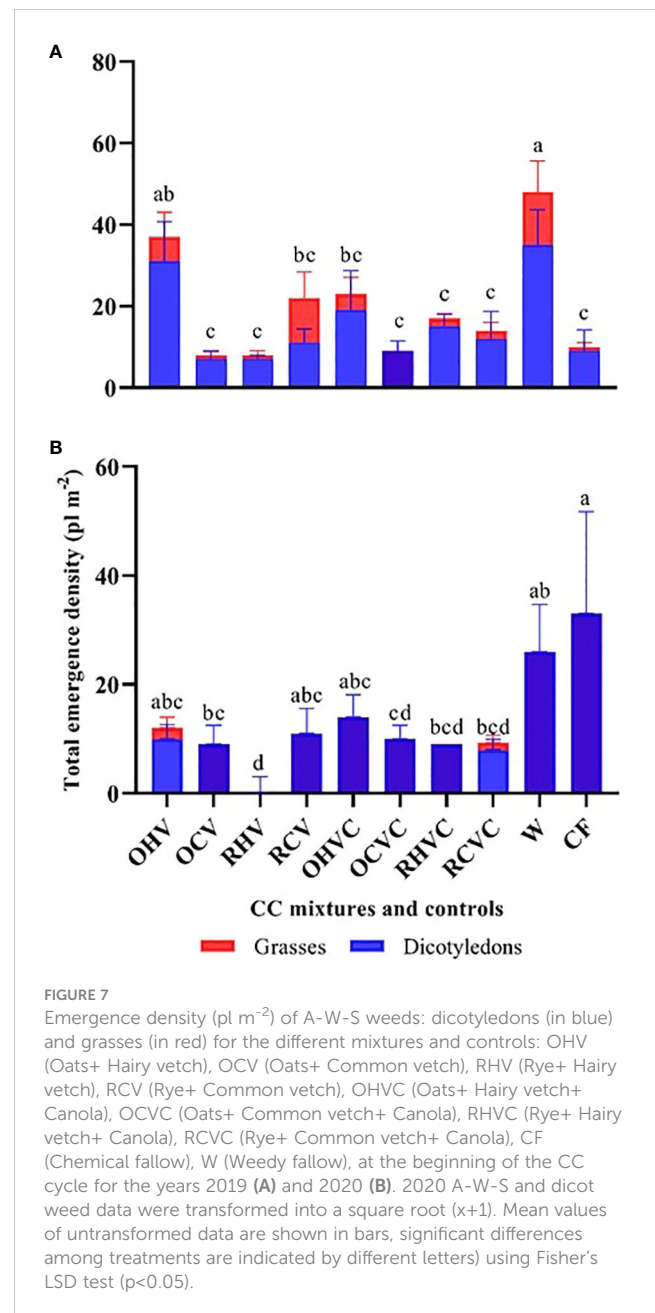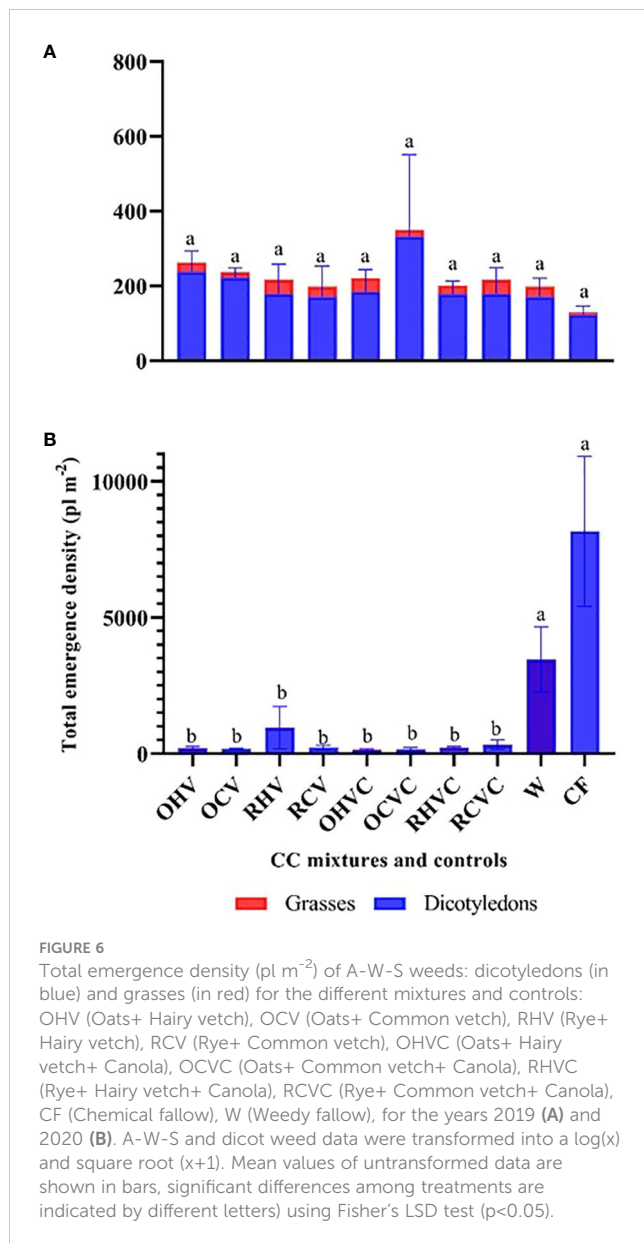
be taken into account when designing CC mixtures if the goal is to achieve a good diversity of its components. Grass seedling rates should be reduced by half to a quarter in a mixture (compared to monocultures) to achieve a balanced ratio with legumes, which tend to be weak competitors and must be planted at the same densities as monocultures to ensure establishment (White et al., 2016). In addition, the vertical orientation of the grass leaves would allow the passage of light through the upper strata (Elhakeem et al., 2021). Therefore, it is important to add other species to the mix with different strategies for using space, mainly those species with horizontal canopy architecture (MacLaren et al., 2019). Thus, the specific composition of the CC mixtures would have a greater influence on the vegetation cover than on the total biomass

production, which would depend mainly on the prevailing environmental conditions, confirming the first hypothesis. The environmental conditions played a pivotal role in the results as indicated by the differences observed between years.

In this contribution we found evidence of an early suppressive effect of the CC mixtures on the emergence dynamics and growth of A-W-S weeds (Figures 6–8). Weed suppression values (66 and 94%) were similar to those provided by chemical methods (see Teasdale and Mohler, 1992; Osipitan et al., 2018) and are considered sufficiently high to prevent seed bank replenishment (Liebman and Nichols, 2020). In addition, an early weed emergence reduction would clearly decrease weed-crop competition (Hock et al., 2006). Based on these results, we decided to accept the second

TABLE 4 Average frequency of occurrence (%) and abundance (%) of the different autumn-winter-spring (A-W-S) and autumn-winter (A-W) weed species: surveyed in all mixtures of CC and controls for 2019 (conventional tillage) and 2020 (non-tillage).

Species	Botanical family	Lifecycle	Abundance (%)		Frequency (%)	
			2019	2020	2019	2020
Anagallis arvensis	Primulaceae	Annual (A-W)	19±9	5±3.5	45.5±12	14±7
Conyza sumatrensis	Asteraceae	Annual (A-W-S)	12±4	13±3	28±7	29±4
Polygonum aviculare	Polygonaceae	Annual (A-W-S)	11±5	21±9	20±8	29±15
Gamochaeta spicata	Asteraceae	Perennial	6±3	4±2	15±5	12.5±3
Lolium **spp.**	Poaceae	Annual (A-W-S)	5±4	1±3	14±8	6±7
Cyclospermum leptophyllum	Apiaceae	Annual (A-W)	7±5	–	15±7	–
Cirsium vulgare	Asteraceae	Annual (A-W-S)	–	6.5±5	–	12±6

FIGURE 6
Total emergence density (pl m^{-2}) of A-W-S weeds: dicotyledons (in blue) and grasses (in red) for the different mixtures and controls: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), CF (Chemical fallow), W (Weedy fallow), for the years 2019 **(A)** and 2020 **(B)**. A-W-S and dicot weed data were transformed into a log(x) and square root (x+1). Mean values of untransformed data are shown in bars, significant differences among treatments are indicated by different letters) using Fisher's LSD test (p<0.05).

FIGURE 7
Emergence density (pl m^{-2}) of A-W-S weeds: dicotyledons (in blue) and grasses (in red) for the different mixtures and controls: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), CF (Chemical fallow), W (Weedy fallow), at the beginning of the CC cycle for the years 2019 **(A)** and 2020 **(B)**. 2020 A-W-S and dicot weed data were transformed into a square root (x+1). Mean values of untransformed data are shown in bars, significant differences among treatments are indicated by different letters) using Fisher's LSD test (p<0.05).
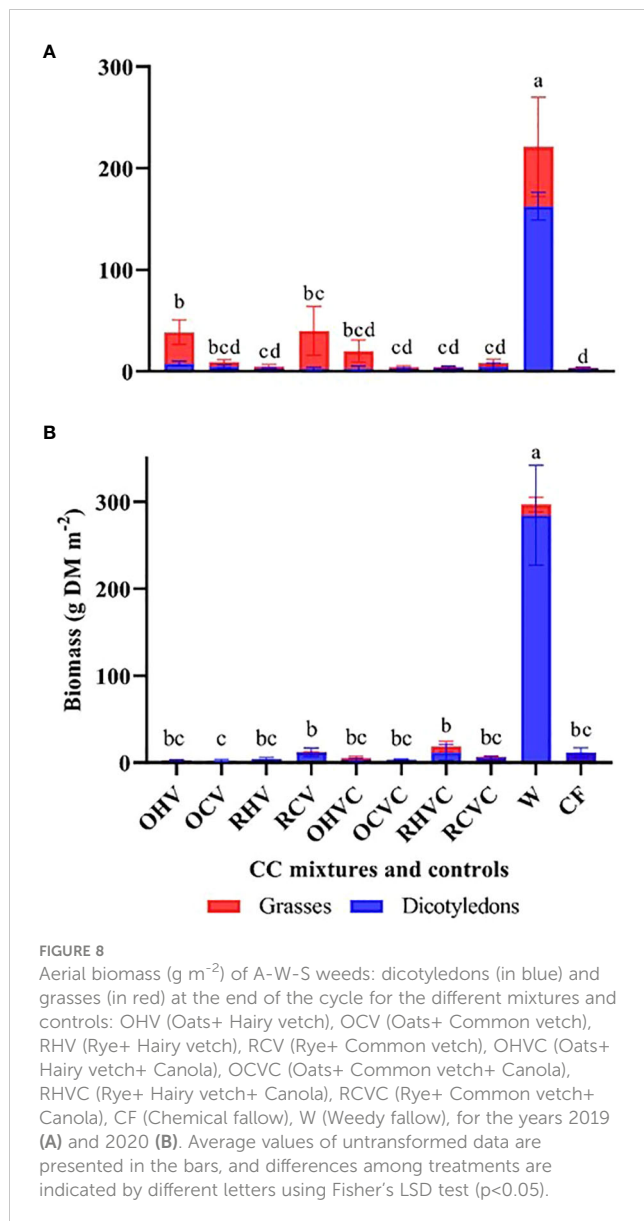
hypothesis. However, the study demonstrates that for both years of the study, CC mixtures were consistently more effective to suppress weed biomass compared to weed density. The latter was only lower than the control treatments in 2020. Despite this fact, both weed biomass production and final density levels were similar to those obtained by chemical fallow. These results are comparable to those reported by Piñeiro et al. (2019) for different types of CC and sites in Argentina. Conversely, Buratovich and Acciaresi (2019) observed a greater reduction in weed biomass in CC compared to the use of herbicides. The benefits of CC mixtures should be addressed within integrated weed management scenarios, considering the potential impact of these results on favoring seedbank depletion (Liebman and Nichols, 2020; Tiwari et al., 2021).

Finally, although many contributions have cited a positive relationship between biomass production (Finney et al., 2016; Florence et al., 2019; MacLaren et al., 2019) or vegetation cover (Kruidhof et al., 2008; Uchino et al., 2011; Dorn et al., 2015;

Elhakeem et al., 2021) and weed suppression, no such relationships were observed in this work. Therefore, we decided to reject the third hypothesis. However, evidence was found that the composition of the mixtures affected the performance of CC. Therefore, these results could indicate that resources complementary among CC species would influence on the weed suppressive capacity of the mixture. However, more studies should be conducted to comprehensively address the effect of CC mixtures on the weed dynamics.

5 Conclusions

The suppression of weed emergence and biomass exerted by CC mixtures were similar to (or even greater than) the chemical-based

FIGURE 8
Aerial biomass (g m^{-2}) of A-W-S weeds: dicotyledons (in blue) and grasses (in red) at the end of the cycle for the different mixtures and controls: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), CF (Chemical fallow), W (Weedy fallow), for the years 2019 **(A)** and 2020 **(B)**. Average values of untransformed data are presented in the bars, and differences among treatments are indicated by different letters using Fisher's LSD test (p<0.05).

effect of different CC mixtures on weed suppression at a community level in a dry subhumid environment of Argentina. It also provides valuable information in a poorly studied field, as weed emergence dynamics in CC. Future research should incorporate new variables in order to understand the relationship between the CC mixtures and weed suppression, such as: (i) the biomass production of both weeds and CC in the initial stages of the crop, (ii) the photosynthetic activity index active radiation as a complement to the vegetal cover, and (iii) the level of the weed seed bank before and after the implementation of the practice.

Data availability statement

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

Author contributions

MM: Writing – original draft. GC: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing. MY: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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control. Average weed biomass figures for all CC mixtures were highly reduced when compared to the weedy control. In this sense, the mixture *S. cereale* + *V. villosa* + canola stood out for presenting the highest performance (productivity and vegetation cover) and high weed suppression. Obtained results support the idea that the use of species with functional differences is a practical recommendation criterion when designing better mixtures. Therefore, the implementation of CC within cropping sequences should be considered as an efficient and complementary tool, to promote IWM tactics and the design of more sustainable agricultural practices in the south-central region of Buenos Aires province, Argentina. From the authors perspective, this contribution provides novel results showing for the first time the

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