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Effects of *Lactobacillus plantarum* on Silage Fermentation and Bacterial Community of Three Tropical Forages

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The fermentation quality and microbial diversity of king grass (K), cassava foliage (C), and *Broussonetia papyrifera* (B) ensiled in the absence of an inoculant (K, C, B) or the presence of *Lactobacillus plantarum* (KL, CL, BL) for 60 days were investigated. The bacterial community was characterized by using the 16S rDNA sequencing technology. The relative abundance of *Lactobacillus* in K was very high, and it decreased after adding *L. plantarum* while *Acinetobacter* increased to some extent. The relative abundance of *Lactobacillus* in group C was also very high, and the inoculant *L. plantarum* enriched it in the CL group. As the second dominant genus of group C, the relative abundance of *Pseudomonas* decreased significantly in CL. *Weissella* and *Enterobacter* were the dominant genera in B and BL, and the relative abundance of *Lactobacillus* decreased in BL. For K, C, and B, the inoculant *L. plantarum* decreased the pH value and NH_3-N content markedly, inhibited the production of butyric acid, increased the content of lactic acid, and significantly improved the fermentation quality. In conclusion, *L. plantarum* affected the bacterial community of C and improved the silage quality of K, C, and B to a certain extent.

Keywords: ***Lactobacillus plantarum*, king grass, cassava foliage, *Broussonetia papyrifera*, silage fermentation, bacterial community**

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INTRODUCTION

Hainan island has a tropical monsoon climate, which is warm and humid all year round, resulting in difficulties in hay preparation. Silage can preserve the fresh plant feed for a long time, reduce nutrient loss, and facilitate animal digestion and absorption (Kung et al., 2018). Silage of excess forage is of great significance to balance the seasonal supply of livestock feed.

King grass [*Pennisetum purpureum Schumacher* × *P. glaucum* (Linnaeus) R. Brown, K], belonging to *Pennisetum*, is widely used as animal feed in tropical and subtropical regions due to its high biomass yield, nutritional values, and palatability in China (Zi et al., 2021). Cassava (*Manihot esculenta* Crantz, C) is a major tropical food crop in the Euphorbiaceae family because of its high protein content and gross energy; its foliage becomes the high-quality protein feed resource

in the tropical areas in China (Napasirth et al., 2015). Using stems and leaves of Cassava to develop ruminant feed can not only reduce the feed cost but also enrich roughage resources and make full use of resources. However, the growth of K depends on high temperature and a rainy environment, resulting in a surplus in summer and a serious shortage in winter (Li et al., 2014). In addition, C is harvested seasonally and needs to be ensiled for long-term preservation. Hence, it is necessary to prepare silage for feeding ruminants in winter. In the tropical gramineous forage, the water-soluble carbohydrates (WSCs) and dry matter (DM) content are low, so it is difficult to produce high-quality silage (Li et al., 2014). At the same time, the content of crude protein (CP) of K is relatively low while fiber is high, which also limits its application in ruminant production (Zhao et al., 2019). *Broussonetia papyrifera* L.Vent. (B) is a fast-growing tree species, belonging to the Moraceae family, and it is widely distributed in Southeast Asia (Tang et al., 2021). It has high nutritional value, strong adaptability, high contents of crude protein and crude fat, and low contents of crude fiber and is rich in various amino acids. It is a high-quality unconventional feed resource. The protein content of B is so high that it has been used as a protein source in animal feed, such as chicken (Zheng et al., 2019) and pig (Wüstholz et al., 2017). However, the content of WSC in B is very low. With its high-buffer capacity, direct silage is difficult to succeed. Inoculants are needed to improve fermentation quality. Adding exogenous microbial agents, especially lactic acid bacteria (LAB), is the most convenient and effective way to improve the silage quality of K. Inoculation of exogenous LAB can inhibit the fungi and aerobic bacteria attached to the forage itself, which are conducive to silage fermentation. In addition, for the problem of low WSC content, it is necessary to select homozymic lactic acid bacteria with higher fermentation efficiency (Muck et al., 2018, p. 3980–4000). The homofermentative lactic acid bacteria represented by *L. plantarum* grow and reproduce rapidly. In the early stage of fermentation, they can quickly produce lactic acid (LA) and reduce pH value, so the fermentation system can quickly form an acidic environment, inhibit the growth and reproduction of harmful microorganisms, and effectively maintain feed nutrition. At present, researchers have inoculated *L. plantarum* in whole crop corn silage (Wang et al., 2018), sugarcane silage (Rabelo et al., 2019), and *Moringa oleifera* leaf silage (Wang et al., 2018). Among them, the research on leaves of sugarcane and *Moringa oleifera* shows that the fermentation effect is better without adding *L. plantarum*; instead, the effect of *L. plantarum* as an inoculant of mixed silage of high momentum amaranth and rice straw is ideal. Inoculants increased the relative abundance of *Lactobacillus*, reduced the relative abundance of *Weissella*, *Pediococcus*, *Lactococcus*, decreased pH, acetic acid (AA), and NH_3-N, and increased LA concentration as compared with no addition silage over the ensiling period (Mu et al., 2020).

However, few studies have focused on the effects of *L. plantarum* on K, C, and B silages. Therefore, in this study, we evaluated the silage effect of K, C, and B in response to the addition of *L. plantarum* in terms of chemical composition, fermentation characteristics, and microbial community.

MATERIALS AND METHODS

Silage Preparation

K (Reyan No. 4), C (South China No. 7), and B were cultivated in the experimental base of the Chinese Academy of Tropical Agricultural Sciences (19°58′E, 19°52′N). The first cut K of the vegetative stage (∼1.5–1.8 m in height) was harvested and chopped into about 2 cm pieces by a grass-shredding machine, and then C and B in the growing stage were cut in the same manner (Donghong No. 1, Donghong Mechanical Equipment Co., Ltd., China). The number of L (Snow Brand Seed Co., Ltd., Japan) was 1.0×10^5 CFU/g (Zi et al., 2021).

In total, six different treatments were carried out in our current research as follows: B: *Broussonetia papyrifera*, BL: *Broussonetia papyrifera* + *L. plantarum*, C: cassava foliage, CL: cassava foliage + *L. plantarum*, K: king grass, and KL: king grass + *L. plantarum.* According to the manufacturer's guidelines, the inoculants were dissolved in sterile distilled water and then mixed thoroughly with the grass. An equal amount of sterile distilled water was added to the control group. Every treatment was carried out in triplicate. Briefly, 200 g of K, C, and B was blended with inoculants, and the mixture was placed in plastic bags (35 × 12 × 5 cm; Guozhong Packing Co., Ltd., Haikou, China). A total of 18 bags (six treatments × three replicates) were sealed by a vacuum packaging machine (DZQ-380B; Ansheng Machinery Co., Ltd., Quanzhou, China) and stored at normal temperature (25∼30°C). The chemical composition, organic acid, and microbial community were determined after 30 days of fermentation.

Chemical and Microbial Analysis

Samples were oven-dried at 65°C for 72 h to determine the DM content, and dried materials were ground for chemical analysis. CP and ether extract (EE) were determined according to the Guidelines of the Association of Official Analytical Chemists (AOAC, 2005). The concentration of neutral detergent fiber (NDF) and acid detergent fiber (ADF) and the content of WSC were determined as described previously (He et al., 2020).

The fermentation quality of silage was determined using distilled water extracts. Briefly, 50 g wet silage was blended with 200 ml distilled water, followed by incubation at 4°C for 24 h and then filtration. The pH (Thunder magnetic phs-3c pH meter) and concentrations of LA, AA, propionic acid (PA), butyric acid (BA), and NH_3-N were measured as established previously (Mao et al., 2014).

Microbial counts were analyzed using the plate count method as described previously (Li et al., 2018). Briefly, 20 g silage samples were mixed with 180 ml sterilized saline, and then LAB, coliform, yeasts, and molds were enumerated on Man, Rogosa, and Sharpe (MRS) agar, violet red bile agar, and rose Bengal agar, respectively.

Microbial Diversity Analyses

DNA Extraction and 16S rRNA Gene Sequencing

The aforementioned extracts were used for the molecular analysis of the microbiota. Using the E.Z.N.A.® Soil DNA

Kit (Omega Bio-Tek, Norcross, GA, United States) to isolate microbial DNA from silage specimens according to the instructions of the manufacturer. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338F (5'-CCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by thermocycler PCR system (Gene Amp 9700, ABI, United States). PCR products were purified and quantified, next-generation sequencing was carried out on an Illumina MiSeq 2500 platform (Illumina, Inc., San Diego, CA, United States), and 250-bp paired-end reads were generated.

Processing and Analysis of Sequencing Data

The filtered reads were assembled into tags with the principles as follows: an overlap between paired-end reads that should be more than 10 bp and <2% mismatch and redundant tags was removed by software MOTHUR (v.1.30) to obtain unique tags (Schloss et al., 2009). Then, the resultant unique tags were employed to determine the relative abundance. The high-quality sequences were clustered into operational taxonomic units (OTUs) defined at a similarity of 97%. Using the core-diversity plug-in within QIIME2[1] to determine diversity metrics (Callahan et al., 2016). The microbial diversity within an individual sample was assessed using the α-diversity indices, including observed OTUs, Chao1 richness estimator, Shannon diversity index, and Simpson and ACE indices. β-diversity was analyzed to assess the structural variation of microbiota across specimens, and then principal coordinates analysis (PCoA) was determined (Vázquez-Baeza et al., 2013). Among samples and groups, appropriate methods, such as LefSe, were employed to identify the bacteria with different relative abundance (Segata et al., 2011). Unless specified earlier, parameters used in the analysis were set as default. The sequencing data were deposited in the Sequence Read Archive (SRA) under the accession number PRJNA556216.

Statistical Analysis

The impacts of *L. plantarum* were investigated using a two-way ANOVA in SAS 9.3 software (SAS Institute Inc., Cary, NC, United States). Duncan's multiple range test was adopted to identify significant differences, and $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chemical and Microbial Compositions of K, C, and B

Table 1 shows the chemical and microbial compositions of C, K, and B. In this study, for C and K, the concentration of NDF and ADF were higher compared with previous reports, while the contents of DM, OM, CP, and ether extract (EE) were lower or comparable to the previous reports (Li et al., 2019). For B, the level of CP was comparable to the previous reports, and the level of NDF was higher compared with previous reports, while the levels of ADF and DM were lower (Si et al., 2018). The content of WSC plays a significant role in assessing fermentation quality. The content of WSC was 5.21, 7.21, and 6.43% in C, K, and B in this study. This can ensure the fermentation quality in theory (Ni et al., 2018). However, in addition to WSC, LAB quantity also needs to reach the threshold. Well-preserved silage needs no less than 10^5 CFU/g FM of LAB (Cai et al., 1998). The number of LAB, coliform, yeasts, and mold in the fresh C was 3.89, 1.53, 2.06, and 0 Log_{10} CFU/g FM, respectively. It was 4.22, 1.77, 2.78, and 3.04 Log_{10} CFU/g FM, respectively, in fresh K and 3.52, 2.08, 2.63, and 0.78 Log_{10} CFU/g FM, respectively, in fresh B. Compared with harmful bacteria, LAB were not dominant in quantity among the three forages, at the beginning of ensiling, yeasts would grow and compete for energy materials with LAB and could not ensure good silage quality, illustrating that silage inoculants, such as LAB inoculants, were imperative for silage.

TABLE 1 | Chemical and microbial compositions of three species of tropical forages.

	Cassava foliage	King grass	*Broussonetia papyrifera*
Dry matter (% DM)	14.56	15.28	19.29
Organic matter (% DM)	90.33	89.84	84.00
Ether extract (% DM)	5.24	6.02	4.30
Water-soluble carbohydrate (% DM)	5.21	7.21	6.43
Crude protein (% DM)	18.11	9.12	15.22
Neutral detergent fiber (% DM)	42.02	76.63	39.23
Acid detergent fiber (% DM)	31.84	49.69	32.38
Lactic acid bacteria (Log_{10} CFU/gFM)	3.89	4.22	3.52
Coliform (Log_{10} CFU/gFM)	1.53	1.77	2.08
Yeast (Log_{10} CFU/gFM)	2.06	2.78	2.63
Mold (Log_{10} CFU/gFM)	0	3.04	0.78

DM, dry matter; FM, fresh matter; CFU, colony-forming unit.

Fermentation Quality of K, C, and B

Table 2 provides the fermentation characteristics of three species of tropical forages. For fermentation quality, the pH of ensiling is the most important key index. The pH was remarkably decreased after fermentation in CL, KL, and BL compared with the control groups. However, the pH values of the inoculant-treated groups were ≥4.2, and related research have indicated a similar pH of *L. plantarum* treatment (Yang et al., 2019; Ren et al., 2020). The reason may be that there was not enough WSC for LAB growth and reproduction, no more LA was produced, and the pH value cannot be reduced to a lower level (Yan et al., 2019). In addition, inoculants reduced the contents of NH_3-N compared with the control group. NH_3-N is a universal index of protein hydrolysis, and it usually reflects the levels of peptide bond hydrolysis and amino acid or peptide deamination. The smaller the value, the less the protein is decomposed, which means a better storage quality (Wang et al., 2019). The remarkably decreased content of NH_3-N in the inoculant-treated silage might be a result of lower pH values, which could inhibit the activities of some harmful

TABLE 2 | Fermentation characteristics of three species of tropical forages.

Item	Cassava foliage		King grass		*Broussonetia papyrifera*		SEM	*P*-value		
	CK	LAB	CK	LAB	CK	LAB		F	L	F × L
pH	4.69^{a}	4.36^{c}	4.51^{b}	4.29^{d}	4.42^{c}	4.20^{d}	0.06	*	*	*
Lactic acid (%DM)	3.72^{d}	4.14^{c}	3.65^{d}	3.69^{d}	4.57^{b}	5.56^{a}	0.28	**	**	**
Acetic acid (%DM)	1.78^{d}	1.83^{d}	2.76^{b}	2.65^{b}	4.89^{a}	2.82^{b}	0.42	**	**	**
Propionic acid (%DM)	1.64^{a}	1.27^{b}	0.84^{c}	0.92^{c}	0.15^{d}	0.26^{d}	0.21	**	**	**
Butyric acid (%DM)	0.15^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00	**	NS	NS
NH_3-N(%DM)	2.09^{c}	1.36^{d}	4.46^{a}	3.65^{b}	3.38^{b}	2.16^{c}	0.43	**	**	**

*F, forages; L, lactic acid bacteria; F×L, the interaction between forages and LAB treatments; SEM, standard error of the mean; a–d means values within the same row with different superscripts in lowercase letters differ significantly from each other at $P < 0.05$; * and **, significant at $P < 0.05$ and 0.01, respectively; NS, not significant.*

TABLE 3 | Alpha-diversity of bacterial diversity of C, K, and B silages.

	B	BL	C	CL	K	KL
Shannon	2.0729	2.0633	1.8216	2.0035	2.1441	2.0004
Simpson	0.2192	0.2308	0.2648	0.2549	0.1894	0.2628
ACE	140.8861	136.1858	109.7787	131.5304	138.2053	137.9635
Chao1	152.9333	133.2786	109.4786	129.3704	145.4143	138.9087

B, Broussonetia papyrifera; BL, Broussonetia papyrifera+ L. plantarum; C, cassava foliage; CL, cassava foliage+ L. plantarum; K, king grass; KL, king grass+ L. plantarum.

bacteria and proteases and effectively conserve nutrients (Wang et al., 2019).

In CL, the content of LA was remarkably elevated, and there was no significant change in acetic acid (AA), besides the content of PA in the inoculant-treated groups reduced by about a fifth compared with the control group. Furthermore, the content of BA was reduced to 0. In KL, the content of LA, AA, and PA was similar to the control group, and BA was never detected. In BL, the content of LA was remarkably increased while AA was significantly decreased than the control group, and there was no significant change in PA. Furthermore, the content of BA was never detected. Furthermore, the content of BA was reduced to 0 in the treatment of all three treatments, manifesting that K, C, and B treated by *L. plantarum* were well-preserved, which was consistent with a previous study (Lv et al., 2020).

These results revealed that the addition of *L. plantarum* in the ensiling process could efficiently promote the fermentation quality.

Microbial Community of K, C, and B Silages

A total of 1,439,466 raw reads were obtained. After splicing and filtering, 1,311,196 clean tags were generated. Each sample produced at least 69,299 clean tags, with an average of 72,844 clean tags.

Table 3 presents the α-diversity of the bacterial diversity of K, C, and B silages. The average Good's coverage for all samples was over 0.99, suggesting that the sequencing process was sufficient to describe the changes in the bacterial community. Shannon and Simpson stand for diversity, and Chao1 and ACE represent richness. **Figure 1** is principal coordinates analysis (PCoA), i.e., principal coordinate analysis, which is a non-constrained data dimensionality reduction analysis method, which can be used to study the similarity or dissimilarity of sample community composition. It was used to search the relevance among the community structures of bacteria during ensiling. Next, the changes in the bacterial community during fermentation were evaluated by a high-throughput sequencing technique based on the 16S ribosomal DNA gene. **Figure 2** presents the relative abundance of bacterial communities in C, K, and B. The linear discriminant analysis (LDA) effect size (LEfSe) method was used in **Figure 3** to examine the discrepancies of microbial communities between groups and probe the specific bacteria in each group (LDA score > 4.0).

In BL, there was no significant change in diversity, while the richness was decreased compared with the control group. PCoA analysis showed the control group and silages treated with *L. plantarum* were not separated, indicating that the microbial community was not changed remarkably during the ensiling process. The combination of α-diversity and β-diversity showed that the effect of bacterial diversity and community structure of *L. plantarum* on B was limited. At the phylum level, the total contents of *Firmicutes* and *Proteobacteria* were more than 90% in both B and BL. Similar results have been found in *Eucalyptus nutans* of four different altitudes over the Tibetan Plateau (Ding et al., 2020). The same phenomenon has been observed in cassava foliage (Li et al., 2020). With inoculant *L. plantarum*, the content of *Firmicutes* was decreased, while the relative abundance of *Proteobacteria* was increased. At the genus level, the dominating bacteria in the B and BL groups were *Weissella* and *Enterobacter*. In addition, *Lactobacillus*, *Pantoea*, *Escherichia-Shigella*, and *Raoultella* were also included. Previous research has shown

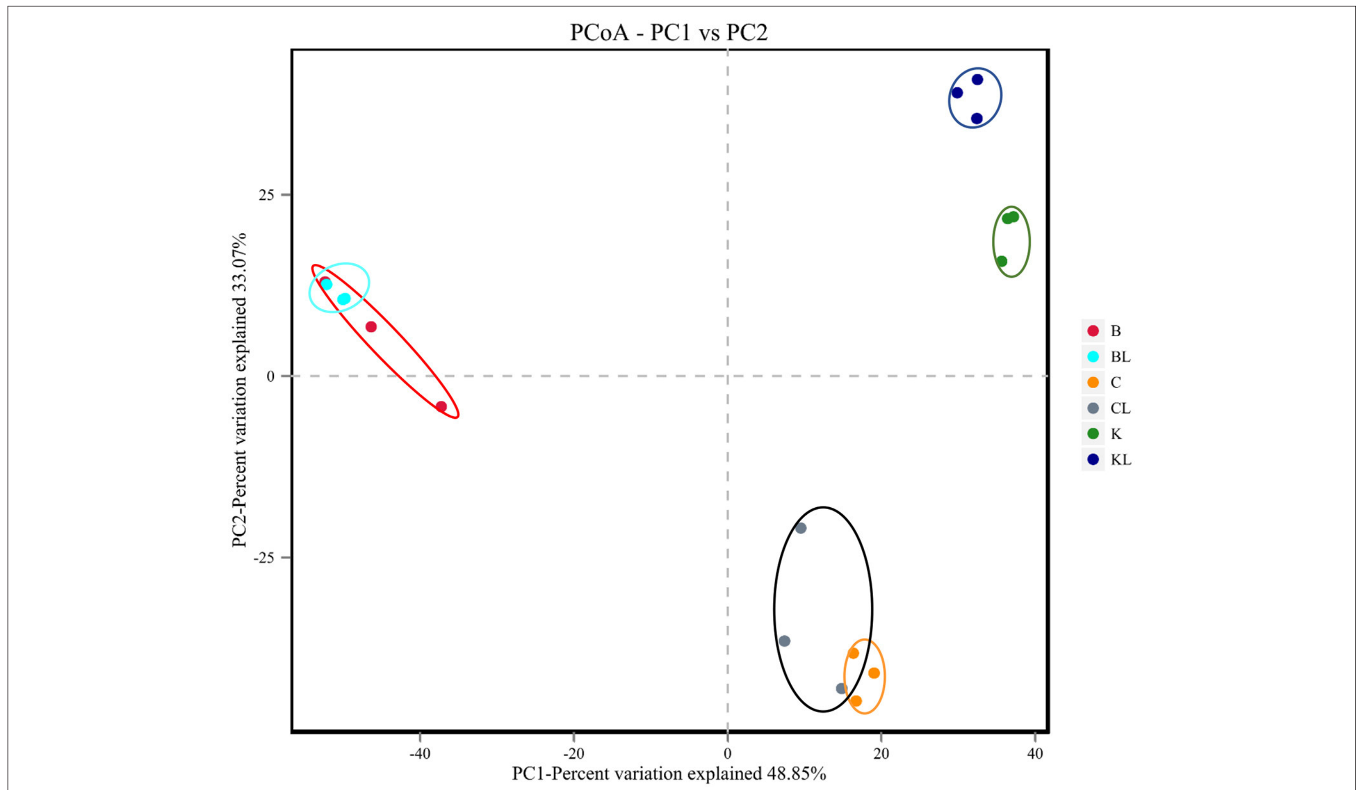

FIGURE 1 | Principal coordinates analysis (PCoA) of the bacterial community of C, K, and B (B, *Broussonetia papyrifera*; BL, *Broussonetia papyrifera*+ *L. plantarum*; C, cassava foliage; CL, cassava foliage+ *L. plantarum*; K, king grass; KL, king grass+ *L. plantarum*).

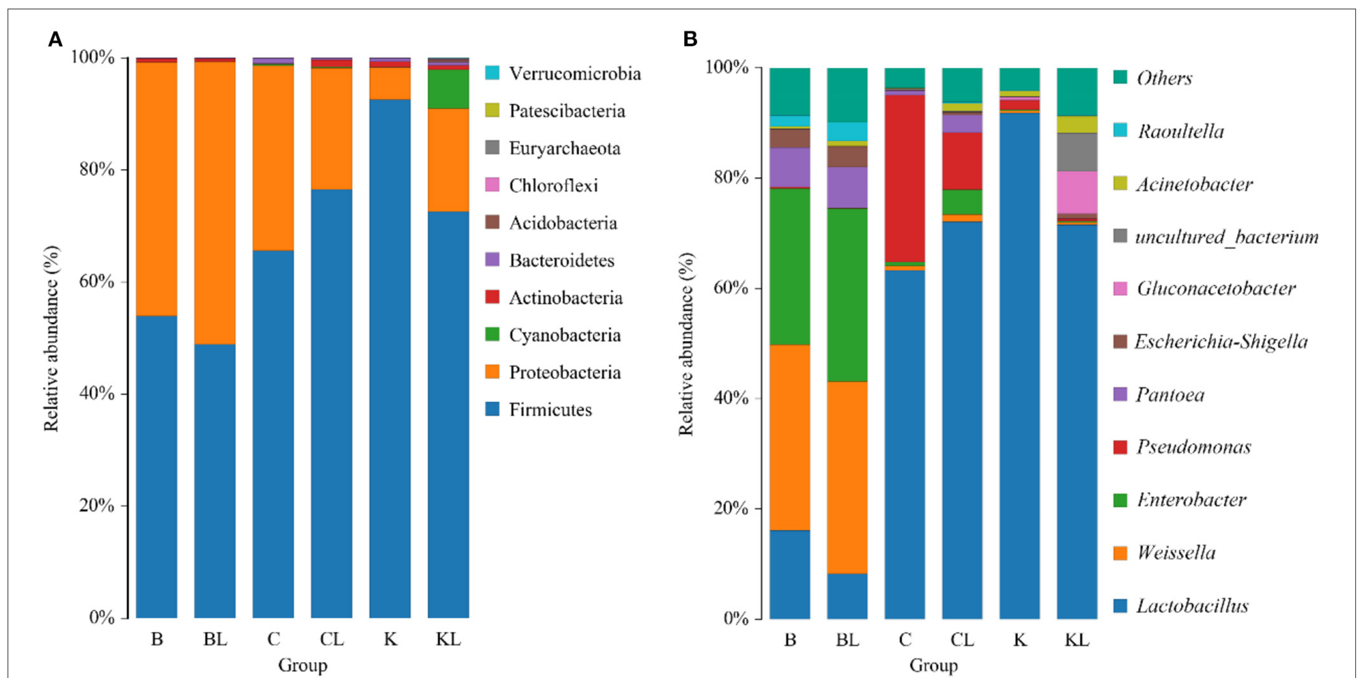

FIGURE 2 | The Silage bacterial community at the phylum **(A)** and genus **(B)** levels of C, K, and B (B, *Broussonetia papyrifera*; BL, *Broussonetia papyrifera*+ *L. plantarum*; C, cassava foliage; CL, cassava foliage+ *L. plantarum*; K, king grass; KL, king grass+ *L. plantarum*).

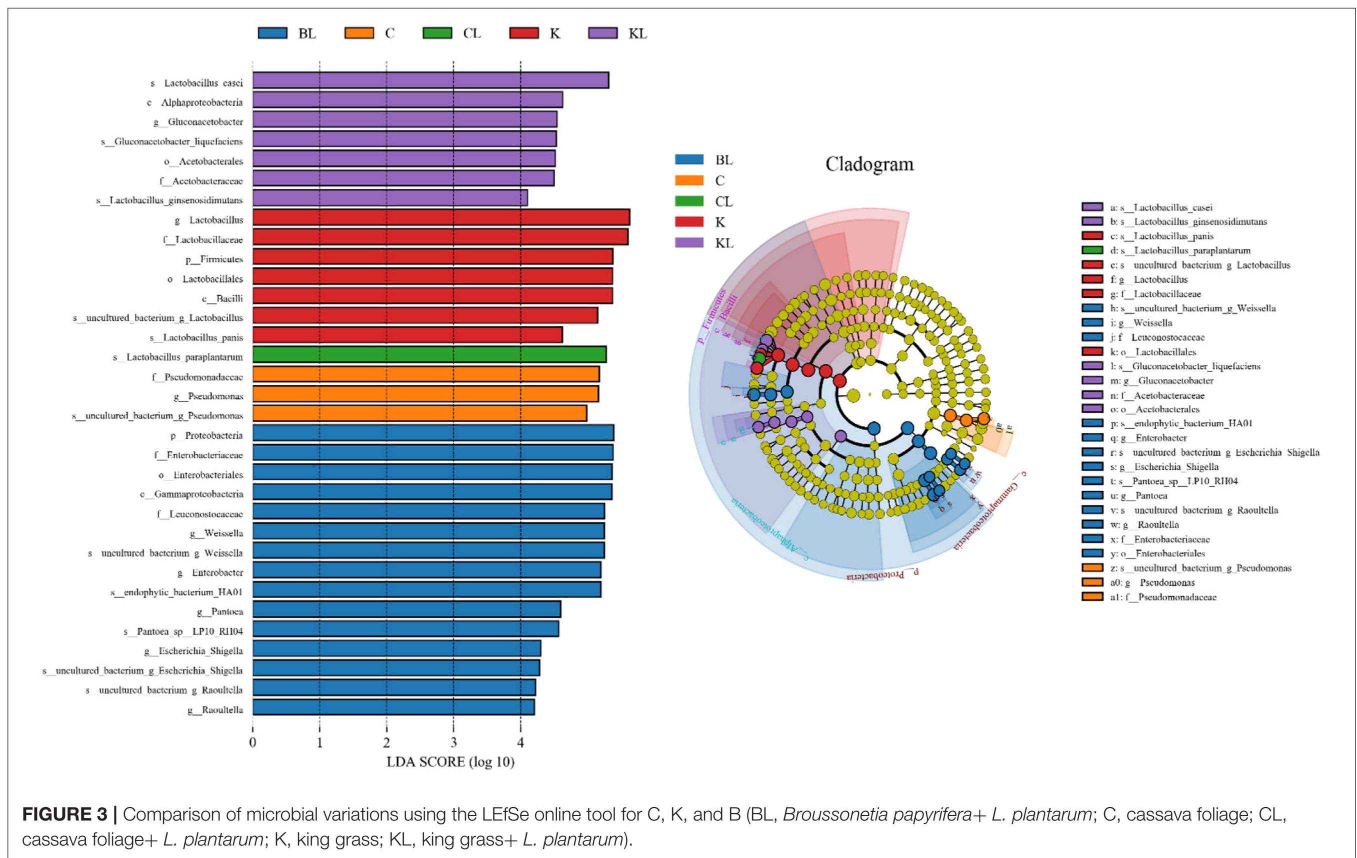

FIGURE 3 | Comparison of microbial variations using the LEfSe online tool for C, K, and B (BL, *Broussonetia papyrifera*+ *L. plantarum*; C, cassava foliage; CL, cassava foliage+ *L. plantarum*; K, king grass; KL, king grass+ *L. plantarum*).

that *Weissella* initiates the silage fermentation, and then the predominant bacteria gradually turn into *Lactobacilli*, which are more tolerant to low pH (Cai et al., 1998). However, *Lactobacillus* was less (16.1–8.3%), and *Enterobacter* was more (28.4–31.4%) compared with the control group. As the core microorganism in the silage process, *Lactobacillus*, the beneficial bacteria, can produce lactic acid and determine the quality of silage (Cai et al., 1998). *Enterobacteriaceae* can grow under anaerobic conditions and trigger protective mechanisms under adverse conditions, including low pH. In this study, *Enterobacter* was the subdominant bacteria after ensiling, existing in silages that are non-pathogenic (Santos et al., 2016). However, their increase is undesirable because they compete with LAB for WSC at the initial stage of ensiling. Other studies also confirmed that *Enterobacter* is harmful to silage because it can compete with LAB for nutrients through fermenting lactic acid into acetic acid and producing ammonia, leading to nutrient loss (Silva et al., 2016). Our finding showed that *L. plantarum* had a weak competitive effect in this treatment, and the high content of oxygen or the high content of *Escherichia coli* attached to the raw materials might lead to the stable period dominated by *Enterobacter* (Chen et al., 2021), which might also explain why there was no significant change in bacterial community composition with or without inoculants. In our study, LAB was not the dominant bacteria in B. It is possible that the competitiveness of LAB already existing or the inoculant *L. plantarum* was weaker than that of the dominant bacteria but the reduction of relative abundance did not affect the fermentation quality of BL. It can be seen from the content of LA and AA. The LDA showed that *Proteobacteria* was the most abundant phylum in the BL group. *Gammaproteobacteria* was the most abundant class, *Enterobacteriaceae* and *Leuconostocaceae* were the most abundant family, *Enterobacteriales* was the most abundant order, and *Weisella*, *Enterobacter*, *Pantoea*, *Escherichia_Shigella*, and *Raoultella* were the most abundant genera. Moreover, *uncultured_bacterium_g_Weisella,endophytic_bacterium_HA01*, *Pantoea_ sp_LI0_RH04*, *uncultured_ bacterium_ g_ Escherichia_ Shigella*, and *uncultured_ bacterium_ g_ Rautella* were the most abundant species.

In CL, the diversity and richness were increased compared with the C group. For β-diversity, **Figure 1** showed that C and CL were separated. The aforementioned diversity analysis showed that L could affect the bacterial diversity and community structure of C silage. At the phylum level, *Firmicutes* and *Proteobacteria* were the dominating bacteria. With inoculant *L. plantarum*, the content of *Proteobacteria* was decreased, while *Firmicutes* was increased. At the genus level, *Lactobacillus* accounts for a very high proportion in C and CL groups. In particular, C and CL groups contained *Pseudomonas*. When *L. plantarum* was added, its relative abundance became less, while the relative abundance of *Lactobacillus* and *Enterobacter* was increased, and *Acinetobacter* appeared. *Pseudomonas* is harmful

to silage quality and nutrient preservation. In this group, the *L. plantarum* treatment notably reduced the relative abundance of *Pseudomonas*, and such a finding was similar to a previous study (Zi et al., 2021). The LDA showed that, in the C group, *Pseudomonadaceae* was the top abundant family, *Pseudomonas* was the most abundant genus, and *uncultured _bacterium_ g_ Pseudomonas* was the most abundant species. In the CL group, *Lactobacillus_ paraplantarum* was the most abundant species.

In KL, the diversity and the richness were decreased. For β-diversity, **Figure 1** indicated that a clear separation of bacterial communities was discovered in K and KL. Therefore, *L. plantarum* could affect the bacterial diversity and community structure of K silage. It could be explained that *L. plantarum* decreased the pH, inhibited the growth of harmful microorganisms, and promoted the growth of LAB species. At the phylum level, *Firmicutes* and *Proteobacteria* had absolute advantages in relative abundance in K and KL, and compared with K, the content of *Firmicutes* was decreased while *Proteobacteria* was increased. Besides, *Cyanobacteria* appeared in the KL group. At the genus level, *Lactobacillus* accounts for a very high proportion in K and KL groups. There were *Gluconacetobacter* and *Acinetobacter* in the KL group, and *Gluconacetobacter* is widely used in wine and vinegar production. It is a bacterial strain that consumes ethanol and sugar to produce acetic acid (Raspor and Goranovic, 2008). Therefore, the higher the relative abundance of *Gluconacetobacter*, the higher the acetic acid content. *Acinetobacter* species are aerobic bacteria. However, previous studies have found that some *Acinetobacter* can survive in an anaerobic environment with acetic acid as substrate (Fuhs and Chen, 1975), which consumes energy from carbohydrates and results in DM loss (Pitt, 1986). Another research has validated this finding, evidenced by increased the content of AA, the abundance of *Acinetobacter*, and the loss of DM in silage treated with *Lactobacillus brucelli* (Oliveira et al., 2017). In this study, when *L. plantarum* was added to K, and the relative abundance of *Acinetobacter* or *Lactobacillus* was increased or decreased, respectively. This finding might be attributed to the high content of acetic acid in the KL group, which provided a suitable growth environment for *Acinetobacter*. The microbial genus level of K had been a relatively single community mainly composed of *Lactobacillus*. When *L. plantarum* was added as an inoculant, the amount of available matrix remains unchanged, which may aggravate intraspecific and interspecific competition so that bacteria with stronger acid production, growth ability, and stress tolerance became dominant bacteria. However, the relative abundance of *Lactobacillus* decreased as a whole. To explore this problem, it is better to identify the community composition to the species level. The LDA showed that, in the K group, *Firmicutes* was the most abundant phylum, *Bacilli* was the most abundant class, *Lactobacillales* was the most abundant order, *Lactobaillaceae* was the most abundant family, *Lactobacillus* was the most abundant genus, and *uncultured_ bacterium_ g_ Lactobailus* and *Lactobacillus_ panis* were the most abundant species. *Alphaproteobacteria* was the most abundant class in the KL group, *Acetobacterales* was the most abundant order, *Acetobacteraceae* was the most abundant family, *Gluconacetobacter* was the most abundant genus, and *Lactobacillus_ Casei*, *Giluconacetobacter_ Liquefaciens*, and *Lactobacillus_ ginsenosidimutans* were the most abundant species.

CONCLUSIONS

Lactobacillus plantarum played a positive role in fermentation quality. The specific performance decreased the pH value, and the NH_3-N content markedly in KL, CL, and BL inhibited the production of BA and increased the content of LA in KL and BL. That shows that the inoculant plays a positive role. Besides, *L. plantarum* were useful to change the bacterial community of C, but it had little effect on K and B. *Lactobacillus* accounts for a very high proportion in K and KL. *Weissella* and *Enterobacter* were the dominant genera in B and BL. The relative abundance of *Lactobacillus* in group C was also very high. In addition, the inoculant *L. plantarum* enriched it in the CL group. As the second dominant genus of group C, the relative abundance of *Pseudomonas* decreased significantly in CL.

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 in the article/supplementary material.

AUTHOR CONTRIBUTIONS

ML and XZ did the experimental design work. YL, TC, RS, XZ, and ML conducted the experiments. YL, ML, and XZ analyzed the data and wrote and revised the manuscript. All the authors read and approved the article.

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