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Addition of Lactic Acid Bacteria Can Promote the Quality and Feeding Value of *Broussonetia papyrifera* (Paper Mulberry) Silage

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Abstract: In this study, the influence of two lactic acid bacteria (LAB) strains [*Lactobacillus rhamnosus* BDy (LR-BDy) and *Lactobacillus buchneri* TSy (LB-TSy)] selected from Southwest China on the fermentation characteristics and in vitro gas production of *Broussonetia papyrifera* (paper mulberry) silage were experimentally explored. The experimental groups were a control group (C), an LB-TSy treatment (LB), an LR-BDy treatment (LR), and an LR-BDy + LB-TSy hybrid group (LR × LB). After the LAB were added, the pH value of paper mulberry silage significantly declined ($p < 0.05$), and the crude protein content was effectively preserved ($p < 0.05$). However, no significant changes were found in the levels of neutral detergent fiber, acid detergent fiber, and crude ash ($p > 0.05$). The lactic acid content in paper mulberry silage was evidently increased ($p < 0.05$). The in vitro gas production in the LR at 36, 48, and 72 h were markedly higher than that in the other treatments ($p < 0.05$). Owing to the addition of LAB, the microbial diversity in paper mulberry silage was reduced, while the relative bacterial abundance of *Lactobacillus* was enhanced. Hence, the addition of LAB selected from the warm and humid region in Southwest China can improve the quality of paper mulberry silage and elevate its feeding value in this region.

Keywords: paper mulberry; silage; in vitro gas production; microbial community; lactic acid bacteria

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1. Introduction

Broussonetia papyrifera (paper mulberry) has been widely applied to feed production, vegetation restoration, and papermaking due to its advantages such as fast growth, strong adaptability, and high yield [1], which is widely distributed in the Asian-Pacific region [2]. Paper mulberry is rich in crude proteins and flavonoids, which have antioxidative and anti-inflammatory effects [3]. It has already been used by scientists to replace Medicago sativa L. to relieve the shortage of high-protein feeds [4]. However, as a ligneous plant with high fiber content [5], paper mulberry shows poor palatability if directly eaten. In addition, paper mulberry is harvested mainly in summer and autumn, so any surplus paper mulberry will be wasted if not well preserved.

As a classical conservation method of green forages, ensiling has been proven capable of softening fibers, improving the palatability, and reducing the protein loss [6]. As indicated by Si et al., adding paper mulberry silage in the daily ration of dairy cows reduces their dry matter (DM) ingestion, strengthens their immunity, and improves the quality of milk while exerting no influence on their physical condition and milk yield [7]. Hao et al. found that adding paper mulberry silage enhanced the antioxidant capacity and immunity of dairy cows without affecting their milk yield, DM digestibility, or intestinal flora [8]. However, lactic acid bacteria (LAB) cannot massively reproduce in paper mulberry due to the high buffering power and low soluble sugar content [5]. Consequently, a satisfactory ensiling effect cannot be achieved for paper mulberry alone. Nevertheless, the initial LAB

content in the silage can be directly increased by inoculating LAB to rapidly reduce the environmental pH, thus achieving feed preservation [9]. Cheng et al. utilized two commercial LAB strains to ferment paper mulberry and found that the fermentation quality of paper mulberry silage improved after the addition of LAB [10]. However, the acid production capacity and growth status of LAB vary between different environments, so the screening of LAB applicable to the ensiling under different climatic conditions is vital [11].

In this study, two LAB strains, *Lactobacillus rhamnosus* BDy (LR-BDy) and *Lactobacillus buchneri* TSy (LB-TSy), were selected for their adaptability to the high-temperature and high-humidity regions in Southwest China. The favorable acid production capacity and high stress resistance in acidic environments of these two strains were verified in the ensiling process of *Medicago sativa* L. [12]. Nevertheless, the fermentation status of the two LAB strains in paper mulberry silage remains unclear. Hence, we use these two LAB strains for paper mulberry ensiling and the nutritional ingredients and fermentation quality were comparatively analyzed. The microbial communities in the paper mulberry silage were analyzed via single-module real-time (SMRT) sequencing technology at the species level, and the feeding value was estimated and evaluated using the in vitro gas production method [13–15].

2. Materials and Methods

2.1. Preparation of Experimental Material

The experiment was carried out on 27 August 2020 using the third-stubble whole-plant hybrid paper mulberry, with the cutting height of 15 cm from the ground. After cutting, paper mulberry was cut into 1.5–2.5 cm segments with a straw chopper. The nutritional ingredients of paper mulberry raw materials are listed in Table 1.

Table 1. Chemical composition of paper mulberry.

Items	Paper Mulberry
Dry Matter (g/kg of FM)	384.78
Crude Protein (g/kg of DM)	164.71
Neutral Detergent Fiber (g/kg of DM)	490.13
Acid Detergent Fiber (g/kg of DM)	240.73
Ash (g/kg of DM)	80.23
WSC (g/kg of DM)	20.62

FM, fresh matter; DM, dry matter; WSC, water-soluble carbohydrate.

The experimental groups were a control group (C; 5 mL/kg distilled water was added), the LR group (5 mL/kg LR-BDy solution was added, with the bacterial count of 1×10^5 CFU/mL), the LB group (5 mL/kg LB-TSy solution was added, with the bacterial count of 1×10^5 CFU/mL), and the hybrid group (LR × LB; 2.5 mL/kg LR-BDy solution and 2.5 mL/kg LB-TSy solution were added, with the bacterial count of 1×10^5 CFU/mL). All bacteria fluid was made up by distilled water. A total of three replicates were set in each treatment. After blending, the mixture was placed into a polyvinyl chloride vacuum sealing bag (30 × 40 cm), followed by vacuum pumping and sealing using a vacuum gauge. Each bag carried 700 g paper mulberry. The bag was unsealed after the storage in a laboratory away from light for 60 d, and the indices were measured.

2.2. Characterization of Used Lactic Acid Bacteria Strains

The LAB strains were LR-BDy, selected from the host-infested maize in Dafang County, Bijie city, in 2017, and LB-TSy, selected from the host-infested maize in Shiqian County, Tongren city, in the same year. Both bacterial strains are preserved at CCTCC. The paper mulberry raw materials used in the experiment were collected from the cultivation base of paper mulberry trees in Changshun County, Guizhou Province (25°43′58.11 N, 106°24′14.18 E, 1019.29 m elevation). All LAB strains were preserved in China Center

for Type Culture Collection (CCTCC, Wuhan, China), LR-BDy's preserving number was CCTCC M 2019986 and LB-TSy's preserving number was CCTCC M 2019987.

2.3. Fermentation Characteristics

The bag was opened after ensiling, and 20 g intermediate samples were taken and blended with 180 g of distilled water, followed by extraction in a 4 °C refrigerator for 24 h and filtering through four layers of gauze and qualitative filter paper to obtain the extract of silage samples. Next, the pH value was determined using a PHS-3C pH meter (Zhejiang SOBO Instrument Co., Ltd. Shaoxing, China), and the levels of lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA) were determined through high-performance liquid chromatography (HPLC) [16]. The HPLC equipment was Agilent 1260LC (Agilent Co., Santa Clara, CA, USA); column, TC-C18 (Agilent Co., Santa Clara, CA, USA); condition, column temperature 50 °C, the first mobile phase, pure methanol, the second mobile phase, 0.01 mol/L KH_2PO_4, pH 2.7, sample injection volume, 10 μL, flow rate, 0.8 mL/min, standard LA, L118492, standard AA, A298827, standard PA, P110446, standard BA, B110438 (Aladdin Co., Shanghai, China), all samples were passed through 0.5 μm filter membrane. Ammonia nitrogen was determined using the method of Broderick and Kang [17].

2.4. Chemical Composition and In Vitro Gas Production

Then, 200 g samples were taken out and placed in an envelope, heated in a 105 °C air-blast drying oven for enzyme deactivation for 30 min, and then transferred to a 65 °C drying oven until reaching a constant weight. Afterwards, the DM content was determined, and the samples were then ground and sieved through a 0.20-mm sieve to determine the nutritional ingredients and in vitro gas production. The CP content was determined in accordance with the method specified in AOAC 990.03. Moreover, the levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined through the filter bag method with reference to Van Soest et al. [18]. Later, water-soluble carbohydrate was assayed according to the anthrone colorimetric method for sugar content determination [19].

Afterwards, the rumen fluid was collected via the in vitro gas production experiment on an Angus bullock (body weight: 600 kg) fitted with permanent rumen cannula. The rumen fluid was collected before feeding in the morning, then filtered with four layers of gauze, and blended with the artificial rumen buffer solution at a proportion of 1:2. The artificial rumen buffer solution was prepared using the method put forward by Menke [20]. The artificial rumen buffer solution was composed of micro element solution ($CaCl_2 \cdot 2H_2O$ 6.60 g, $CoCl_2 \cdot 6H_2O$ 0.50 g, and $FeCl_3 \cdot 6H_2O$ 4.00 g, dissolved in 50 mL distilled water), buffer solution (NH_4HCO_3 0.80 g and $NaHCO_3$ 7.00 g, dissolved in 200 mL distilled water), macro element solution (Na_2HPO_4 1.14 g, KH_2PO_4 1.24 g, and $MaSO_4 \cdot 7H_2O$ 0.12 g, dissolved in 100 mL distilled water), indicator solution (Resazurin 0.1 g dissolved by 100 mL distilled water), and deoxidizer solution (NaOH 0.16 g and $Na_2S \cdot 9H_2O$ 0.625 g, dissolved by 100 mL distilled water). During the preparation, CO_2 was continuously let in until the culture solution became bluish green. Then, part of the mixture was placed in a syringe. The syringe contained 60 mL mixture fluid and 0.50 g of dried feed powders. This mixture was cultured in a 39 °C thermostatic shaker (50 rpm/min) for 72 h, and the gas production reads at different time points (0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72 h) were recorded from the scale on the syringe [20]. Gas production was calculated using the following formula:

$$GP_t = \frac{V_t - V_0 - GP_c}{W}$$

GP_t (mL) was the per 1.0 g feed gas production at t hours, V_t (mL) was the scale reading of the syringe of the fermentation at t hours, V_0 (mL) was the initial scale reading, GP_c (mL) was the gas production of control group (none feed added), W (g) was feed weight.

During the experiment, the Angus bullock was feed twice a day at 8:00 a.m. and 3:00 p.m.; feed 8 kg of dry matter per day, concentrate and roughage ratio of 1:1, contents

whole plant corn silage 2.2 kg, wheat straw 1 kg, bean curd residue 0.8 kg, and mixture concentrate 4 kg.

2.5. Genetic Analysis

The full-length 16S rRNA gene was amplified by PCR for SMRT sequencing using the forward primer 27F(AGRGTTTGATYNTGGCTCAG) and the reverse primer1492R (TASGGHTACCTTGTTASGACTT). The PCR program was as follows: 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, with a final extension of 72 °C for 7 min. After the individual quantification step, amplicons were pooled in equal amounts. Libraries were established using SMRTbell^{TM} Template Prep Kit (PacBio) following manufacturer's recommendations, and then sequenced on the PacBio Sequel platform.

Raw data were processed using the protocol RS_Read-sofinsert.1 in SMRT Link version 8.0 software (Pacific Biosciences, Menlo Park, CA, USA). Low-quality sequences were removed using the Quantitative Insights Into Microbial Ecology (QIIME) package (ver. 1.7). Sequences with the similarity $\geq$97% were distributed to the same operational taxonomic unit (OTU) using the UCLUST algorithm. The SILVA database (release 132) was implemented to classify different OTUs and annotate the taxonomic information for each OTU representative sequence based on Bergey's taxonomy at the genus, family, order, class, and phylum levels, according to classification at an 80% minimum bootstrap threshold [21].

2.6. Statistical Analysis

Statistical analysis of one-way ANOVA was performed using R (v.22). Duncan's HSD test was employed to determine the differences of the treatment means, significance was set at $p < 0.05$, and the data were expressed as mean and accompanied by standard error of the mean (SEM). Based on OTU results, α-diversity (Chao and Shannon indices and Coverage), β-diversity (principal component analysis, PCA; Heatmap; redundancy analysis, RDA; and β-nearest taxon index, β-NTI) were performed using BMKCloud (www.biocloud.net, accessed date on 1 July 2021).

3. Results

3.1. Fermentation Quality and Chemical Composition of Paper Mulberry Silage

At 60 d of fermentation, the chemical composition and fermentation quality of paper mulberry silage are displayed in Table 2. The pH, LA, PA, and Lactic Acid/Acetic Acid (LA/AA) of paper mulberry silage were significantly affected by the LAB added ($p < 0.05$). The highest pH value and the lowest LA content existed in the C group ($p < 0.05$). The LA content in the LR group was significantly higher than that in the LB group and the LR $\times$ LB group ($p < 0.05$), but no significant differences in pH or LA/AA was found between the three groups ($p > 0.05$). After the addition of LAB, the CP content was evidently higher than that in the C group ($p < 0.05$), and the CP content in the LB group was markedly higher than that in the LR group ($p < 0.05$). However, the DM, DM loss, NDF, ADF, ash, and AA were not significantly influenced by the addition of LAB ($p > 0.05$). C group showed higher ammonia nitrogen than other treatments ($p < 0.05$).

Table 2. The chemical composition and fermentation quality of paper mulberry silage.

	C	LB	LR	LR × LB	SEM	*p*-Value
Chemical composition						
Dry matter (g/kg of FM)	368.13 a	375.70 a	371.64 a	372.30 a	2.61	0.106
Crude Protein (g/kg of DM)	122.57 c	147.63 a	139.14 b	146.35 ab	3.13	***
Neutral Detergent Fiber (g/kg of DM)	490.72 a	502.24 a	490.58 a	499.31 a	6.51	0.248
Acid Detergent Fiber (g/kg of DM)	241.68 a	243.39 a	237.08 a	242.63 a	5.6	0.686
Ash (g/kg of DM)	83.84 a	85.84 a	73.71 a	78.33 a	5.97	0.245
DM loss (%)	4.33 a	2.36 a	3.42 a	3.24 a	0.68	0.106
Fermentation characteristics						
pH	4.65 a	4.19 b	4.20 b	4.20 b	0.05	***
Lactic acid (g/kg of DM)	19.19 d	42.27 c	63.32 a	52.16 b	2.32	***
Acetic acid (g/kg of DM)	10.47 a	9.09 a	9.82 a	8.99 a	1.51	0.746
Propionic acid (g/kg of DM)	7.97 c	2.76 d	12.28 b	26.17 a	1.58	***
Butyric acid (g/kg of DM)	ND	ND	ND	ND	ND	ND
Ammonia nitrogen (g/kg of FM)	3.71 a	3.10 b	2.48 c	3.04 bc	0.26	0.007
Lactic acid/acetic acid	1.83 b	4.68 a	6.75 a	5.93 a	0.89	0.003

a, b, c, where there is a significant interaction, values within a row with different letters differ significantly at $p < 0.05$; ***: $p < 0.001$; C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy; SEM: standard error of the mean; FM, fresh matter; DM, dry matter; ND, not detected.

3.2. In Vitro Gas Production of Paper Mulberry Silage

As seen in Table 3, LR showed great gas production potential, the gas production of LR was significantly higher than that in the other treatment at 36, 48, and 72 h ($p < 0.05$).

Table 3. In vitro gas production of paper mulberry silage.

Treatments	C	LB	LR	LR × LB	SEM	*p*-Value
Gas production per 1 g (mL)						
GP2	58.07 a	59.46 a	58.25 a	56.84 a	1.56	0.468
GP4	93.69 a	95.25 a	94.36 a	91.25 b	0.97	0.017
GP6	130.08 b	134.69 a	131.22 ab	127.48 b	1.62	0.014
GP8	161.46 b	169.11 a	167.23 a	161.74 b	2.23	0.017
GP10	184.35 c	193.99 ab	194.93 a	188.57 bc	2.41	0.007
GP12	205.58 c	215.23 ab	219.05 a	210.40 bc	2.75	0.006
GP24	275.62 b	282.42 ab	290.81 a	280.28 b	3.86	0.025
GP36	303.21 b	308.05 b	318.42 a	307.11 b	3.98	0.026
GP48	317.16 b	323.68 b	333.89 a	321.66 b	4.00	0.017
GP72	329.59 b	337.18 b	347.54 a	334.99 b	3.95	0.011

a, b, c, where there is a significant interaction, values within a row with different letters differ significantly at $p < 0.05$; C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy; SEM: standard error of the mean; GP: in vitro gas production, 2, 4, 6 . . . 72 means in vitro rumen fermentation time.

3.3. Microbial Community and Correlation Analysis of Paper Mulberry Silage

The alpha diversity of paper mulberry silage is presented in Table 4, the coverage ranged from 0.9965 to 0.9979, indicating that the vast majority of microbiota were fully captured. After the addition of LAB, the number of OTU, Ace index, and Chao1 index presented a declining trend compared with those in the C group.

Table 4. Alpha diversity of paper mulberry silage.

	OTU	Ace	Chao1	Simpson	Shannon	Coverage
C	128	163.37	157.58	0.62	2.09	0.9965
LB	109	131.84	133.89	0.54	2.16	0.9973
LR	85	106.50	108.86	0.62	2.06	0.9979
LR × LB	107	133.44	138.98	0.50	2.19	0.9972

C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy.

The microbial communities of paper mulberry silage is displayed in Figure 1. In Figure 1a, the main microbiota in C group were Firmicutes (81.85), when LAB was inoculated, Firmicutes content increased by up to 90.98% (LB), 95.66% (LR), and 95.51% (LR × LB). In Figure 1b, after LAB were added, the content of LAB rose from 68.95% (C group) to 82.24%, 87.52%, and 88.06% (LB, LR, and LR × LB groups, respectively). In Figure 1c, we can see the main LAB were *Lactobacillus pentosus* (68.09%) in the C group and *Lactobacillus pentosus* (66.00%), *L. rhamnosus* (8.80%), and *L. buchneri* (7.04%) in the LB group. In the LR group, the proportion of *Lactobacillus pentosus* declined to 39.95%, *L. rhamnosus* accounted for 39.41%, and *L. buchneri* accounted for 7.26%. In the LR × LB group, *Lactobacillus pentosus* accounted for 49.34%, *L. buchneri* for 31.44%, and *L. rhamnosus* for 6.37%.

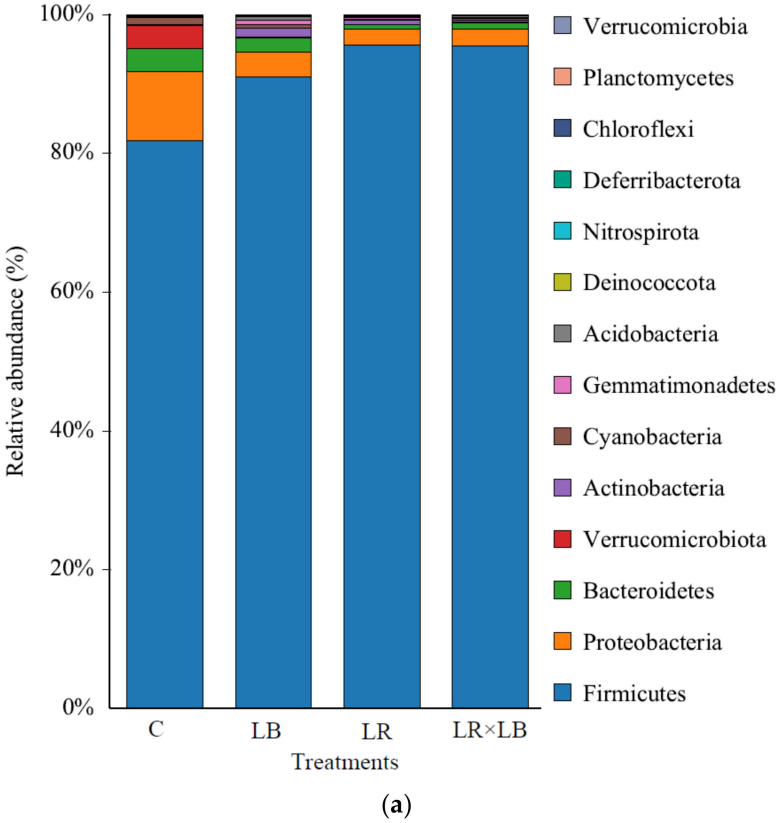
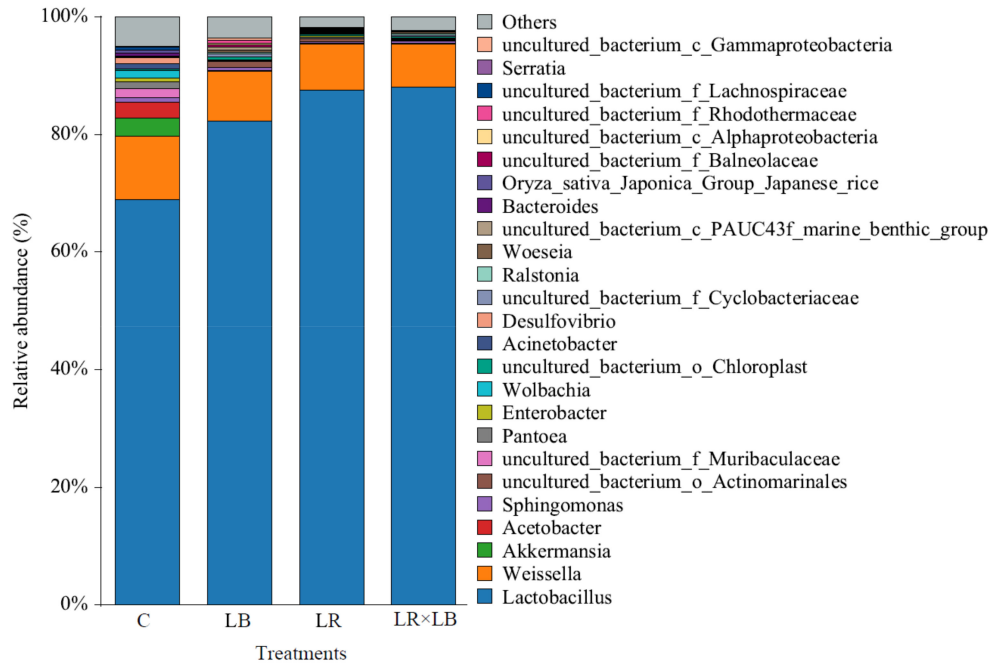
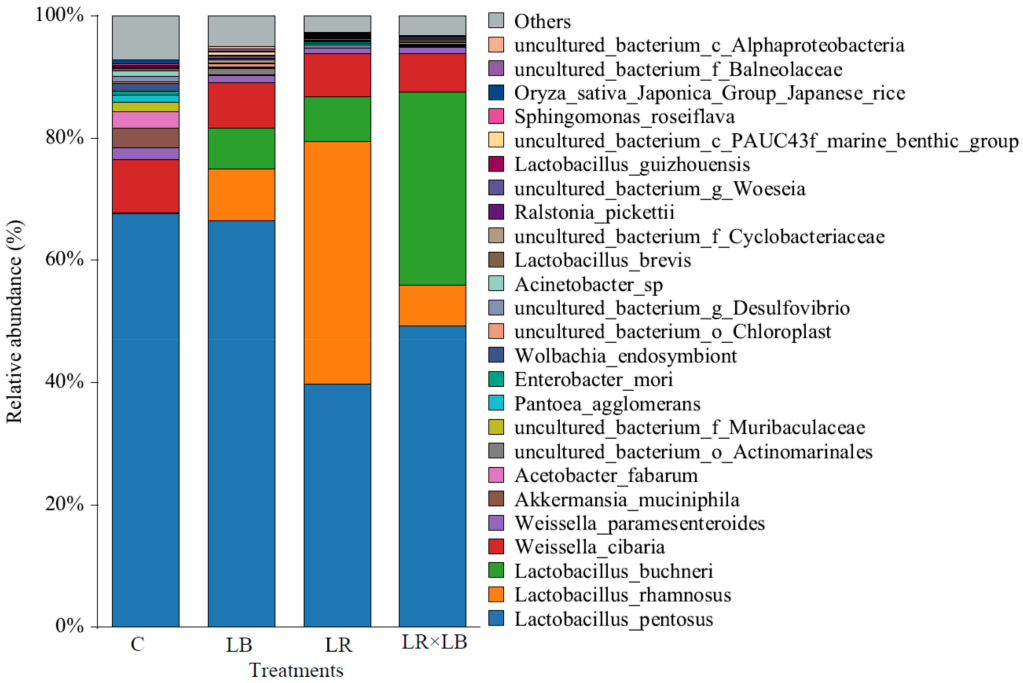


Figure 1. *Cont.*



(**b**)



(**c**)

Figure 1. Relative abundances of paper mulberry silage bacterial communities. (**a**) In phylum level; (**b**) in genus level; (**c**) in species level; C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy.

It can be seen from Figure 2 (correlation heatmap) and Figure 3 (redundancy analysis, RDA) that the levels of *L. buchneri* and *L. rhamnosus* were positively correlated with those of LA and PA but negatively correlated with the pH.

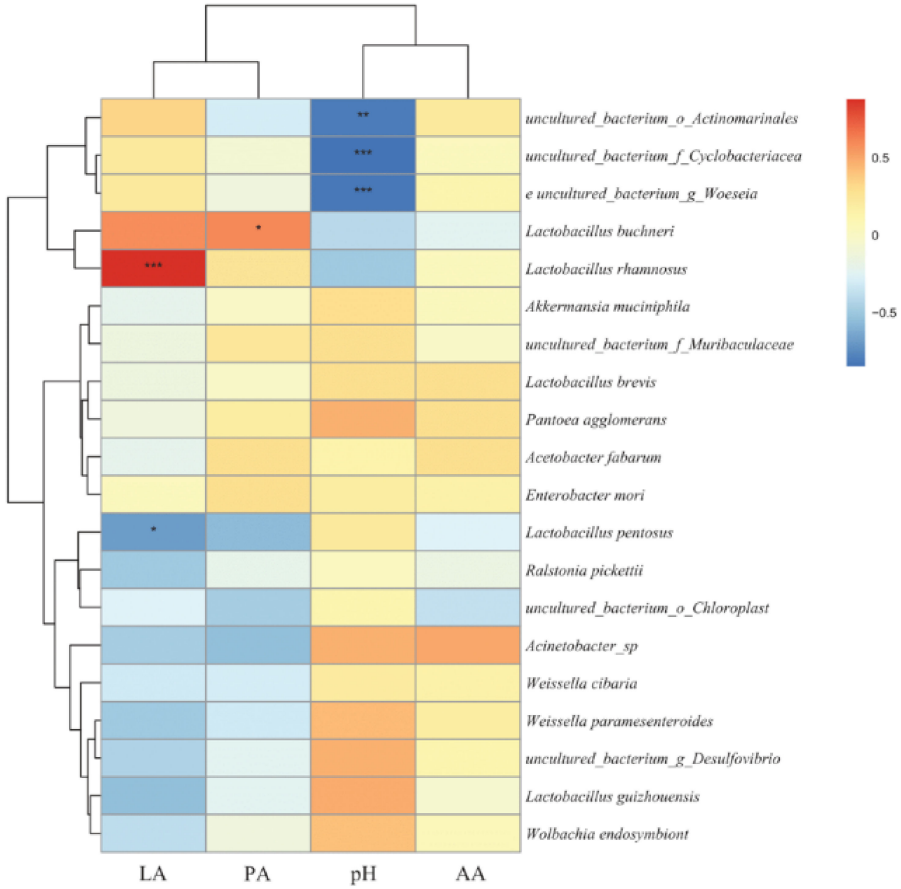


Figure 2. Correlations between fermentation characteristics and microbial community. * significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$.

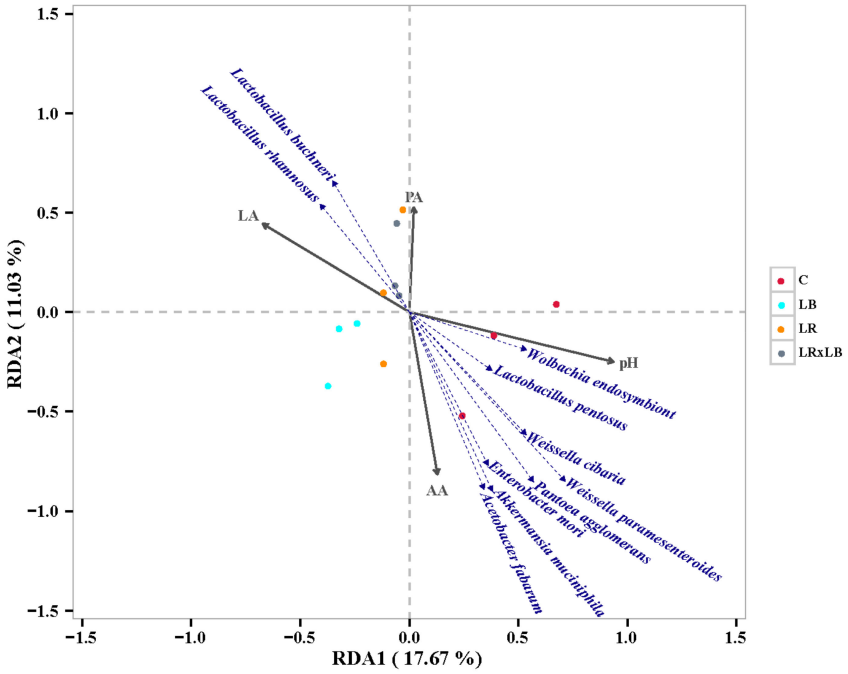


Figure 3. Redundancy analysis between fermentation characteristics and microbial community. C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy.

The principal component analysis (PCA) chart of paper mulberry silage is shown in Figure 4. PCA showed that the difference in the bacterial community succession was evident between the LR and LR × LB groups, but that between the C and LB groups was minor. The β-NTI was calculated for the assembly process of bacterial communities in paper mulberry silage (Figure 5). As revealed by the organizational taxonomic unit clustering of paper mulberry silage, the β-NTI score mostly exceeded 2 points in the C and LR groups (51.52% and 54.29%, respectively), showing that the deterministic process played a dominant role in the two groups. The β-NTI score mostly ranged from −2 to 2 points in the LB and LR × LB groups (71.88% and 76.47%, respectively), indicating that the dominant role of deterministic processes was even more evident in these two groups.

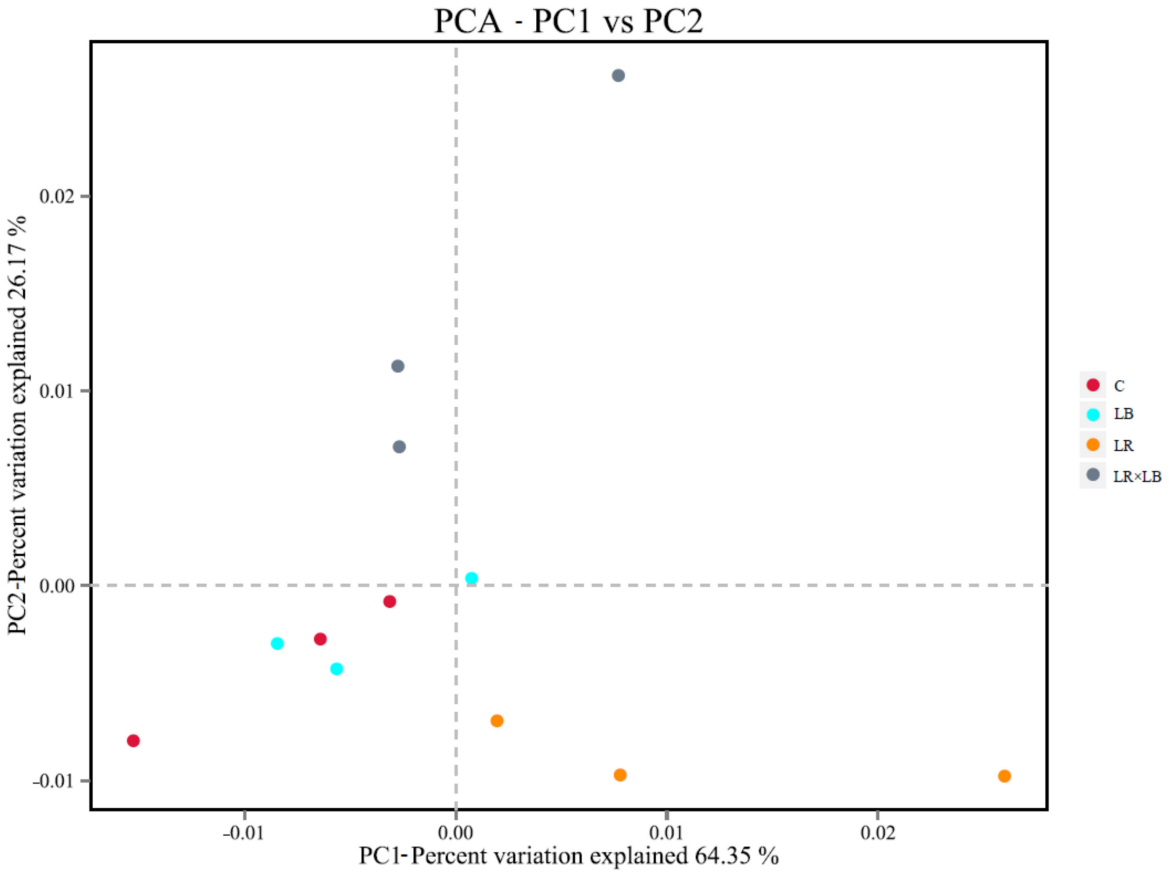


Figure 4. Principal component analysis (PCA) graph of the bacterial community succession between studied samples of paper mulberry silage. C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy.

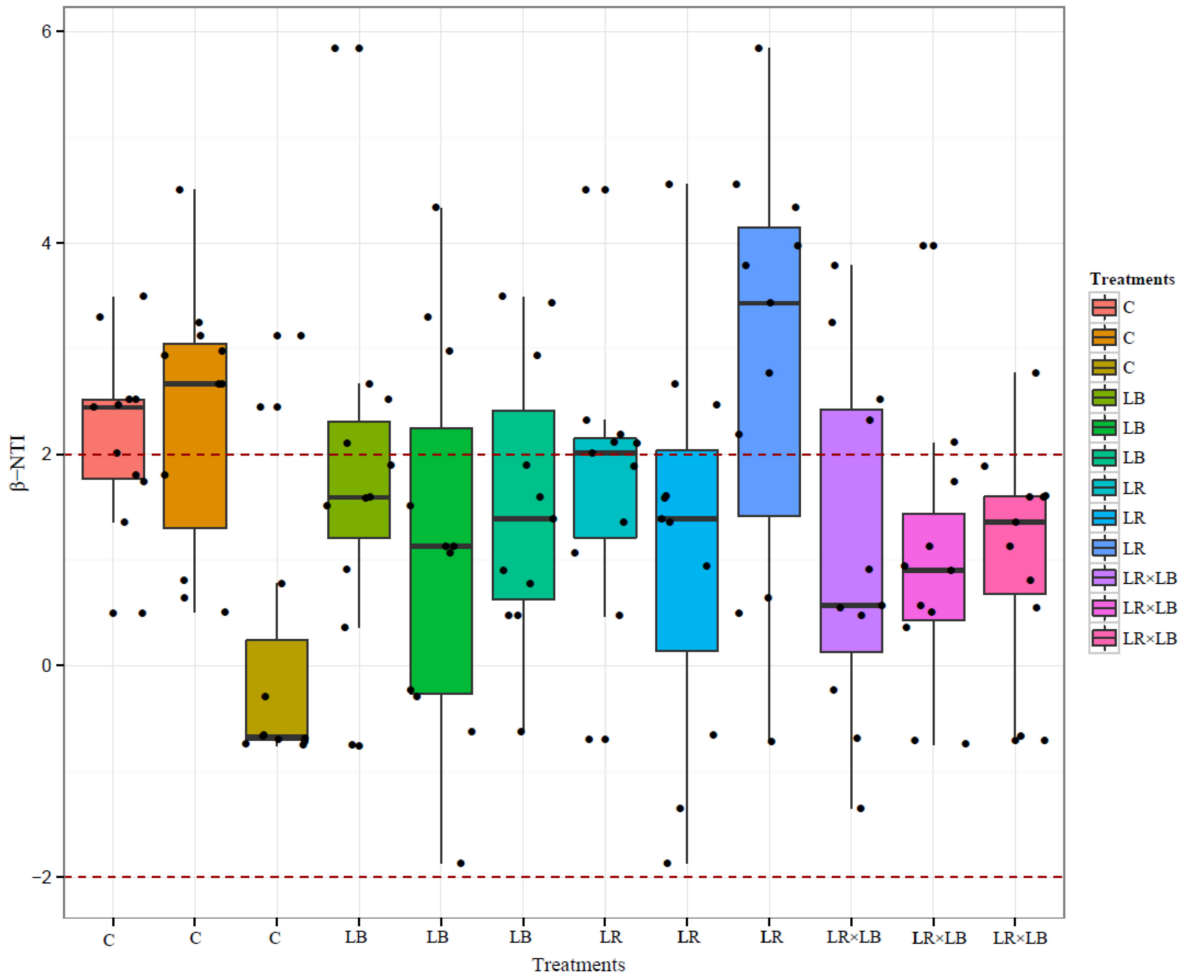
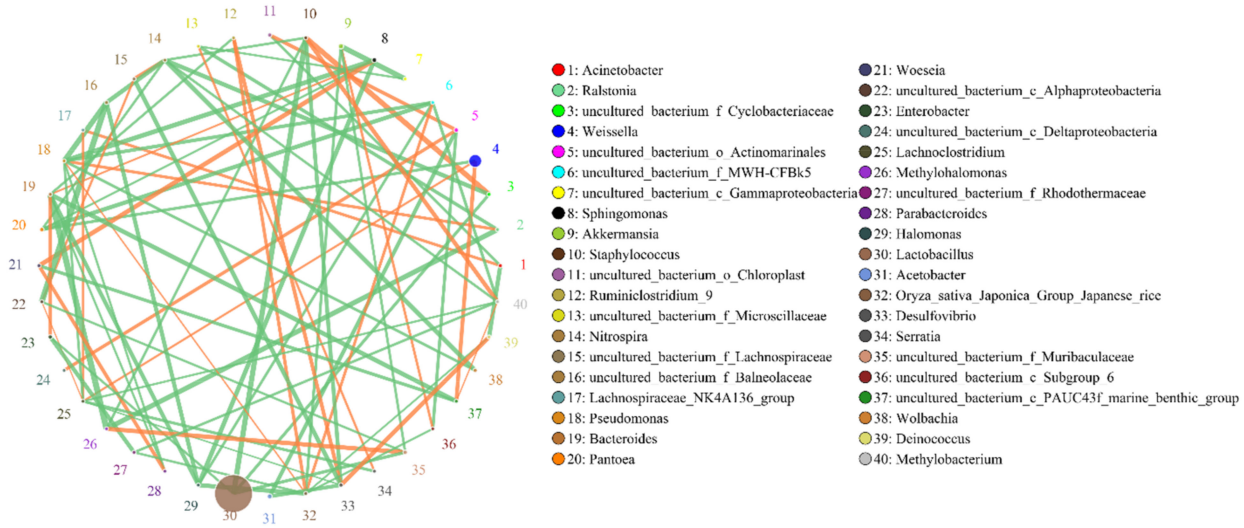
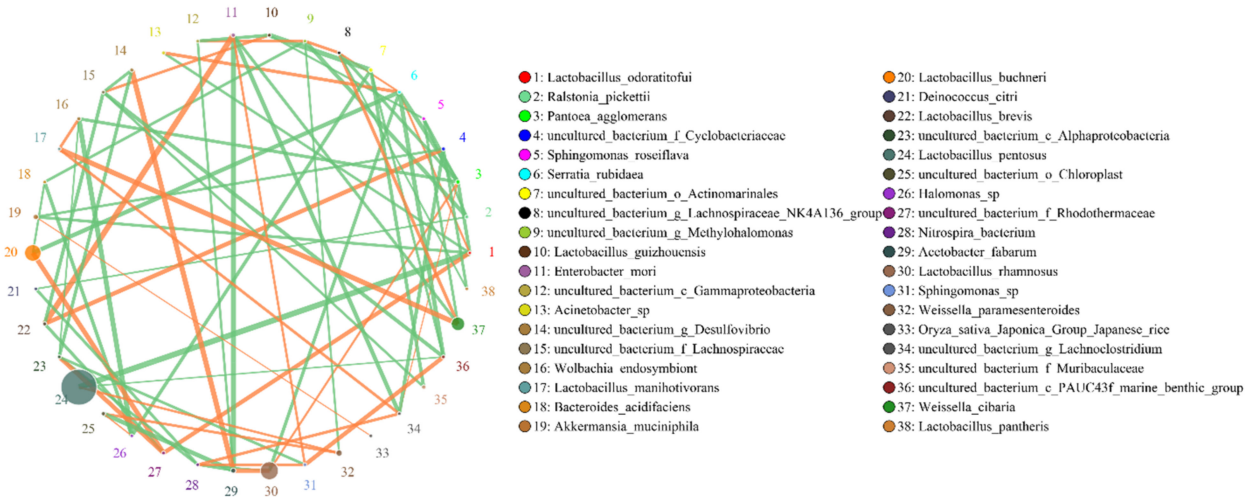


Figure 5. β-NTI of paper mulberry silage. C: silage with distilled water; LB: silage with LB-TSy; LR: silage with LR-BDy; LR × LB: silage with LB-TSy and LR-BDy.

The microbial Spearman correlation coefficient at the genus level is presented in Figure 6a. The microbial abundance of *Lactobacillus* was the highest, which was negatively correlated with *Staphylococcus* and *Enterobacter* and repressed the growth of other microorganisms. The microbial Spearman correlation coefficient at the species level is displayed in Figure 6b. *L. pentosus*, *L. rhamnosus*, *L. buchneri*, and *Weissella cibaria* were the most abundant. *L. pentosus* was negatively correlated with *L. odoratitofui* and positively correlated with *Weissella paramesenteroides*. *L. rhamnosus* was positively correlated with *Acetobacter fabarum* and *Pantoea agglomerans* and negatively correlated with *Serratia rubidaea*. *L. buchneri* had a positive correlation with the family *Rhodothermaceae* and negative correlation with *Serratia rubidaea* and *Bacteroides_acidifaciens*. *Weissella cibaria* was positively associated with *Lactobacillus manihotivorans* and negatively associated with *Sphingomonas roseiflava* and *Enterobacter mori*.



(**a**)



(**b**)

Figure 6. Spearman correlation analysis of paper mulberry silage. (**a**) In genus level; (**b**) in species level.

4. Discussion

4.1. Fermentation Quality, Chemical Composition and Correlation between Silage and Bacterial Community of Paper Mulberry Silage

DM loss is an important index used to evaluate the nutrition retention of silage, should be minimized to retain more nutritional ingredients to facilitate ensiling [22]. With complex fibrous structures, NDF and ADF can be hardly degraded in silage [23]. It was discovered in this study that the levels of NDF and ADF slightly increased after paper mulberry ensiling. The same phenomenon was found by Nair et al., which might be attributed to the reduction in DM content [24]. Adding substances such as cellulase in the ensiling process is a common method specific to NDF and ADF [25].

Silage can be well preserved only when the pH value, the most basic index evaluating the fermentation quality of silage, is kept at approximately 4.20 [26], while pH values lower than 5 are also acceptable for crop feedstocks with sugar contents during the ensiling process [27]. Through anaerobic fermentation for 60 d, the pH declined to approximately 4.2 in LB, LR, and LR × LB groups, while that in the C group was 4.65, indicating that the paper mulberry ensiling was facilitated by adding LAB. Because of the microbial vital activities in the silage, DM degrades into exhaust gas and waste fluid [28]. However, in this experiment, all samples were kept in good condition, hence the exhaust gas and waste fluid were less, so it explained why the DM loss was low (2.36% to 4.33%).

Unsuccessful ensiling will have a severely negative impact on the nutrition retention of feeds. Yang et al. found that proteolysis can occur more easily in *Medicago sativa* L. silage without inoculation of microbial inoculum, and the CP degradation in the silage is mainly caused by infectious microbes such as *Clostridium* and *Enterobacter* [29]. We observed from the microbial species that *Clostridium* and *Enterobacter* accounted for extremely small proportions in all the treatment groups, indirectly indicating that CP has been effectively protected in the silage during the experiment. Furthermore, the microbial sequencing analysis found that the proportion of LAB was not low in the C group, but except for *Lactobacillus pentosus*, the levels of minor anaerobic bacteria such as *Weissella cibaria*, *Weissella paramesenteroides*, *Akkermansia muciniphila*, and *Acetobacter fabarum* in the C group were all higher than those in the other three groups. This might be because the reproduction of other aerobic bacteria and yeasts was well inhibited by the good sealing conditions, laying a foundation for the dominance of LAB. However, a small quantity of LAB adhered to the paper mulberry in the C group, which slowed down the fermentation progress of the silage, and organic matter was utilized by the other anaerobic bacteria, leading to a higher pH value and a lower concentration of LA in the C group.

PA, an antifungal substance, can enhance the aerobic stability of silage. Silva et al. found that inoculated *L. buchneri* can stimulate microorganisms to transform 1,2-propanediol into PA [30]. It might be explained why the PA content was increased in the LR × LB group. However, the concentration of PA in the LB group was lower than that in the LR × LB group, since the reproduction of *L. buchneri* was inhibited in the LB group. Regarding the bacterial community structure in the LB group, though repressed, the added *L. buchneri* still increased the content of LAB in paper mulberry silage.

When ensiling sweet potato stems, Romero et al. found that adding LAB with two different fermentation types can markedly increase the LA content without significantly influencing the AA content in the silage, so the expected dual effect on increasing the levels of LA and AA did not appear [31]. This dual effect was also not observed in this experiment. We speculate that the two different types of LAB generated a synergistic effect or antagonistic action after being blended in the silage. When LB-TSy was added, *L. pentosus* exerted an antagonistic effect on the growth of LB-TSy. In the LR group, the content of *L. rhamnosus* was second only to that of *L. pentosus*. We guess that LR-BDy is capable of mutualistic symbiosis with *L. pentosus*. After LR-BDy and LB-Sy were blended in the silage in the LR × LB group, *L. buchneri* accounted for a much larger proportion than *L. rhamnosus*. A possible reason is that in the initial ensiling phase, *L. rhamnosus* promoted the reproduction of *L. buchneri*. In the later phase, however, *L. buchneri* was still active due to the declining pH and *L. rhamnosus* activity [32,33]. As a result, the content of *L. buchneri* was far higher than that of *L. rhamnosus* in the LR × LB group. Moreover, as the reproduction of *Clostridium butyricum* can be facilitated by high moisture, the moisture should be reasonably regulated in the ensiling process [34]. In this experiment, the moisture content was low in paper mulberry raw materials in all groups, which to some extent restrained the BA fermentation in *Clostridium*, thus keeping the good flavor of the silage.

4.2. In Vitro Gas Production of Paper Mulberry Silage

In vitro gas production, which is positively correlated with the feed degradability, can serve as a numerical basis for the actual feed digestibility of animals [35]. In vitro gas

production in the rumen results from multiple factors. On the one hand, the microbial vital activities in the rumen generate gases [36]. On the other hand, when feeds are digested in the rumen, organic matter will be decomposed to generate gases such as CO_2 and CH_4 [37]. Therefore, the gas production can reflect whether the energy in silage is well preserved. Shah et al. explored the influence of additives on the fermentation of SuMu No.2 *Pennisetum purpureum* in the ensiling process, and the results revealed that the additives preserved the organic matter and promote gas production in the silage [38]. In this experiment, the potential gas production and GP72 in the LR group were significantly higher than those in the other groups, indicating that LR-BDy can obviously inhibit the effect of other microorganisms on the carbohydrates in the silage, thus exerting a positive effect on their preservation.

4.3. Microbial Community of Paper Mulberry Silage

The ACE, Chao1, and Shannon indices were low in the LR, LB, and LR × LB groups, meaning there was low bacterial diversity in these groups, i.e., the growth of infectious microbes was effectively suppressed by the LAB added, which was consistent with the results obtained by Zi et al. [39]. As pointed out by McAllister et al., the higher abundance of the microbial composition in silage will lead to the reproduction of many kinds of unfavorable microorganisms in the ensiling process, thus damaging the silage fermentation quality [40]. LAB-aided fermentation can directly increase the abundance of LAB in silage and reduce the proliferation of infectious microbes, thus effectively facilitating the fermentation [41]. Firmicutes is a dominant phylum in the fermentation of silage since it includes the vast majority of LAB species [42–44]. After the addition of LAB, the proportion of Firmicutes rose from 81.85% to over 90%, while that of *Lactobacillus*, which plays a dominant role in fermentation, rose from 68.95% to over 82%, thus consolidating the dominant position of *Lactobacillus* and showing the enhancing effect of LAB on the microbial community structure in paper mulberry silage [23,24].

5. Conclusions

The addition of LAB isolated from the warm and humid region of Southwest China can improve the quality of paper mulberry silage, and inoculation with LR-BDy has a potential application in enhancing the feeding value of paper mulberry silage.

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