fermentation

MDPI

Article

Fermentation Characteristics, Microbial Compositions, and Predicted Functional Profiles of Forage Oat Ensiled with *Lactiplantibacillus plantarum* or *Lentilactobacillus buchneri*

Yanzi Xiao [1,2], Lin Sun [3], Zhijun Wang [4], Wei Wang [2], Xiaoping Xin [1,*], Lijun Xu [1,*] and Shuai Du [5,*]

[1] Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Science, Hulunber Grassland Ecosystem Observation and Research Station, Beijing 100081, China
[2] College of Agriculture and Forestry, Hulunbuir University, Hulunber 021000, China
[3] Inner Mongolia Academy of Agricultural Science & Animal Husbandry, Hohhot 010031, China
[4] Key Laboratory of Forage Cultivation, Processing and High Efficient Utilization of Ministry of Agriculture, Key Laboratory of Grassland Resources, College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Ministry of Education, Hohhot 010019, China
[5] National Engineering Laboratory of Biological Feed Safety and Pollution Prevention and Control, Key Laboratory of Molecular Nutrition, Ministry of Education, Key Laboratory of Animal Nutrition and Feed, Ministry of Agriculture and Rural Affairs, Key Laboratory of Animal Nutrition and Feed Science of Zhejiang Province, Institute of Feed Science, Zhejiang University, Hangzhou 310058, China
* Correspondence: xinxiaoping@caas.cn (X.X.); xulijun@caas.cn (L.X.); nmgdushuai@zju.edu.cn (S.D.)

Abstract: This study aimed to investigate the effects of lactic acid bacteria (LAB) inoculants on the fermentation quality, microbial compositions, and predicted functional profiles of forage oat. The forage oat was inoculated with distilled water, *Lentilactobacillus buchneri* (LB), and *Lactiplantibacillus plantarum* (LP) as the control (CON), LB and LP treatments, respectively, and the addition of *Lentilactobacillus buchneri* (LB) or *Lactiplantibacillus plantarum* (LP) resulted in 1×10^6 colony-forming units/g of fresh weight. After 30 days of fermentation, the lowest pH (4.23) and the lowest content of ammoniacal nitrogen (NH_3-N) in dry matter (DM, 4.39%) were observed in the LP treatment. Interestingly, there was a significant ($p < 0.05$) difference in lactic acid (LA) concentration among the three treatments. The LP treatment had the highest lactate concentration (7.49% DM). At the same time, a markedly ($p < 0.05$) elevated acetic acid (AA) concentration (2.48% DM) was detected in the LB treatment. The Shannon and Chao1 indexes of bacterial and fungal communities in all the silage samples decreased compared to those in the fresh materials (FM). Proteobacteria was the dominant phylum in the FM group and shifted from Proteobacteria to Firmicutes after ensiling. *Lactobacillus* (64.87%) and *Weissella* (18.93%) were the predominant genera in the CON, whereas *Lactobacillus* dominated the fermentation process in the LB (94.65%) and LP (99.60%) treatments. For the fungal community structure, the major genus was *Apiotrichum* (21.65% and 60.66%) in the FM and CON groups after 30 days of fermentation. *Apiotrichum* was the most predominant in the LB and LP treatments, accounting for 52.54% and 34.47%, respectively. The genera *Lactococcus*, *Pediococcus*, and *Weissella* were negatively associated with the LA content. The genus *Ustilago* and *Bulleromyces* were positively associated with the LA content. These results suggest that the addition of LAB regulated the microbial community in oat silage, which influenced the ensiling products, and LP was more beneficial for decreasing the pH and NH_3-N and increasing the LA concentration than LB in forage oat silage.

Keywords: lactic acid bacteria; fermentation quality; microbial community; forage oat silage

Citation: Xiao, Y.; Sun, L.; Wang, Z.; Wang, W.; Xin, X.; Xu, L.; Du, S. Fermentation Characteristics, Microbial Compositions, and Predicted Functional Profiles of Forage Oat Ensiled with *Lactiplantibacillus plantarum* or *Lentilactobacillus buchneri*. *Fermentation* **2022**, *8*, 707. https://doi.org/10.3390/fermentation8120707

Academic Editor: Donatella Cimini

Received: 03 November 2022
Accepted: 01 December 2022
Published: 4 December 2022

1. Introduction

Forage oat (*Avena sativa*), one of the most important feed resources worldwide with the features of high yield, abundant nutrition, and high adaptability, is considered an indispensable basic forage crop for the livestock industry [1,2]. Ensiling, an important,

routine, and reliable forage and crop preservation technique for animal husbandry, has attracted increasing attention, especially in developed countries [3,4]. The preservation of silage from forages and crops relies on microbial or ecological diversity. Of the various microbiome components, lactic acid bacteria (LAB) play a critical role in the conservation of silage with high quality. Microbial inoculants can alter different aspects of silage fermentation, such as the production of lactic acid and other beneficial organic acids and the lowering of pH to inhibit the proliferation of decaying microorganisms [5–7]. In addition, silage preservation using LAB inoculants is widely regarded as a reliable and convenient storage method for fresh forage in biorefining and animal production and has been widely applied to improve the retention of forage nutrition and ensiling performance [8,9].

The application of LAB inoculants is determined by their growth performance and the ability to rapidly induce acidification, increasing aerobic stability and silage digestibility [10]. Based on natural plant biomass fermentation, two categories of LAB inoculants, homofermentative and heterofermentative LAB, are mostly considered for silage production. *Lactiplantibacillus plantarum* is a homologous fermentation lactic acid bacteria, which can be widely reported to reduce pH quickly, inhibit pathogenic microbial growth, and preserve plant proteins [7]. *Lentilactobacillus buchneri* is a heterologous lactic acid bacteria, which can produce additional compounds from hexoses other than lactic acids, such as acetic acid, ethanol, and CO_2 that can produce significant concentrations of acetic acid through the conversion of lactic acid during fermentation [11]. Nevertheless, not all LAB inoculants markedly improve silage quality due to the diversity of LAB inoculants, and it is impossible to create universal LAB-based products [12]. Additionally, the alterations of microbial communities are not significantly determined by fermentation, but these microorganisms are connected to silage quality [13]. The changes in the bacterial community worldwide have been investigated in alfalfa [14], corn [15], barley [16], wheat [17], and other forage silages. Previous studies also indicate that LAB inoculation could improve the fermentation quality of oat silage by reconstructing the bacterial community composition [18,19], whereas research on the changes in the fungal community of forage oat silage during the fermentation process is limited.

Therefore, the current study aimed to explore the effects of *L. plantarum* or *L. buchneri* on ensiling performance and the microbiome of forage oat silage. Additionally, the associations between fermentation characteristics and the microbiome were also assessed.

2. Materials and Methods

2.1. Substrate and Silage Preparation

Forage oat was collected from Hulunber Grassland Ecosystem National Observation and Research Station Chinese Academy of Agricultural Sciences, Hulunber, China (49°23′13″ N, 120°02′47″ E; altitude, 627–635 m; annual temperature, −2.4 °C). On 30 July 2021, fresh forage oat was harvested at the milk maturity stage, spread for 3 h after harvest, and cut into 30 mm segments with a shredder (Fulida Tool Co., Ltd., Linyi, China). In total, 400 g of freshly collected forage oat was stored in a sterilized 15 L freezer (15L, Shaners Equipment, Guangzhou, China) and was quickly sent and rushed to the laboratory for raw material analysis.

The *Lactiplantibacillus plantarum* (MTD-1) and *Lentilactobacillus buchneri* (40788) additives were purchased from the Jiangsu Lvke Biotechnology Company and were utilized as LAB additives. The forage oat was inoculated with distilled water, *L. buchneri* and *L. plantarum* as the CON, LB, and LP treatments, respectively, and the addition of *L. plantarum* or *L. buchneri* was dissolved into the same volume of distilled water with the same volume in the CON treatment and was added at 1×10^6 colony-forming units/g of fresh weight are shown in Table 1. Approximately 250 g of chopped forage oat was placed in a polyethylene bag (32 × 26 cm) and vacuum sealed. The samples were equally distributed and kept at an ambient temperature (20 °C–25 °C) for 30 days of fermentation.

Table 1. The design of the experiment.

Fresh Materials	Treatment	Distilled Water	LAB Additives
Forage oat	CON	10ml/kg FM	0
	LB	10ml/kg FM	*L. buchneri* (1×10^6 CFU/g)
	LP	10ml/kg FM	*L. plantarum* (1×10^6 CFU/g)

CON, control group; LB, *Lentilactobacillus buchneri* inoculated treatment; LP, *Lactiplantibacillus plantarum* inoculated treatment; FM, fresh weight; CFU, colony-forming units.

2.2. Analyses of Fermentation Characteristics and Chemical Compositions

For sampling, clean sterilized containers were used to collect the fresh material (FM), and the forage oat silage was blended uniformly. The dry matter (DM) content was tested after drying a subsample in an oven for 72 h at 65 °C (WP-25AB, Taisite Instrument, Tianjin, China). The specimens were ground through a 1 mm screen (FW100, Taisite Instrument, Tianjin, China) for further chemical composition analysis. Water-soluble carbohydrates (WSC) were detected by the anthrone method [20]. A method developed by the Association of Official Analytical Chemists was applied to assess the crude protein (CP) contents [21]. The ANKOM A200i Fiber Analyser (ANKOM Technology, Macedon, NY, USA) was utilized to assess the fiber compositions of the lignin, neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents [22,23]. In total, 10 g of silage specimens was added to 90 mL of deionized water and kept at 4 °C for a 24 h extraction. Then, the extracts were filtered through a four-layer cheesecloth to analyze fermentation characteristics. A glass-electrode pH meter was used to measure the filtrates' pH. Ammonia nitrogen (NH_3-N) was detected as described in a previous report [24]. Organic acids, including lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), in the forage oat silage were quantitated as described in a previous report [25]. Microbial populations (LAB, yeasts, and molds) in the FM were assessed as described in a previous report [25].

2.3. DNA Extraction, PCR and Sequencing

To analyze the microbiome of forage oat silage, all specimens were stored at –80 °C until DNA extraction. The HiPure Stool DNA Kit (Magen, Guangzhou, China) was used to extract microbial DNA from the FM and silage samples based on the manufacturer's protocol. To assess the bacterial communities, amplicons covering the 16S rRNA V5–V7 hypervariable regions were obtained with the primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1193R (5′-ACGTCATCCCCACCTTCC-3′) [26]. Similarly, amplicons covering the ITS region of fungal DNA were obtained with the primers ITS1_F_KYO2 (5′-TAGAGGAAGTAAAAGTCGTAA-3′) and ITS86R (5′-TTCAAAGATTCGATGATTCAC-3) [27]. The sequences of these samples have been uploaded into the public database with the accession number PRJNA863426.

2.4. Microbial Community Analyses

Editing, unique sequence selection, chimera identification, read assembly, amplicon sequence variant (ASV) detection, and grouping were carried out with the divisive amplicon denoising algorithm (DADA2) in R version 3.5.1 [28]. The ASV classification into organisms used the naïve Bayesian model according to the RDP classifier (http://rdp.cme.msu.edu/) based on the SILVA (bacteria) [29] or ITS2 (for fungi) [30] database and applied a confidence threshold of 80%. The alpha diversity indicator Chao1 and Good's coverage were calculated with QIIME v1.9.1 [31]. Principal coordinate analysis (PCoA) was performed with unweighted or weighted UniFrac distance in the R package (2.5.3) (San Diego, CA, United States). Krona v2.6 was applied by visualizing taxon abundance [32]. The bacterial community compositions were visualized with R v2.2.1 [33], and the results are depicted in stacked bar plots. The phylum and genus abundance levels of the fungi were obtained with Circos v0.69-3 [34], and the results were shown as circular layouts. The phylum and genus abundances were graphed with the heatmap package

v1.0.12, and these results are expressed by the heatmap [35]. Spearman correlation coefficients for genera were assessed with R v1.8.4 [36], and a network for the correlation coefficients was obtained with the online platform (http://www.omicsmart.com). An online tool (https://www.omicstudio.cn/tool/60) was used to carry out linear discriminant analysis (LDA) effect size (LEfSe) analyses, with LDA score >3 and $p < 0.05$ as the thresholds.

2.5. Statistical Analysis

The ensiling performance data (DM, WSC, pH, LA, AA, PA, and NH_3-N parameters) were expressed as the mean ± standard error of triplicate measurements. The impacts of additives on silage quality were analyzed by SAS 9.0 software (SAS Inc.). The data were assessed with the additive effect by the general linear models: $Y_{ij} = \mu + \alpha_i + \beta_j + (a \times \beta)_{ij} + \varepsilon_{ij}$, where μ is the overall mean, α_i is the additive effect, and ε_{ij} is the residual error [6].

3. Results

3.1. Characteristics of Forage Oat Prior to Ensiling

Table 2 shows the chemical compositions and microbial populations of the forage oat prior to ensiling. The DM content was 426.92 g/kg FM. The WSC, CP, NDF, and ADF contents were 163.26, 124.64, 533.33, and 340.51 DM, respectively. The numbers of LAB, yeasts and mold were 4.57, 4.67, and 4.13 lg_{10} CFU/g in FM, respectively.

Table 2. Chemical compositions and microbial populations of forage oat by plate culture before ensiling.

Item	Forage Oat
DM (%)	426.92 ± 1.26
WSC (g/kg DM)	163.26 ± 0.36
CP (g/kg DM)	124.64 ± 0.12
NDF (g/kg DM)	533.33 ± 1.57
ADF (g/kg DM)	340.51 ± 0.71
LAB (lg10 CFU/g FM)	4.57 ± 0.07
Yeasts (lg10 CFU/g FM)	4.67 ± 0.11
Molds (lg10 CFU/g FM)	4.13 ± 0.09

DM-dry matter; WSC-water-soluble carbohydrates; CP-crude protein; NDF-neutral detergent fiber; ADF-acid detergent fiber; CFU-colony-forming units; LAB-lactic acid bacteria.

3.2. Fermentation Parameters of Forage Oat Silage Treated without or with Additives

The fermentation characteristics of forage oat silages inoculated without or with additives are summarized in Table 3. Following a 30-day fermentation process, the DM content decreased in the LP treatment compared with the CON and LB treatments, whereas a higher DM content was observed in the CON group compared with the LB treatment. Similarly, the lowest WSC content was also found in the LP treatment, followed by the LB and CON treatments. As expected, the lowest pH value was found in the LP treatment, and there was no significant difference in the pH value between the CON and LB treatments. Meaningfully, the NH_3-N content was significantly ($p < 0.05$) lower in the LP treatment than in the CON and LB treatments, and no significant difference was observed between the CON and LB treatments. Interestingly, there was a significant ($p < 0.05$) difference in the LA concentration among the three treatments, and the order was the LP, LB, and CON treatments. At the same time, a markedly ($p < 0.05$) elevated AA concentration was detected in the LB treatment among the three treatments, followed by the CON and LP treatments. In addition, a significantly ($p < 0.05$) higher PA concentration was also found in the LB treatment compared to the CON and LP treatments, and there were no significant ($p > 0.05$) differences between the CON and LP treatments. Notably, BA was not detected in any of the treatments.

Table 3. Fermentation quality of the forage oat silage.

Items	Treatments		
	CON	LB	LP
DM (%)	43.13 ± 0.05 a	42.93 ± 0.35 a	41.17 ± 0.47 b
WSC (% DM)	25.66 ± 1.08 a	17.50 ± 0.51 b	14.33 ± 0.57 c
pH	4.67 ± 0.13 a	4.57 ± 0.03 a	4.23 ± 0.08 b
NH_3-N (% DM)	6.13 ± 0.11 a	5.96 ± 0.33 a	4.39 ± 0.88 b
LA (% DM)	4.47 ± 0.32 c	5.51 ± 0.22 b	7.49 ± 0.27 a
AA (% DM)	1.74 ± 0.12 b	2.48 ± 0.17 a	1.22 ± 0.05 c
PA (% DM)	0.09 ± 0.03 b	0.15 ± 0.02 a	0.05 ± 0.02 b
BA (% DM)	ND	ND	ND

DM, dry matter; WSC, water-soluble carbohydrates; NH_3-N, ammonia nitrogen; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; ND, not detected; CON, control group; LB, *Lentilactobacillus buchneri* inoculated treatment; LP, *Lactiplantibacillus plantarum* inoculated treatment. Means in the same column with different letters indicate significant differences ($p < 0.05$).

3.3. Bacterial Community Compositions

As shown in Table 4, this study obtained 1,853,774 high-quality reads with a coverage of approximately 0.99 for all samples. The number of ASVs identified by the overall 16S rRNA gene sequencing was 486, and the lowest ASV was found in the LP treatment. The Chao1 and Shannon indexes of bacterial diversity were affected by additives. The Chao1 index of LB was significantly higher than that of LP ($p < 0.05$). Compared with the CON treatment, the LP treatment significantly ($p < 0.05$) reduced the Shannon index.

Table 4. Characteristics of amplicon libraries in the bacterial community.

Characteristic	FM	CON	LB	LP	Total No.
No. of sequences	112,652 ± 2668 c	180,449 ± 2986 a	170,449 ± 3186 ab	17,429 ± 10,062 b	1,853,774
No. of ASV	37 ± 1 ab	51 ± 7 a	46 ± 3 a	27 ± 2 b	486
Chao1 index	47.60 ± 2.11 ab	57.65 ± 10.49 ab	60.72 ± 9.19 a	32.14 ± 6.06 b	
Shannon index	1.18 ± 0.34 b	2.12 ± 0.11 a	1.46 ± 0.07 b	0.06 ± 0.01 c	
Good's coverage	0.99	0.99	0.99	0.99	

ASV, amplicon sequence variant; FM, fresh material; CON-control group; LB, *Lentilactobacillus buchneri* inoculated treatment; LP, *Lactiplantibacillus plantarum* inoculated treatment; Means with different letters in each row differ at $p < 0.05$.

A Venn diagram depicts the common and unique ASVs in different treatments. In total, four ASVs, as core genera, were present in all treatments (Figure 1A). Principal component analysis (PCoA) showed that the FM, CON, LB, and LP treatments were overtly separated and distinct (Figure 1B).

Figure 1C shows the phylum-level compositions of the microbiomes in FM and forage oat silage. Proteobacteria (99.90%) were the dominant phylum in the FM group. After the fermentation process, Firmicutes dominated the fermentation (Figure 1C). Genus-level compositions of the bacterial community are displayed in the FM and forage oat silage in Figure 1D. The major genus in the FM group was *Erwinia*, followed by *Pesudomnas*. After ensiling, the dominant genera varied in the CON, LB, and LP treatments. *Lactobacillus* and *Weissella* were the predominant genera in the CON treatment, whereas *Lactobacillus* dominated the fermentation process in the LB and LP treatments (Figure 1D). The LEfSe data revealed substantially different taxonomic features between the FM and treatments (Figure 1E). *Erwinia* was concentrated in the FM group. After the fermentation process, *Weissella* was enriched in the CON treatment, whereas *Halomonas* and *Lactobacillus* were enriched in the LB and LP treatments, respectively.

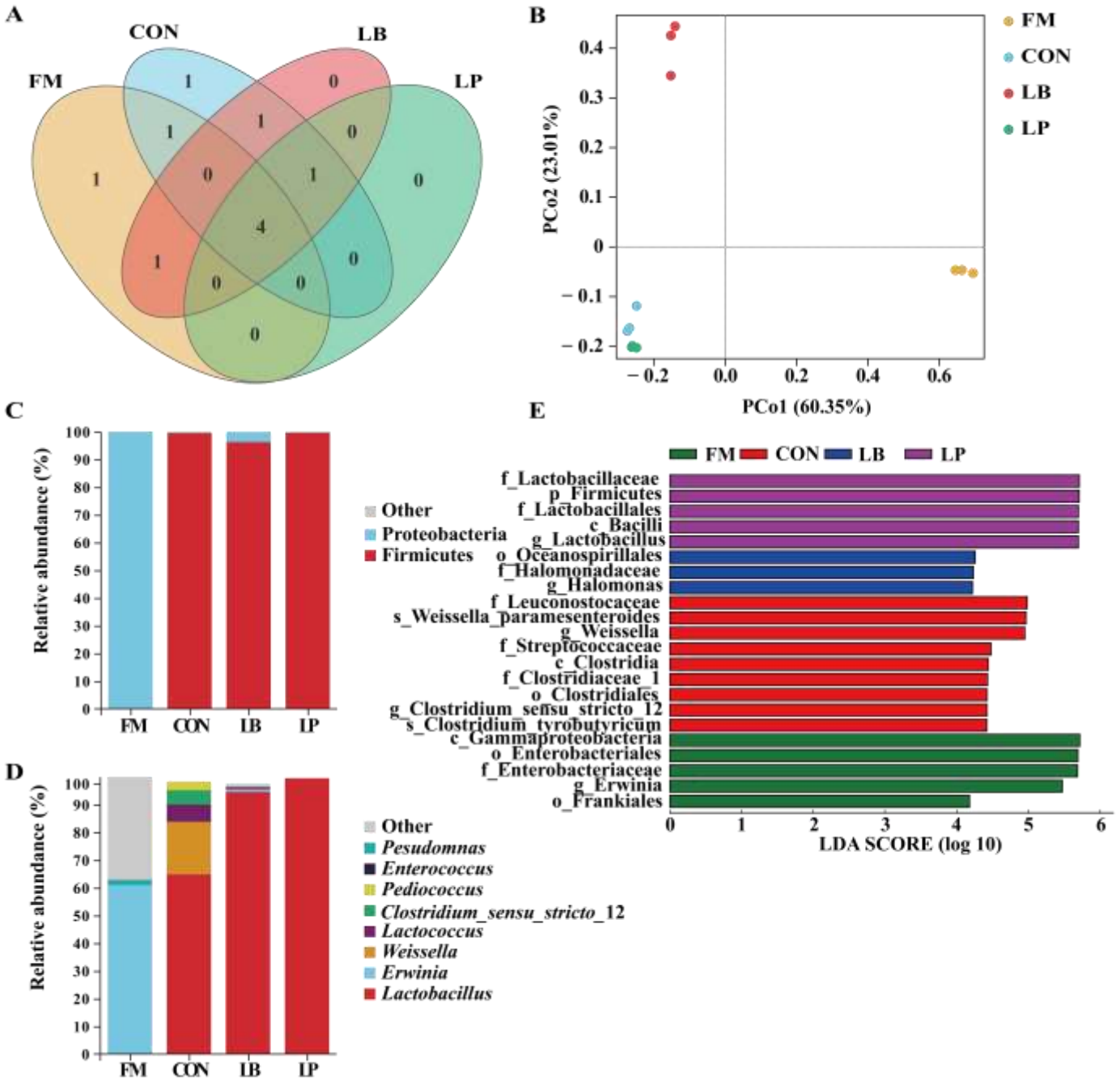

Figure 1. Bacterial diversity and community structure in fresh materials and silages ($n = 3$). (**A**) Venn diagram representing the common and unique ASVs found in fresh materials and silages. (**B**) Principal component analyses (PCoA) of samples conducted based on Bray UniFrac distance. (**C**) The relative abundance (%) of bacterial phyla (1% at least in one group) of FM and forage oat silage at the phylum level. (**D**) The relative abundance (%) of bacterial phyla (1% at least in one group) of FM and forage oat silage at the genus level. (**E**) Linear discriminant analysis (LDA) effect size (LEFSe) histogram showing the LDA scores (>4.0) computed for features at the OTU level. The length of the histogram represents the LDA score of different species. FM, fresh material; CON, control group; LB, *Lactiplantibacillus buchneri* inoculated treatment; LP, *Lentilactobacillus plantarum* inoculated treatment.

3.4. Fungal Community Compositions

A total of 771,465 high-quality sequences were collated by ITS gene amplification and were based on 97% sequence similarity; the number of ASVs identified was 602, and the lowest ASVs were found in the CON group (Table 5). The Chao1 and Shannon indexes of

the LP treatment were the lowest and were significantly lower than those of the FM treatment ($p < 0.05$). Good's coverage index was higher than 0.99 in all treatments, indicating that the sequencing depth was suitable for analyzing fungal communities in these samples.

Table 5. Characteristics of amplicon libraries in the fungal community.

Characteristic	FM	CON	LB	LP	Total No.
No. of sequences	65,210 ± 1001	64,992 ± 2456	65,227 ± 1927	61,725 ± 362	771, 465
No. of ASV	51 ± 9 b	36 ± 1 b	43 ± 4 b	69 ± 2 a	602
Chao1 index	55.33 ± 7.80 a	42.20 ± 2.04 b	45.08 ± 5.05 b	41.06 ± 2.24 b	
Shannon index	3.26 ± 0.43 a	2.43 ± 0.07 b	2.28 ± 0.30 bc	2.05 ± 0.14 c	
Good's coverage	0.99	0.99	0.99	0.99	

ASV, amplicon sequence variant; FM, fresh material; CON, control group; LB, *Lentilactobacillus buchneri* inoculated treatment; LP, *Lactiplantibacillus plantarum* inoculated treatment. Means with different letters in each row differ at $p < 0.05$.

A Venn diagram depicts the common and unique ASVs in different treatments. In total, 14 ASVs, as the core genera, were shared by all treatments at the same time 37, 25, 31, and 61 ASVs belonged to the FM, CON, LB, and LP treatments, respectively (Figure 2A). The PCoA plot shows that the FM, CON, LB, and LP treatments were overtly separated and distinct (Figure 2B). Figure 1C shows the phylum-level compositions of the microbiomes in the FM and forage oat silage. *Basidiomycota* was the dominant phylum in the FM and silages, followed by Ascomycota (Figure 2C). The genus-level compositions of the fungal community of FM and forage oat silage are displayed in Figure 2D. The fungal composition structure in the FM and silage varied. In the FM and CON groups, the major genus was *Apiotrichum*, followed by *Bulleromyces*, *Sporobolomyces,* and *Filobasidium*. After fermentation, *Apiotrichum* dominated the LB and LP treatments.

The LEfSe data revealed substantially different taxonomic features among the FM and various treatments (Figure 2E). The genera *Bulleromyces* and *Filobasidium* were enriched in the FM group. After ensiling, the genus *Apiotrichum* was concentrated in the LB treatment, and the major genus *Ustilago* was concentrated in the LP treatment.

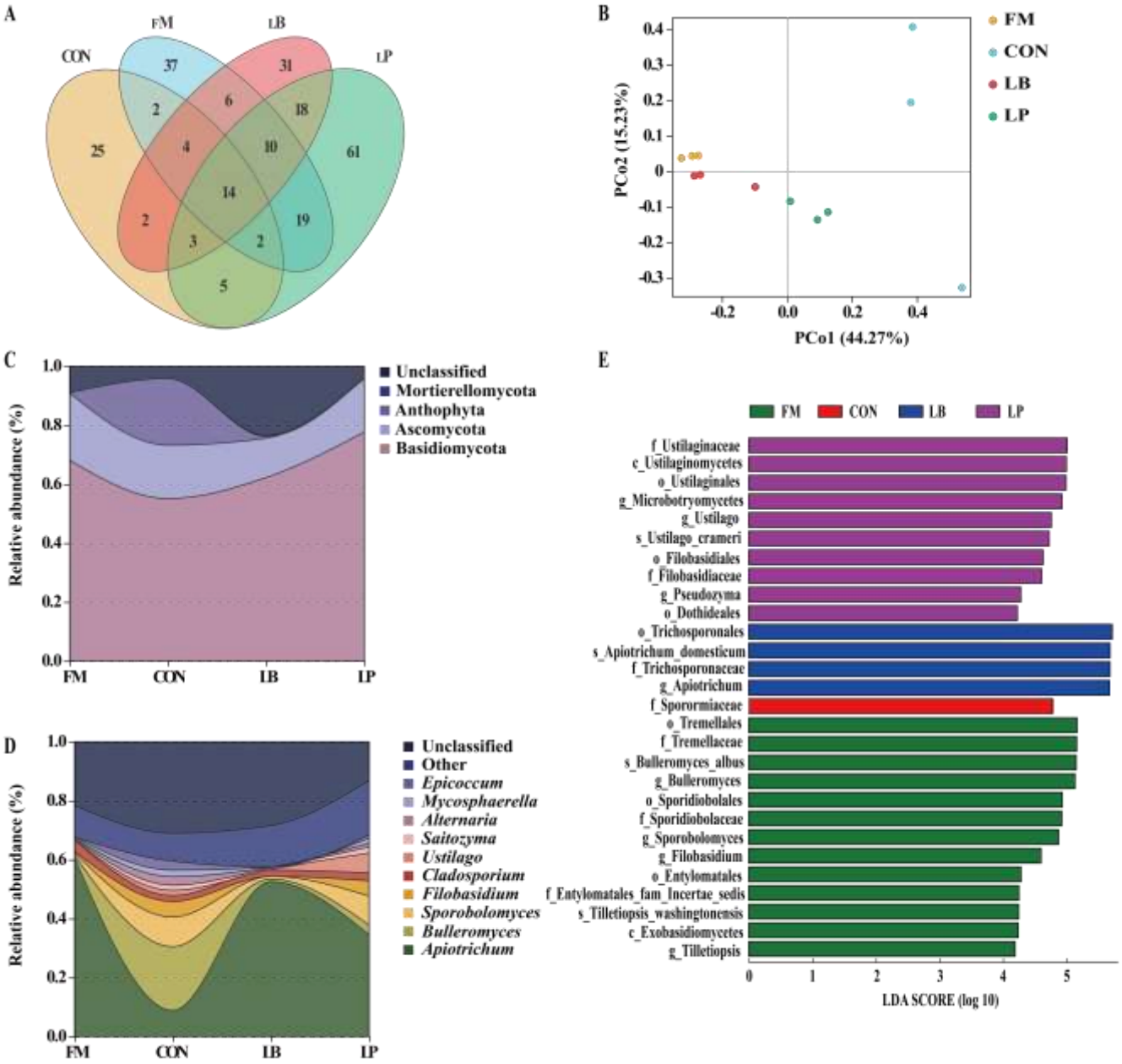

Figure 2. Fungal diversity and community structure in fresh materials and silages (n = 3). (**A**) Venn diagram representing the common and unique ASVs found in fresh materials and silages. (**B**) Principal component analyses (PCoA) of samples conducted based on Bray UniFrac distance. (**C**) Streamgraph (%) of fungal phyla (1% at least in one group) of FM and forage oat silage at the phylum level. (**D**) The streamgraph (%) of bacterial phyla (1% at least in one group) of FM and forage oat silage at the genus level. (**E**) Significantly different genera with an LDA score greater than the estimated value (default score = 4). The length of the histogram represents the LDA score of different genera. FM-fresh material; CON, control treatment; LB, *Lactiplantibacillus buchneri* inoculated treatment; LP, *Lentilactobacillus plantarum* inoculated treatment. Letters indicate the taxonomy of the bacteria: p, phylum, c, class; o, order; f, family; g, genus.

3.5. Association between Microbial Community and Fermentation Features in Forage Oat Silage

In the current study, a heatmap was used to evaluate the correlations between the microbial genus (top 10) and fermentation profile based on the Spearman analysis (Figure 3). The LA content was significantly associated with *Lactobacillus* (rho = 0.967; p = 0.0002), and was negatively correlated with DM, NH_3-N, pH, and WSC (rho = −0.695, p = 0.038; rho = −0.8, p = 0.013; rho = −0.917, p = 0.001; rho = −0.962, p < 0.0001; respectively).

Interestingly, the genera *Lactococcus*, *Pediococcus,* and *Weissella* were negatively associated with LA content (rho = −0.867, p = 0.005; rho = −0.933, p < 0.001; rho = −0.917, p = 0.001; respectively), and was positively associated with the pH (rho = 0.783, p = 0.017; rho = 0.933, p < 0.001; rho = 0.8, p = 0.014) and WSC (rho = 0.904, p < 0.001; rho = 0.979, p < 0.001; rho = 0.904, p < 0.001). The correlations between the fungal community and fermentation quality are shown in Figure 3B. The genera *Ustilago* and *Bulleromyces* were positively associated with the LA content (rho = 0.803, p = 0.009; rho = 0.833, p = 0.008, respectively). Nevertheless, the genus *Ustilago* had a negative association with the pH (rho = −0.840, p = 0.005), NH_3-N (rho = −0.858, p = 0.003), and WSC (rho = −0.880, p = 0.002) contents. Additionally, the genera *Mycosphaerella* and *Filobasidium* were negatively correlated with the AA (rho = −0.842, p = 0.004; rho = −0.757, p = 0.018, respectively) and PA (rho = −0.673, p = 0.047; rho = −0.705, p = 0.033, respectively) contents.

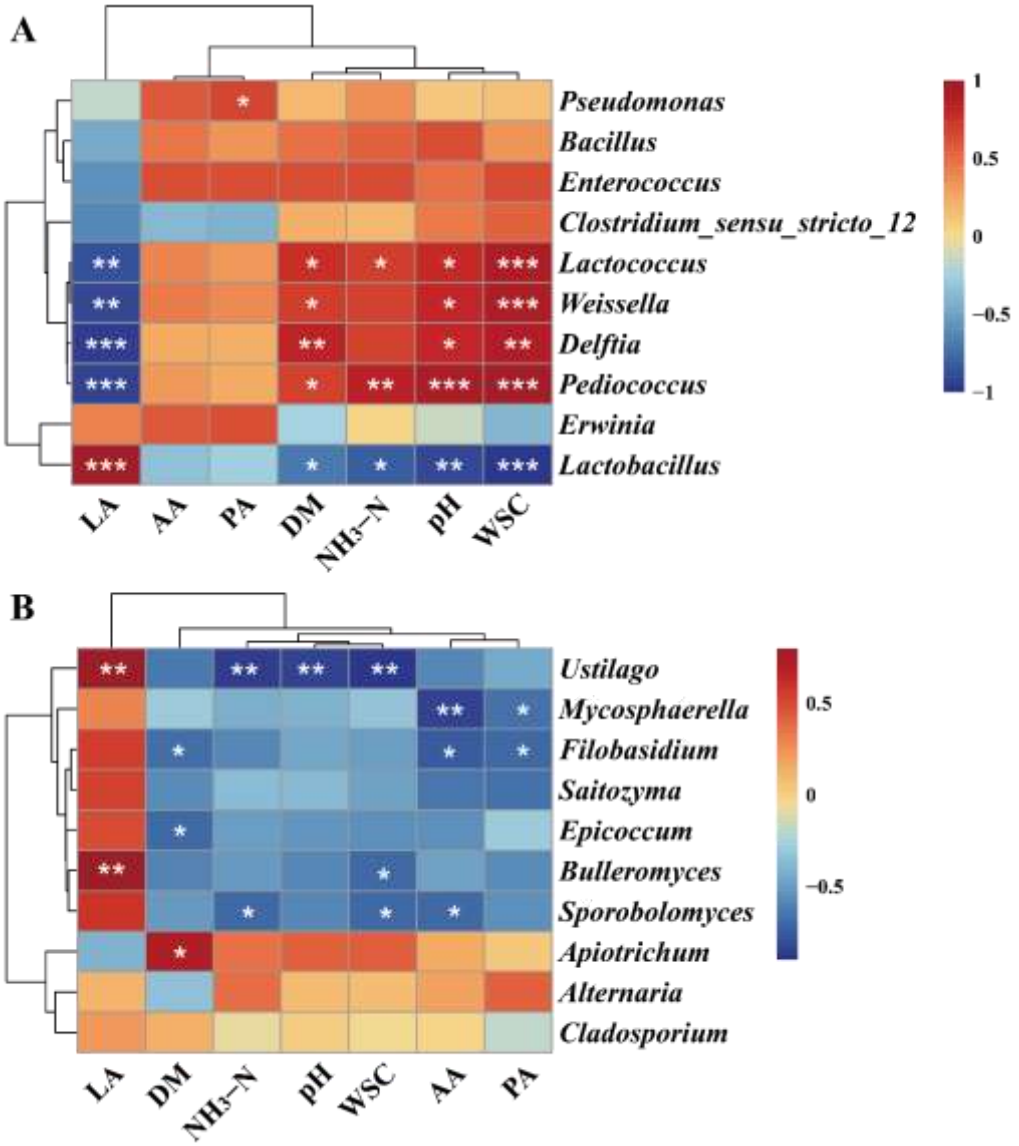

Figure 3. Spearman correlation heatmap of the microbial community with fermentation characteristics at the genus level. (**A**) Correlation between dominant bacterial genera and fermentation characteristics; (**B**) Correlation between dominant fungal genera and fermentation characteristics. A positive correlation is indicated in red color, and a negative correlation is indicated in blue. * Indicates significance at p < 0.05, ** indicates significance at p < 0.01, and *** indicates significance at p < 0.001.

3.6. Predicted Functions of the Microbial Community in Forage Oat Silage

The bacterial metabolic functions were determined by PICRUSt according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. As shown in Figure 4A, a total of six different metabolic pathways were displayed in the FM and different treatments. The main predicted genes were assigned to the metabolism, accounting for approximately 80% of FM and silage, followed by genetic information processing, environmental information processing, and cellular processes. As seen in Figure 4B, the top 15 metabolic functions are displayed, and the majority of the metabolic pathways were assigned to the metabolism with nine pathways. Figure 4C shows the top 30 metabolic pathways at the third pathway level. Carbohydrate metabolism and amino acid metabolism were the critical metabolic pathways at level 3. Interestingly, the pentose phosphate pathway, pyruvate metabolism, and glycolysis/gluconeogenesis were enriched after the fermentation process, especially in the LP treatment. Interestingly, some pathways of the amino acid metabolism, energy metabolism, and metabolism of cofactors and vitamins remained stable after ensiling. The predicted functional profiles of the fungal community were also determined based on FUNGuild (Figure 4D). Importantly, the metabolic pathways of the animal pathogen-undefined saprotrophs, animal endosymbiont-animal pathogen-endophyte-plant pathogen-undefined saprotrophs, endophyte-lichen parasite-plant pathogen-undefined saprotrophs, fungal parasite-plant pathogen-plant saprotroph, and animal pathogen-endophyte-plant pathogen-wood saprotrophs predicted were inhibited after the fermentation process.

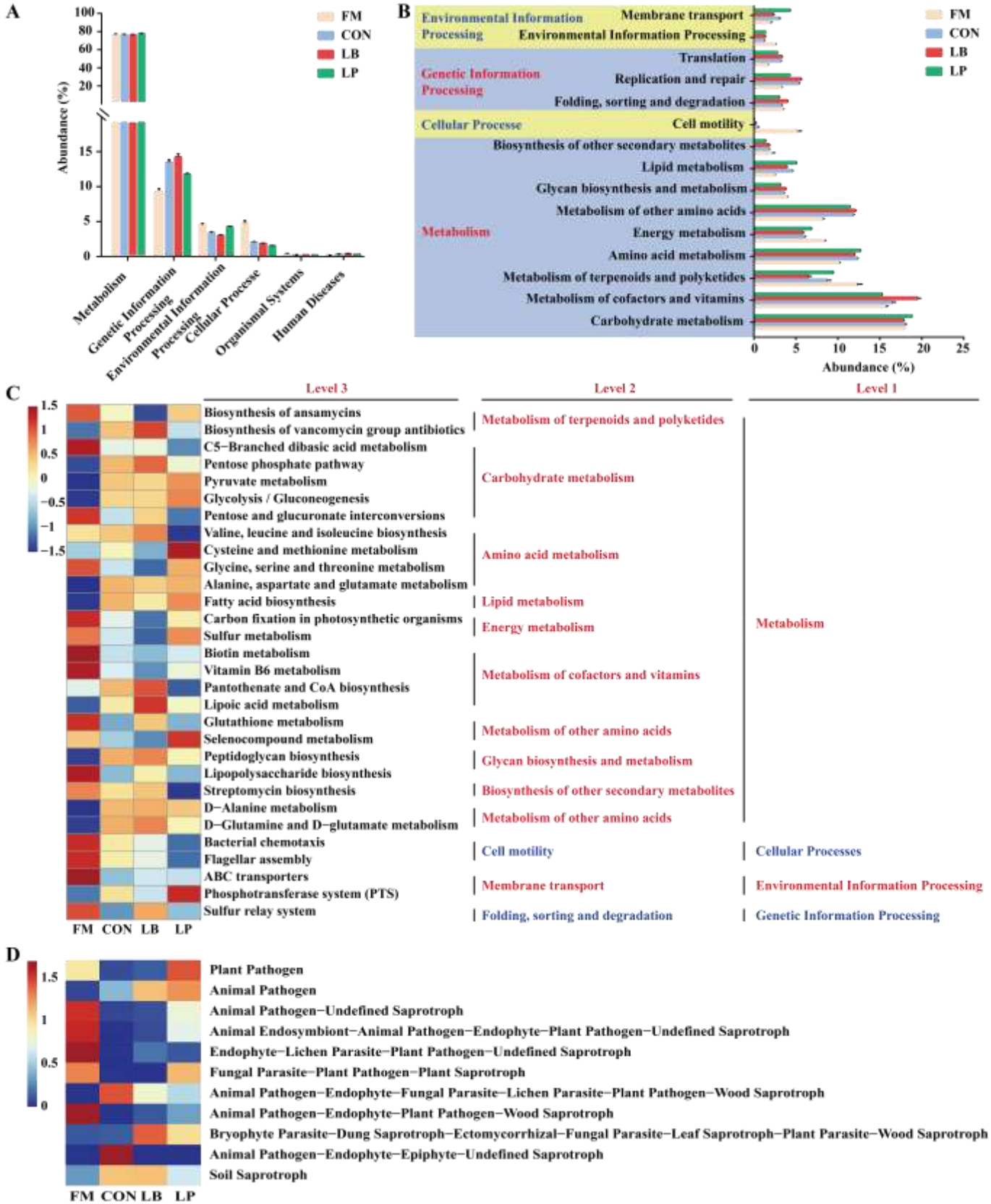

Figure 4. Dynamics of predicted microbial functional profiles in FM and forage oat silage analyzed by PICRUSt for bacteria and FUNGuild for fungi (n = 3). (**A**) Level 1 metabolic pathways. (**B**) Level 2 Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologue functional predictions of the relative abundances of the top 20 metabolic functions. (**C**) Level 3 KEGG orthologue functional predictions of the relative abundances of the top 30 metabolic functions. (**D**) Variations in the composition

of fungal functional groups inferred by FUNGuild. FM, fresh material; CON, control group; LB, *Lentilactobacillus buchneri* inoculated treatment; LP, *Lactiplantibacillus plantarum* inoculated treatment.

4. Discussion

In the current work, the ensiling performances and microbial communities of forage oat treated with *L. plantarum* and *L. buchneri*, respectively, were characterized by performing many physicochemical analyses in combination with 16S and ITS rRNA gene sequencing, which is helpful for understanding the effect of LAB on forage oat silage.

The ensiling performance of silage is mainly influenced by the DM content, WSC content, and the number of LAB in the raw material [37]. The DM content in FM is a critical factor that directly affects the silage quality [38]. In this study, forage oat was harvested at the milk maturity stage with a DM content of >300 g/kg FM, which is in accordance with a previous report that the optimal DM values ranging between 300 and 400 g/kg FM are beneficial for yielding high-quality silage [39]. The NDF and ADF contents of fresh forage oat samples were similar to the data reported previously [39], whereas the DM, WSC, and CP contents were relatively higher, which could result from the plant genotype, sowing density, and harvest time [40]. In general, the amounts and species of epiphytic LAB constitute critical factors for controlling silage fermentation and determining whether inoculant addition is required [41]. For well-preserved silage, the number of LAB should be 10^5 CFU/g of FM or higher in forage materials [42]. Here, the LAB amounts in the FM were lower than the requirements, and the addition of LAB was necessary for obtaining high-quality forage oat silage.

After 30 days of the fermentation process, the DM and WSC concentrations in the LAB-inoculated silage were reduced compared to the CON treatment, especially in the *L. plantarum*-inoculated silage, which could be the WSC as the main substrate for the growth of LAB in silage [43]. pH is generally regarded as the key monitoring parameter of silage quality [44]. Previous researchers have found that spoilage bacteria could be inhibited by a lower acidic environment, and a pH of 4.2 was considered an indicator of well-conserved silage [45,46]. In this research, the addition of *L. plantarum* significantly decreased the pH compared to that in the CON and LB treatments, which could contribute to the addition of homofermentative generally resulting in lower pH because there are often greater products of LA [5]. Additionally, a higher pH was also found in the *L. buchneri*-inoculated silage, which is in agreement with a previous study that found a moderately higher pH in silage treated with *L. buchneri* [47] because of the degradation of LA to AA and 1,2-propanediol by *L. buchneri* [48]. Therefore, the lower DM and WSC contents and pH were tested by the LP treatment, and the greater LA and AA contents were also detected in the LP and LB treatments. In addition, *L. buchneri*-inoculated forage oat silage also had a higher level of PA, probably due to *L. diolivorans* which could convert the 1,2-propanediol to PA and 1-propanol [49]. This is in accordance with the findings that the production of BA was not detected or was lower than the detected level in the present study [47], suggesting low numbers of undesirable microorganisms [44].

High-throughput sequencing was performed to assess the changes in bacterial communities and structures, as well as in the predicted metabolic functions. The Chao1 and Shannon indexes mirror the bacterial richness and species diversity [50]. In this study, significant differences were observed in the bacterial diversity between the FM and forage oat silage based on the Shannon index, which is in agreement with previous reports that a decreased alpha diversity was observed because undesirable microorganisms were inhibited by the pH and were gradually replaced by LAB [6,7]. Here, a coverage exceeding 0.99 was determined for FM and all the forage oat silage samples, suggesting that sequencing could accurately reflect the bacterial communities [14]. In the PCoA plot, the FM, CON, LB, and LP treatments in this study were well separated, indicating that different additives had certain effects on the bacterial communities, which were similar to the

corroborated report that additives increased the variability of bacterial communities and could explain the differences in silage quality [51].

The phylum Proteobacteria is the dominant phylum in FM, accounting for approximately 99% of all bacterial species. In the forage oat silage, the primary phylum shifted from Proteobacteria to Firmicutes, in agreement with the findings by Du and colleagues [52]. At the genus level, there were significant changes among the microorganisms between the FM and forage oat silage. It is well known that the dominant bacterial community involved in well-preserved silage comprises the phylum Firmicutes and the genera *Lactobacillus*, *Lactococcus*, *Pedicoccus*, *Weissella*, and *Leuconostoc*, which are common in standing plants and contribute to the initial pH reduction in silages [53]. After the fermentation process, the abundance of *Erwinia* markedly decreased in this research. The abundance of *Erwinia* might be inhibited in an acidic environment (pH < 5.40) during anaerobic conditions [54]. The present study also revealed that the abundance of *Lactobacillus* was statistically increased with the LB and LP treatments compared to that of the CON treatment. These results could be explained by the addition of LAB, which created an environment that was beneficial for the growth of LAB [25]. Species of *Pediococcus*, such as *Pediococcus pentosaceus*, are used as silage inoculants because they can dominate the early stages of ensiling and promote a decrease in pH [55]. Moreover, the abundance of *Pediococcus* was significantly decreased in the LB and LP treatments after 30 days of ensiling. This probably explains the negative correlation between the *Pediococcus* and LA content, as *Pediococcus* are outcompeted by *Lactobacillus* at a low pH due to LA accumulation and the lower acidic environment [56]. *Weissella* is a gram-positive, catalase-negative, heterofermentative bacterium [57]. *Weissella* also plays a critical role in the early phase of silage. In the present study, the abundance of *Weissella* was replaced by *Lactobacillus* in the LB and LP treatments, which is similar to the result that *Weissella* is progressively suppressed with a decreasing pH during lactate fermentation [7].

Early studies carried out on fermentation systems have focused on the description of the bacterial community, especially the structure and functions of LAB. Nevertheless, there is currently a growing interest associated with fungal diversity in fermentation functions [58,59]. The fungal community is mainly concerned with the aerobic decay of silage, but there is currently no satisfactory explanation for the fungal community and its functions in forage oat silage. In this research, the ITS amplification sequence was utilized to assess the changes in the fungal community in forage oat silage in different treatments after 30 days of ensiling. The Good's coverage in all the treatments for the fungal community was higher than 0.99, which was similar to that of the bacterial community, suggesting an optimal depth of ITS amplicon sequencing for the reliable analysis for all silage samples [60]. The Shannon index is a commonly used parameter that is directly proportional to species diversity. In this work, the Shannon index was dropped after the fermentation process; these results suggest that the consumption of oxygen by aerobic microorganisms to produce an anaerobic and acidic environment was unsuitable to support the reproduction and survival of the fungi [61,62].

At the phylum level, the predominant ITS-detected sequence belonged to Basidiomycota and was followed by lower Ascomycota, which is in disagreement with the results which found that Ascomycota was the primary phylum in corn silage [63,64], which could be explained by the raw materials, processing technology, and environmental conditions [65]. Compared to the FM group, the abundance of *Apiotrichum* was increased in forage oat silage, and these results were in disagreement with other reports on the dominant genes in elephant grass silage [64], fermented defatted rice bran [60], and sainfoin silage [66]. These results could be explained by a series of catabolic reactions that provide the essential nutrients to support the growth of these genera [62]. Species of *Bulleromyces* have been characterized by the widest variety of substrates, including leaves and fermented foods [67]. In this research, *Bulleromyces* had a high relative abundance in FM and a lower abundance in forage oat silage samples, which is inconsistent with previous reports, which found that *Geotrichum* was the dominant fungus in maize materials [68].

Bulleromyces were enriched in the postharvest samples, where a special environment might be the main reason [69].

To illustrate the relationship between the identified microorganisms and the measured ensiling products, the ten most influential microbial genera were displayed by the heatmap according to Spearman analysis. In agreement with these findings, the LA content was significantly associated with *Lactobacillus*, and the DM, NH_3-N, pH, and WSC were negatively associated with *Lactobacillus* [70]; this was because forage oat could provide sufficient WSC for the fermentation of ensiling [6]. On the other hand, the genera *Lactococcus*, *Pediococcus,* and *Weissella* were negatively associated with the LA content but were positively associated with pH and WSC. These microorganisms are sensitive acid bacteria that can be inhibited by an acidic environment, and therefore, their abundance decreases at low pH levels [6]. Additionally, the genera *Mycosphaerella* and *Filobasidium* were negatively connected with the AA content, and AA can inhibit fungal propagation and prolong the aerobic stability of silage, which might be the main reason [6].

It is of immense interest to determine the predicted function profiles and metabolic pathways of the microbiota involved in silage. The predicted functional profiles of the bacterial community from levels one to three were evaluated based on the KEGG databases by PICRUST. After fermentation progressed, the abundance of the majority of the metabolic pathways during carbohydrate metabolism and amino acid metabolism gradually increased, which is in accordance with a previous report [71]. On the one hand, carbohydrate metabolism, including the pentose phosphate pathway, gluconeogenesis, glycolysis metabolism, and pyruvate metabolism, can produce many compounds for the growth of bacteria [62]. On the other hand, amino acid metabolism, such as valine, leucine, and isoleucine biosynthesis; glycine, serine, and threonine metabolism; and energy metabolism, for example, carbon fixation, in photosynthetic organisms and sulfur metabolism were suppressed, indicating that undesirable bacteria were inhibited and nutritional compositions were well-preserved [6]. The source of the external fungi was the spores present on the raw material, which remained at a high level [62]. The high number of animal pathogen-undefined saprotroph, animal endosymbiont-animal pathogen-endophyte-plant pathogen-undefined saprotrophs, endophyte-lichen parasite-plant pathogen-undefined saprotrophs, fungal parasite-plant pathogen-plant saprotrophs, and animal pathogen-endophyte-plant pathogen-wood saprotrophs showed the broad range and diversity of the fungi in FM, which was similar to previous results [62]. This could contribute to the lower pH and organic acids (LA and AA) and can inhibit fungal propagation [6].

5. Conclusions

The results show that the addition of *L. plantarum* and *L. buchneri* effectively improves the fermentation quality and *Lactobacillus* abundance of oat silage, especially *L. plantarum*, which is more conducive to reducing the pH and NH_3-N in the silage and increasing the lactic acid content. In the future, adding *L. plantarum* will be a potential way to improve the quality of the silage.

Author Contributions: Conceptualization, Investigation, Visualization, Methodology, Formal analysis, Funding acquisition, Writing—original draft, Y.X.; Investigation, L.S., Z.W., and W.W.; Supervision, Writing—review and editing, Funding acquisition, X.X. and L.X.; Methodology, Validation, Data curation, Writing—Original draft, Writing—Review and editing, S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the selection and mechanism of low temperature resistant lactic acid bacteria in natural forage silage in Hulunbuir (NJZY21238), Key Cultivation Technology and Development Program of High Yield and Quality in Hulunbuir Area (GH202001), Grassland and Grass Industry Scientific Data Resource Construction and Sharing Service (2022-22), and China Agriculture Research System (CARS-34).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The sequences of these samples have been uploaded into the public database with the accession number PRJNA863426.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Xiong, Y.; Xu, J.; Guo, L.; Chen, F.; Jiang, D.; Lin, Y.; Guo, C.; Li, X.; Chen, Y.; Ni, K.; et al. Exploring the Effects of Different Bacteria Additives on Fermentation Quality, Microbial Community and In Vitro Gas Production of Forage Oat Silage. *Animals* **2022**, *12*, 1122.
2. Zhao, M.; Feng, Y.; Shi, Y.; Shen, H.; Hu, H.; Luo, Y.; Xu, L.; Kang, J.; Xing, A.; Wang, S.; et al. Yield and quality properties of silage maize and their influencing factors in China. *Sci. China Life Sci.* **2022**, *65*, 1655–1666.
3. Lin, H.; Lin, S.; Awasthi, M.K.; Wang, Y.; Xu, P. Exploring the bacterial community and fermentation characteristics during silage fermentation of abandoned fresh tea leaves. *Chemosphere* **2021**, *283*, 131234.
4. Zhou, Y.M.; Chen, Y.P.; Guo, J.S.; Shen, Y.; Yang, J.X. The correlations and spatial characteristics of microbiome and silage quality by reusing of citrus waste in a family-scale bunker silo. *J. Clean. Prod.* **2019**, *226*, 407–418.
5. Muck, R.E.; Kung, L., Jr. Effect of silage additives on ensiling. In Proceedings from the Silage: Field to Feedbunk North American Conference, Hershey, PA, USA, 11–13, 1997; NRAES-99.
6. Ren, H.; Sun, W.; Yan, Z.; Zhang, Y.; Wang, Z.; Song, B.; Zheng, Y.; Li, J. Bioaugmentation of sweet sorghum ensiling with rumen fluid: Fermentation characteristics, chemical composition, microbial community, and enzymatic digestibility of silages. *J. Clean. Prod.* **2021**, *294*, 126308.
7. Okoye, C.O.; Wang, Y.; Gao, L.; Wu, Y.; Li, X.; Sun, J.; Jiang, J. The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. *Microbiol. Res*. **2022**, *266*, 127212.
8. Xu, D.; Wang, N.; Rinne, M.; Ke, W.; Weinberg, Z.G.; Da, M.; Bai, J.; Zhang, Y.; Li, F.; Guo, X. The bacterial community and metabolome dynamics and their interactions modulate fermentation process of whole crop corn silage prepared with or without inoculants. *Microb. Biotechnol.* **2021**, *14*, 561–576.
9. da Silva, É.B.; Liu, X.; Mellinger, C.; Gressley, T.F.; Stypinski, J.D.; Moyer, N.A.; Kung, L., Jr. Effect of dry matter content on the microbial community and on the effectiveness of a microbial inoculant to improve the aerobic stability of corn silage. *J. Dairy Sci.* **2022**, *105*, 5024–5043.
10. Jung, J.S.; Ravindran, B.; Soundharrajan, I.; Awasthi, M.K.; Choi, K.C. Improved performance and microbial community dynamics in anaerobic fermentation of triticale silages at different stages. *Bioresour. Technol.* **2022**, *345*, 126485.
11. Ni, K.K.; Yang, H.X.; Wei, H.U.A.; Wang, Y.P.; Pang, H.L. Selection and characterisation of lactic acid bacteria isolated from different origins for ensiling *Robinia pseudoacacia* and *Morus alba* L. leaves. *J. Integr. Agric*. **2016**, *15*, 2353–2362.
12. Fijałkowska, M.; Przemieniecki, S.W.; Purwin, C.; Lipiński, K.; Kurowski, T.P.; Karwowska, A. The effect of an additive containing three Lactobacillus species on the fermentation pattern and microbiological status of silage. *J. Sci. Food Agr.* **2020**, *100*, 1174–1184.
13. Zhang, L.; Zhou, X.K.; Gu, Q.C.; Liang, M.Z.; Mu, S.L.; Zhou, B.; Huang, F.; Lin, B.; Zou, C.X. Analysis of the correlation between bacteria and fungi in sugarcane tops silage prior to and after aerobic exposure. *Bioresour. Technol.* **2019**, *291*, 121835.
14. Yang, L.; Yuan, X.; Li, J.; Dong, Z.; Shao, T. Dynamics of microbial community and fermentation quality during ensiling of sterile and nonsterile alfalfa with or without *Lactobacillus plantarum* inoculant. *Bioresour. Technol.* **2019**, *275*, 280–287.
15. Gharechahi, J.; Kharazian, Z.A.; Sarikhan, S.; Jouzani, G.S.; Aghdasi, M.; Hosseini Salekdeh, G. The dynamics of the bacterial communities developed in maize silage. *Microb. Biotechnol.* **2017**, *10*, 1663–1676.
16. Liu, B.Y.; Huan, H.L.; Gu, H.R.; Xu, N.X.; Shen, Q.; Ding, C.L. Dynamics of a microbial community during ensiling and upon aerobic exposure in lactic acid bacteria inoculation-treated and untreated barley silages. *Bioresour. Technol.* **2019**, *273*, 212–219.
17. Keshri, J.; Chen, Y.R.; Pinto, R.; Kroupitski, Y.; Weinberg, Z.G.; Saldinger, S.S. Bacterial dynamics of wheat silage. *Front. Microbiol.* **2019**, *10*, 1532.
18. Jia, T.; Yu, Z. Effect of temperature and fermentation time on fermentation characteristics and biogenic amine formation of oat silage. *Fermentation* **2022**, *8*, 352.
19. Wang, S.; Li, J.; Zhao, J.; Dong, Z.; Dong, D.; Shao, T. Effect of epiphytic microbiota from napiergrass and Sudan grass on fermentation characteristics and bacterial community in oat silage. *J. Appl. Microbiol.* **2022**, *132*, 919–932.
20. Thomas, A.T. An automated procedure for the determination of soluble carbohydrates in herbage. *J. Sci. Food Agr.* **1977**, *28*, 639–642.
21. AOAC. *Official Methods of Analysis*, 18th ed.; AOAC International: Gaithersburg, MD, USA, 2005.
22. Van Soest, P.V.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597.
23. Li, Y.; Wang, Z.; Li, T.; Jiang, S.; Sun, Z.; Jiang, H.; Qian, M.; Zhou, H.; Xu, Q. Changes in microbial community and methanogenesis during high-solid anaerobic digestion of ensiled corn stover. *J. Clean. Prod.* **2020**, *242*, 118479.
24. Broderick, G.A.; Kang, J.H. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* **1980**, *63*, 64–75.

25. You, S.; Du, S.; Ge, G.; Wan, T.; Jia, Y. Microbial community and fermentation characteristics of native grass prepared without or with isolated lactic acid bacteria on the Mongolian Plateau. *Front. Microbiol.* **2021**, *12*, 731770.
26. Beckers, B.; Beeck, M.O.D.; Thijs, S.; Truyens, S.; Weyens, N.; Boerjan, W.; Vangronsveld, J. Performance of 16s rDNA primer pairs in the study of Rhizosphere and Endosphere bacterial microbiomes in metabarcoding studies. *Front. Microbiol.* **2016**, *7*, 650.
27. Scibetta, S.; Schena, L.; Abdelfattah, A.; Pangallo, S.; Cacciola, S.O. Selection and experimental evaluation of universal primers to study the fungal microbiome of higher plants. *Phytobiomes J.* **2018**, *2*, 225–236.
28. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583.
29. Pruesse, E.; Quast, C.; Knittel, K.; Fuchs, B.M.; Ludwig, W.; Peplies, J.; Glöckner, F.O. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **2007**, *35*, 7188–7196.
30. Ankenbrand, M.J.; Keller, A.; Wolf, M.; Schultz, J.; Forster, F. ITS2 database V: Twice as much. *Mol. Biol. Evol.* **2015**, *32*, 3030–3032.
31. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336.
32. Ondov, B.D.; Bergman, N.H.; Phillippy, A.M. Interactive metagenomic visualization in a Web browser. *BMC Bioinform.* **2011**, *12*, 385.
33. Wickham, H. ggplot2. Wiley Interdisciplinary Reviews. *Comput. Stat.* **2011**, *3*, 180–185.
34. Krzywinski, M.; Schein, J.; Birol, I.; Connors, J.; Gascoyne, R.; Horsman, D.; Jones, S.J.; Marra, M.A. Circos: An information aesthetic for comparative genomics. *Genome Res.* **2009**, *19*, 1639–1645.
35. Kolde, R.; Kolde, M.R. *Package 'Pheatmap'*; R Foundation for Statistical Computing, Vienna, Austria, 2018.
36. Revelle, W.; Revelle, M.W. Package 'psych'. *Compr. R Arch. Netw.* **2015**, *337*, 338.
37. Borreani, G.; Chion, A.R.; Colombini, S.; Odoardi, M.; Paoletti, R.; Tabacco, E. Fermentative profiles of field pea (*Pisum sativum*), faba bean (*Vicia faba*) and white lupin (*Lupinus albus*) silages as affected by wilting and inoculation. *Anim. Feed Sci. Technol.* **2009**, *151*, 316–323.
38. Nadeau, E. Effects of plant species, stage of maturity and additive on the feeding value of whole-crop cereal silage. *J. Sci. Food Agric.* **2007**, *87*, 789–801.
39. Wang, S.; Zhao, J.; Dong, Z.; Li, J.; Kaka, N.A.; Shao, T. Sequencing and microbiota transplantation to determine the role of microbiota on the fermentation type of oat silage. *Bioresour. Technol.* **2020**, *309*, 123371.
40. Vasco-Correa, J.; Li, Y. Solid-state anaerobic digestion of fungal pretreated *Miscanthus sinensis* harvested in two different seasons. *Bioresour. Technol.* **2015**, *185*, 211–217.
41. Yan, Y.; Li, X.; Guan, H.; Huang, L.; Ma, X.; Peng, Y.; Li, Z.; Nie, G.; Zhou, J.; Yang, W.; et al. Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. *Bioresour. Technol*. **2019**, *279*, 166–173.
42. Cai, Y. Identification and characterization of *Enterococcus* species isolated from forage crops and their influence on silage fermentation. *J. Dairy Sci.* **1999**, *82*, 2466–2471.
43. McDonald, P.; Henderson, A.R.; Heron, S.J.E. *The Biochemistry of Silage*; Chalcombe Publications: Southampton, UK, 1991.
44. da Silva, N.C.; Nascimento, C.F.; Nascimento, F.A.; De Resende, F.D.; Daniel, J.L.P.; Siqueira, G.R. Fermentation and aerobic stability of rehydrated corn grain silage treated with different doses of *Lactobacillus buchneri* or a combination of Lactobacillus *plantarum* and *Pediococcus acidilactici. J. Dairy Sci.* **2018**, *101*, 4158–4167.
45. Ahmadi, F.; Lee, Y.H.; Lee, W.H.; Oh, Y.K.; Park, K.; Kwak, W.S. Long-term anaerobic conservation of fruit and vegetable discards without or with moisture adjustment after aerobic preservation with sodium metabisulfite. *Waste Manag.* **2019**, *87*, 258–267.
46. Guo, L.; Wang, X.; Lin, Y.; Yang, X.; Ni, K.; Yang, F. Microorganisms that are critical for the fermentation quality of paper mulberry silage. *Food Energy Secur.* **2021**, *10*, 304.
47. Kleinschmit, D.H.; Kung, L., Jr. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. *J. Dairy Sci*. **2006**, *89*, 3999–4004.
48. Hu, W.; Schmidt, R.J.; McDonell, E.E.; Klingerman, C.M.; Kung, L., Jr. The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. *J. Dairy Sci.* **2009**, *92*, 3907–3914.
49. Krooneman, J.; Faber, F.; Alderkamp, A.C.; Elferink, S.O.; Driehuis, F.; Cleenwerck, I.; Swings, J.; Gottschal, J.C.; Vancanneyt, M. *Lactobacillus diolivorans* sp. nov.; a 1, 2- propanediol- degrading bacterium isolated from aerobically stable maize silage. *Int. J. Syst. Evol. Microbiol.* **2002**, *52*, 639–646.
50. Guan, H.; Yan, Y.; Li, X.; Li, X.; Shuai, Y.; Feng, G.; Ran, Q.; Cai, Y.; Li, Y.; Zhang, X. Microbial communities and natural fermentation of corn silages prepared with farm bunker-silo in Southwest China. *Bioresour. Technol.* **2018**, *265*, 282–290.
51. Ni, K.; Wang, F.; Zhu, B.; Yang, J.; Zhou, G.; Pan, Y.I.; Tao, Y.; Zhong, J. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. *Bioresour. Technol.* **2017**, *238*, 706–715.

52. Du, S.; You, S.; Jiang, X.; Li, Y.; Jia, Y. Dynamics of the fermentation quality and *microbiota* in *Ephedra sinica* treated native grass silage. *J. Appl. Microbiol.* **2022**, *133*, 3465–3475.
53. Dong, Z.; Li, J.; Chen, L.; Wang, S.; Shao, T. Effects of freeze–thaw event on microbial community dynamics during red clover ensiling. *Front. Microbiol.* **2019**, *10*, 1559.
54. McGarvey, J.A.; Franco, R.B.; Palumbo, J.D.; Hnasko, R.; Stanker, L.; Mitloehner, F.M. Bacterial population dynamics during the ensiling of *Medicago sativa* (alfalfa) and subsequent exposure to air. *J. Appl. Microbiol.* **2013**, *114*, 1661–1670.
55. Ogunade, I.M.; Jiang, Y.; Cervantes, A.P.; Kim, D.H.; Oliveira, A.S.; Vyas, D.; Weinberg, Z.G.; Jeong, K.C.; Adesogan, A.T. Bacterial diversity and composition of alfalfa silage as analyzed by Illumina MiSeq sequencing: Effects of Escherichia coli O157: H7 and silage additives. *J. Dairy Sci.* **2018**, *101*, 2048–2059.
56. Lin, C.; Bolsen, K.K.; Brent, B.E.; Fung, D.Y.C. Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. *J. Appl. Bacteriol.* **1992**, *73*, 375–387.
57. Ni, K.; Zhao, J.; Zhu, B.; Su, R.; Pan, Y.; Ma, J.; Zhou, G.; Tao, Y.; Liu, X.; Zhong, J. Assessing the fermentation quality and microbial community of the mixed silage of forage soybean with crop corn or sorghum. *Bioresour. Technol.* **2018**, *265*, 563–567.
58. Zou, W.; Ye, G.; Zhang, K. Diversity, function, and application of Clostridium in Chinese strong flavor baijiu ecosystem: A review. *J. Food Sci.* **2018**, *83*, 1193–1199.
59. Du, H.; Song, Z.; Zhang, M.; Nie, Y.; Xu, Y. The deletion of Schizosaccharomyces pombe decreased the production of flavor-related metabolites during traditional Baijiu fermentation. *Food Res. Int.* **2021**, *140*, 109872.
60. Su, W.; Jiang, Z.; Wang, C.; Xu, B.; Lu, Z.; Wang, F.; Zong, X.; Jin, M.; Wang, Y. Dynamics of defatted rice bran in physicochemical characteristics, microbiota and metabolic functions during two-stage co-fermentation. *Int. J. Food Microbiol.* **2022**, *362*, 109489.
61. Yan, K.; Abbas, M.; Meng, L.; Cai, H.; Peng, Z.; Li, Q.; El-Sappah, A.H.; Yan, L.; Zhao, X. Analysis of the fungal diversity and community structure in sichuan dark tea during pile–fermentation. *Front. Microbiol.* **2021**, *12*, 706714.
62. Su, W.; Jiang, Z.; Hao, L.; Li, W.; Gong, T.; Zhang, Y.; Du, S.; Wang, C.; Lu, Z.; Jin, M.; et al. Variations of soybean meal and corn mixed substrates in physicochemical characteristics and microbiota during two-stage solid-state fermentation. *Front. Microbiol.* **2021**, *12*, 688839.
63. Romero, J.J.; Joo, Y.; Park, J.; Tiezzi, F.; Gutierrez-Rodriguez, E.; Castillo, M.S. Bacterial and fungal communities, fermentation, and aerobic stability of conventional hybrids and brown midrib hybrids ensiled at low moisture with or without a homo-and heterofermentative inoculant. *J. Dairy Sci.* **2018**, *101*, 3057–3076.
64. Vu, V.H.; Li, X.Y.; Wang, M.Y.; Liu, R.M.; Zhang, G.J.; Liu, W.; Xia, B.X.; Sun, Q. Dynamics of fungal community during silage fermentation of elephant grass (*Pennisetum purpureum*) produced in northern Vietnam. *Asian-Australas. J. Anim. Sci.* **2019**, *32*, 996–1006.
65. Hu, S.; He, C.; Li, Y.; Yu, Z.; Chen, Y.; Wang, Y.; Ni, D. Changes of fungal community and non-volatile metabolites during pile-fermentation of dark green tea. *Food Res. Int.* **2021**, *147*, 110472.
66. Huang, H.C.; Chang, W.T.; Wu, Y.H.; Yang, B.C.; Xu, M.R.; Lin, M.K.; Chen, H.J.; Cheng, J.H.; Lee, M.S. Phytochemicals levels and biological activities in *Hibiscus sabdariffa* L. were enhanced using microbial fermentation. *Ind. Crop. Prod.* **2022**, *176*, 114408.
67. Hui, F.L.; Niu, Q.H.; Ke, T.; Li, Y.X. *Cryptococcus nanyangensis* sp. nov.; a new *Basidiomycetous* yeast isolated from the gut of wood-boring larvae. *Curr. Microbiol.* **2012**, *65*, 617–621.
68. Grazia, L.; Suzzi, G.; Romano, P. Isolation and identification of moulds in maize silage. *Inf. Agrar.* **1990**, *46*, 57–59.
69. Kim, M.S.; Park, E.J. Postharvest-induced microbiota remodeling increases fungal diversity in the phyllosphere mycobiota of broccoli florets. *Postharvest Biol. Technol.* **2021**, *181*, 111693.
70. Fang, D.; Dong, Z.; Wang, D.; Li, B.; Shi, P.; Yan, J.; Zhuang, D.; Shao, T.; Wang, W.; Gu, M. Evaluating the fermentation quality and bacterial community of high-moisture whole-plant quinoa silage ensiled with different additives. *J. Appl. Microbiol.* **2022**, *132*, 3578–3589.
71. Bai, J.; Ding, Z.; Ke, W.; Xu, D.; Wang, M.; Huang, W.; Zhang, Y.; Liu, F.; Guo, X. Different lactic acid bacteria and their combinations regulated the fermentation process of ensiled alfalfa: Ensiling characteristics, dynamics of bacterial community and their functional shifts. *Microb. Biotechnol.* **2021**, *14*, 1171–1182.