agriculture

MDPI

Article

Effects of Cellulase and *Lactiplantibacillus plantarum* on Chemical Composition, Fermentation Characteristics, and Bacterial Community of *Pennisetum giganteum* z.x.lin Silage

Zhili Wu [1,2,†], Xiongfei Zhang [1,2,†], Rongnuo Li [1,2], Jingtao Hui [1,2], Lu Deng [1,2], Inho Kim [3], Jie Wei [4], Junhu Yao [1,2] and Xinjian Lei [1,2,*]

[1] College of Animal Science and Technology, Northwest A&F University, Xianyang 712100, China; wzl1295354914@163.com (Z.W.); xiongfeizhang2024@nwafu.edu.cn (X.Z.); lrn000310@163.com (R.L.); 19939369481@163.com (J.H.); denglu128128@163.com (L.D.); yaojunhu2008@nwafu.edu.cn (J.Y.)
[2] Key Laboratory of Livestock Biology, Northwest A&F University, Xianyang 712100, China
[3] Department of Animal Resource and Science, Dankook University, Cheonan 31116, Republic of Korea; inhokim@dankook.ac.kr
[4] Xi'an Architectural Design and Research Institute Co., Ltd., Xi'an 710000, China; 18829075888@163.com
* Correspondence: leixinjian@nwafu.edu.cn
† These authors contributed equally to this work.

Abstract: In order to explore the effects of additives on the chemical composition, fermentation characteristics, and bacterial community of *Pennisetum giganteum* z.x.lin silage, *Pennisetum giganteum* z.x.lin was ensiled with no additives (CON), cellulase (CE), *Lactiplantibacillus plantarum* (LP), or the combination of cellulase and *Lactiplantibacillus plantarum* (LPCE) at room temperature for 60 days, respectively. The results indicated that LPCE had the highest dry matter (DM) content. Compared with CON, LP exhibited higher ($p < 0.05$) levels of water-soluble carbohydrate (WSC), crude protein (CP), and lactic acid (LA), along with a higher ($p < 0.05$) ratio of LA/acetic acid (AA). Meanwhile, silage inoculated with cellulase (CE and LPCE) showed lower ($p < 0.05$) contents of acid detergent fiber (ADF) and neutral detergent fiber (NDF) than CON. Furthermore, additive treatments improved the bacterial community composition of silage, and *Lactobacillus* was abundant in LPCE (LDA score > 4.0). Compared with CE and LP, LPCE more effectively promoted the transformation of microbial functions, resulting in an upregulated ($p < 0.05$) carbohydrate metabolism and a downregulated ($p < 0.05$) membrane transport. In conclusion, cellulase or *Lactiplantibacillus plantarum* improved the silage quality of *Pennisetum giganteum* z.x.lin by reducing the fiber content or enhancing LA fermentation, and their combination exhibited a powerful ability to establish a bacterial community dominated by *Lactobacillus*, which facilitated the production of high-quality silage.

Keywords: bio-additives; ensiling; enzyme-bacteria synergy; microbial diversity; *Pennisetum giganteum* z.x.lin

Academic Editor: Siran Wang

Received: 5 December 2024
Revised: 25 December 2024
Accepted: 26 December 2024
Published: 3 January 2025

Citation: Wu, Z.; Zhang, X.; Li, R.; Hui, J.; Deng, L.; Kim, I.; Wei, J.; Yao, J.; Lei, X. Effects of Cellulase and *Lactiplantibacillus plantarum* on Chemical Composition, Fermentation Characteristics, and Bacterial Community of *Pennisetum giganteum* z.x.lin Silage. *Agriculture* **2025**, *15*, 97. https://doi.org/10.3390/agriculture15010097

1. Introduction

As people's living standards improve and the population size continuous to grow, people's demand for high-quality meat and dairy products is also on the rise. However, the efficient production of livestock products depends on a continuous supply of high-quality feed, which is not available in many regions worldwide. For instance, in northwest China and southwest Botswana, land degradation has hampered forage production, making local

high-quality forage scarce [1,2]. In addition, drought, a key environmental factor threatening plant growth, has become increasingly frequent and severe due to climate warming [3]. Therefore, it is urgent to develop novel feed resources to address the current and future challenges of feed shortages caused by environmental factors. *Pennisetum giganteum* z.x.lin is a typical C_4 plant, characterized by a strong environmental stress resistance, well-developed root system, and high biomass [4]. Currently, *Pennisetum giganteum* z.x.lin is extensively utilized for improving soil quality [5] and producing biomass fuel [6]. Meanwhile, *Pennisetum giganteum* z.x.lin is also used as livestock feed due to its high yielding ability and strong environmental adaptability [4]. However, the high fiber content, unstable supply, and low water-soluble carbohydrate (WSC) content of *Pennisetum giganteum* z.x.lin deeply limit its application in animal husbandry.

Ensiling is an effective approach to preserving green forage. The application of various additives has further diversified feed resources and improved silage quality. Lactic acid bacteria (LAB) inoculants are extensively employed to enhance lactic acid (LA) fermentation and prevent silage spoilage. For example, *Lactiplantibacillus plantarum*, which belongs to the group of homofermentative LAB, has been proven to benefit the silage quality of various forage crops, including alfalfa [7]. Additionally, *Lactiplantibacillus plantarum* have been shown to enhance the microbial community of whole-plant corn silage, reducing the levels of certain mycotoxins and the relative abundance of harmful bacteria [8]. However, LAB inoculants have little effect on the nutrient digestibility of silage with a high fiber content. Cellulase can break down structural carbohydrates into oligosaccharides and monosaccharides, which can be used by LAB for generating LA and reducing the pH of silage [9]. Similar to *Pennisetum giganteum* z.x.lin, *Caragana korshinskii* has the characteristics of a well-developed root system, rapid growth, and high fiber content [10]. A previous study showed that inoculation with cellulase and *Lactiplantibacillus plantarum* could effectively enhance the fermentation quality of *Caragana korshinskii* silage [11]. However, the palatability and digestibility of *Caragana korshinskii* silage are still poor due to the presence of thorns and other factors [12]. *Pennisetum giganteum* z.x.lin is widely planted and applied in northwest China. Surprisingly, in areas affected by soil erosion, the fresh weight yield of *Pennisetum giganteum* z.x.lin can reach up to 178 t per hectare [6]. Although certain characteristics of *Pennisetum giganteum* z.x.lin are not conducive to silage fermentation, with the application of appropriate additives, it has the potential to become a practical silage material, particularly in regions with poor soil and arid climates. But few studies have evaluated the effects of cellulase and *Lactiplantibacillus* on the silage quality of *Pennisetum giganteum* z.x.lin.

Silage quality is closely linked to its bacterial community. In order to investigate the role of silage additives in the fermentation process of *Pennisetum giganteum* z.x.lin, analyzing the bacterial community is essential. With the advancement of next-generation sequencing technology, 16s rRNA gene sequencing has been extensively and deeply applied to the analysis of bacterial community [13]. At present, there are insufficient data on the effects of cellulase and *Lactiplantibacillus plantarum* on the bacterial community of *Pennisetum giganteum* z.x.lin silage. Therefore, this experiment was conducted to explore the effects of cellulase and *Lactiplantibacillus plantarum* on the chemical composition, fermentation characteristics, and bacterial community of *Pennisetum giganteum* z.x.lin silage, with the hypothesis that additive treatments could improve the silage quality.

2. Materials and Methods

2.1. Silage Preparation

Pennisetum giganteum z.x.lin was cultivated at a plot (about 1000 m^2) of the "JunCao" planting base of Shaanxi Fengqing Ecological Development Co., Ltd. (Xi'an, China) (a

warm, temperate continental climate, N 33°44′50″–35°10′30″, E 109°20′17″–109°54′48″, altitude 554 m, Pucheng, China), with no fertilizers, irrigation, or herbicides. *Pennisetum giganteum* z.x.lin cultivated at the base was about 2.5 m in height (elongation stage) and was harvested using a silage harvester (4QZ-18B; Henan Changjun Agricultural Machinery Co., Ltd., Xinxiang, China), leaving a stubble of about 15 cm. During the harvesting process, the *Pennisetum giganteum* z.x.lin was chopped into 2–3 cm pieces by the silage harvester for making silage. The initial characteristics of pre-ensiled *Pennisetum giganteum* z.x.lin are presented in Table 1.

Table 1. Characteristics (of g/kg DM) of the pre-ensiled *Pennisetum giganteum* z.x.lin.

DM	CP	NDF	ADF	Hemicellulose	WSC
of g/kg FM					
245.2	99.7	608.4	343.9	264.5	38.0

ADF, acid detergent fiber; CP, crude protein; DM, dry matter; FM, fresh matter; NDF, neutral detergent fiber; and WSC, water-soluble carbohydrate.

Four treatments were established, as follows: (i) control (CON), treated with 5 mL of double-distilled water/kg fresh material (FM); (ii) cellulase (CE, with an application dose of 100 IU/g FM) [14]; (iii) *Lactiplantibacillus plantarum* (LP, with an application dose of 1.0×10^6 colony-forming units (CFU)/g FM) [13]; and (iv) cellulase + *Lactiplantibacillus plantarum* (LPCE, with application doses of cellulase and *Lactiplantibacillus plantarum* of 100 IU/g FM and 1.0×10^6 CFU/g FM, respectively). The cellulase powder (5×10^5 IU/g) and *Lactiplantibacillus plantarum* (Lp194, a native strain isolated from rumen contents; 1×10^{11} CFU/g) were obtained from Guangdong VTR Bio-Tech Co., Ltd. (Zhuhai, China) and Jiangsu Wecare Biotechnology Co., Ltd. (Suzhou, China), respectively. The additives were mixed in 15 mL of double-distilled water and uniformly applied to a pile of ensiling material weighing 3 kg. Then, each pile of forage was thoroughly blended and placed into 30 × 40 cm polyethylene plastic bags with 1 kg of forage per bag. After that, each polyethylene plastic bag was drained of air and sealed using a vacuum laminator (DLYS891; Deli Group Co., Ltd., Ningbo, China). Then, 12 bags (4 treatments × 3 replicates) were kept at room temperature for 60 days. Upon fermentation's completion, each bag of silage samples was split into three equal portions, as follows: the first portion served for nutritional value analysis, the second for fermentation parameter analysis, and the third for microbial diversity analysis.

2.2. Chemical Analysis

The silage samples from the first portion were dried by an oven (DHG-9030A, Shanghai Yiheng Scientific Instruments Co., Ltd., Shanghai, China) at 65 °C for 48 h. Then, the dried silage samples were ground via a mill (HK-08B; Guangzhou Xulang Machinery Equipment Co., Ltd., Guangzhou, China) to pass over a 1 mm sieve. The dry matter (DM; method 930.15) and crude protein (CP; method 990.03) of the silage samples were assessed following the guidelines established by the Association of Official Analytical Chemists [15]. The CP content was converted by total nitrogen (TN) with a conversion factor of 6.25. As described by Van Soest et al. [16], neutral detergent fiber (NDF) was measured using a heat-stable α amylase, and then acid detergent fiber (ADF) was determined with acid detergent. The contents of NDF and ADF were inclusive of the residual ash. The hemicellulose content was derived by subtracting ADF from NDF. According to the manufacturer' instructions, the WSC content was measured through a plant-soluble sugar content test kit (A145-1-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The second portions of the silage samples (10 g) were homogenized in 90 mL of double-distilled water and stored at 4 °C for 24 h [17]. Once fully mixed, the liquid extracts were

filtered through a quantitative filtered paper. The pH value of each filtrate was assessed with a pH meter (HI 90s24C; HANNA Instruments, Woonsocket, RI, USA). Subsequently, the filtrate was passed through a 0.45 μm membrane, and then the concentrations of acetic acid (AA), propionic acid (PA), and butyric acid (BA) were analyzed via gas chromatography (GC7890A; Agilent, Santa Clara, CA, USA), as detailed by Wang et al. [18]. The LA concentration was measured by an LA assay kit (A019-2-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The ammonia nitrogen (NH_3-N) content was assessed with reference to the guidelines of Broderick and Kang [19]. The contents of WSC, LA, and NH_3-N were all measured by colorimetric methods with a microplate reader (UV-2300; Shimadzu, Kyoto, Japan).

2.3. Bacterial Community Analysis

The total bacterial DNA from the third portion was extracted through a MagAtrract PowerSoil Pro DNA Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol. Next, the quality of DNA was assessed by 1% agarose gel electrophoresis. Following that, the concentration and purity of DNA were assessed by NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA). Then, polymerase chain reaction (PCR) was conducted to amplify the 16S rRNA gene (V5–V7) with a pair of specific primers, 799F (5′-AACMGGATTAGATACCCKG-3′) and 1993R (5′-ACGTCATCCCCACCTTCC-3′), using a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) [20]. The products of PCR were analyzed through 2% agarose gel electrophoresis and then processed with a PCR Clean-Up Kit (Shanghai Meiji Yuhua Biomedical Technology Co., Ltd., Shanghai, China). Next, the amplified fragments were quantified by Qubit 4.0 (Thermo Fisher Scientific, Waltham, MA, USA) for following sequencing on the NovaSeq platform (Illumina, San Diego, CA, USA). Based on QIIME2 [21], the original 16S rRNA gene reads were filtered, merged, screened, and denoised using DADA2 to obtain the representing sequence and abundance information of amplicon sequence variants (ASVs). The ASVs were analyzed for taxonomic annotation by RDP Classifier (v11.5) and the SILVA 16S rRNA database (v138) with a confidence coefficient of 0.7. Five commonly used alpha-diversity-related indices, consisting of Chao, Ace, Shannon, Simpson, and Coverage, were derived using Mothur (v1.30.2) to assess the diversity and richness of the bacterial community. Based on the weighted Unifrac distance, the beta diversity was assessed with principal coordinate analysis (PCoA) to identity the differences between the bacterial community structures among treatments with the ANOSIM test. According to the results of taxonomic analysis, bar charts of the bacterial community composition at the phylum and genus were constructed using R (v3.3.1). The bacterial differences among treatments were identified via linear discriminant analysis effect size (LEfSe). Using PICRUSt2 (v2.2.0) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, functional genes were predicted and then matched to their corresponding metabolic pathways.

2.4. Statistical Analysis

The basic data on chemical composition, fermentation characteristics, and bacterial community were organized by Excel 2019 and assessed through the one-way analysis of variance procedure of SPSS 27.0 (IBM Corp, Armonk, NY, USA), with each fermentation bag considered as an experimental unit (n = 3 per treatment). The statistical model was as follows:

$$Y_i = \mu + \alpha_i + \varepsilon_i \quad (1)$$

where Y_i is the is the observed value, μ is the overall mean, α_i is the treatment effect (i = CON, CE, LP, and LPCE), and ε_i is the experimental error. Tukey's test was employed to compare the mean values. The data on the bacterial community were processed through the resources

available on the Majorbio Cloud Platform (https://cloud.majorbio.com/page/tools/, accessed on 10 May 2024). Statistical significance was declared when the p value < 0.05.

3. Results

3.1. Effects of Additives on Chemical Composition and Fermentation Characteristics of Pennisetum giganteum z.x.lin Silage

The chemical composition of *Pennisetum giganteum* z.x.lin silage is given in Table 2. LP showed a higher ($p < 0.05$) CP content than CON, but a lower ($p < 0.05$) DM content than LPCE. Both CE and LPCE showed a lower ($p < 0.05$) NDF content compared with CON and LP. In addition, CE, LP, and LPCE all exhibited a lower ($p < 0.05$) ADF content compared with CON. Meanwhile, LPCE showed a lower ($p < 0.05$) ADF content compared with LP. However, no significant difference ($p > 0.05$) was observed in hemicellulose among the four treatments. Moreover, LP exhibited a higher ($p < 0.05$) WSC content than CON.

Table 2. Effects of additives on chemical composition of *Pennisetum giganteum* z.x.lin silage.

Item	Treatments				SEM	*p*-Value
	CON	CE	LP	LPCE		
DM (g/kg FM)	223.8 ^{ab}	226.9 ^{ab}	217.1 ^{b}	229.5 ^{a}	1.73	0.031
CP (g/kg DM)	95.2 ^{b}	101.5 ^{ab}	104.8 ^{a}	103.5 ^{ab}	1.38	0.032
NDF (g/kg DM)	607.2 ^{a}	537.4 ^{b}	588.1 ^{a}	548.1 ^{b}	9.34	0.001
ADF (g/kg DM)	390.7 ^{a}	334.7 ^{bc}	344.6 ^{b}	306.1 ^{c}	9.81	<0.001
Hemicellulose (g/kg DM)	216.5	202.7	243.5	242.0	7.43	0.125
WSC (g/kg DM)	23.6 ^{b}	29.8 ^{ab}	34.2 ^{a}	24.2 ^{ab}	1.613	0.030

$^{a–c}$ Values with different letters in the same row are significantly different ($p < 0.05$). CON, no additives; CE, added cellulase (100 IU/g FM); LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM). ADF, acid detergent fiber; CP, crude protein; DM, dry matter; FM, fresh matter; NDF, neutral detergent fiber; SEM, standard error of mean; and WSC, water-soluble carbohydrate.

The fermentation characteristics of *Pennisetum giganteum* z.x.lin silage are presented in Table 3. Interestingly, there was no significant difference ($p > 0.05$) in pH among the four treatments. Additionally, LP had the highest LA content ($p < 0.05$) and a higher ($p < 0.05$) ratio of LA/AA than CON. However, the additive treatments had little impact ($p > 0.05$) on the AA content. LPCE exhibited a higher ($p < 0.05$) NH_3-N content compared with CON. Meanwhile, the contents of PA and BA were low and even undetectable in all treatments.

Table 3. Effects of additives on fermentation characteristics of *Pennisetum giganteum* z.x.lin silage.

Item	Treatments				SEM	*p*-Value
	CON	CE	LP	LPCE		
pH	3.75	3.69	3.73	3.79	0.03	0.672
LA (g/kg DM)	9.10 ^{b}	10.98 ^{b}	22.56 ^{a}	11.59 ^{b}	1.69	<0.001
AA (g/kg DM)	21.55	15.26	20.21	12.12	1.56	0.089
PA (g/kg DM)	0.23	0.25	0.20	ND	-	-
BA (g/kg DM)	0.08	ND	ND	ND	-	-
Ratio of LA/AA	0.42 ^{b}	0.80 ^{ab}	1.16 ^{a}	0.98 ^{ab}	0.10	0.037
NH_3-N (g/kg TN)	81.3 ^{b}	114.0 ^{ab}	114.0 ^{ab}	165.3 ^{a}	11.31	0.034

^{a,b} Values with different letters in the same row are significantly different ($p < 0.05$). CON, no additives; CE, added cellulase (100 IU/g FM); LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM). AA, acetic acid; BA, butyric acid; DM, dry matter; LA, lactic acid; ND, no detected; NH_3-N, ammonia nitrogen; PA, propionic acid; SEM, standard error of mean; and TN, total nitrogen.

3.2. Effects of Additives on Microbial Diversity of *Pennisetum giganteum* z.x.lin Silage

The bacterial alpha-diversity-related indices of the *Pennisetum giganteum* z.x.lin silage are presented in Table 4. The coverage values of all silage samples were more than 99.9%. Meanwhile, there was no significant difference ($p > 0.05$) in Chao and Ace values among the four treatments. Both CE and LPCE showed a lower ($p < 0.05$) Shannon value compared with CON, while LPCE also exhibited a lower ($p < 0.05$) Shannon value compared with LP. In addition, both CE and LPCE exhibited a higher ($p < 0.05$) Simpson value than CON. The PCoA plot showed that PCoA1 and PCoA2 explained the changes in the bacterial community structure by 85.63% and 6.71%, respectively (Figure 1). In the ANOSIM test, $p = 0.005$ and R = 0.6543, indicating that the bacterial community structure of the silage was significantly changed after the addition of additives. Meanwhile, the bacterial community structure of CON was apparently separated from that of other treatments through PCoA1.

Table 4. The alpha diversity of bacterial community in *Pennisetum giganteum* z.x.lin silage.

Items	Treatments				SEM	*p*-Value
	CON	CE	LP	LPCE		
Chao	250.3	184.4	287.0	193.4	17.82	0.097
Ace	251.4	186.1	291.4	196.5	17.48	0.102
Shannon	3.008 ^{a}	2.226 ^{bc}	2.670 ^{ab}	2.137 ^{c}	0.1149	0.001
Simpson	0.1155 ^{b}	0.2803 ^{a}	0.1966 ^{ab}	0.2609 ^{a}	0.0223	0.008
Coverage	0.9998	0.9998	0.9993	0.9996	0.0001	-

$^{a–c}$ Values with different letters in the same row are significantly different ($p < 0.05$). CON, no additives; CE, added cellulase (100 IU/g FM); LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); and LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM). SEM, standard error of mean.

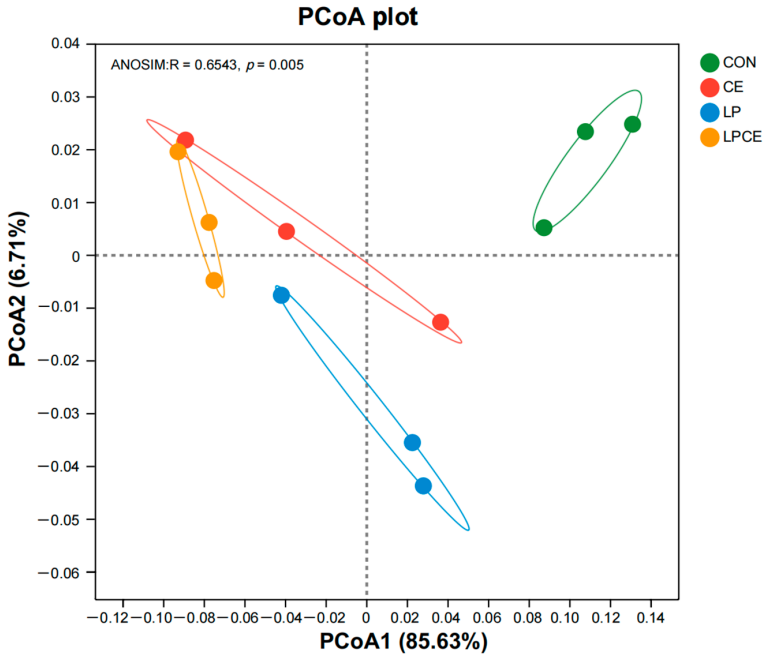

Figure 1. Principal coordinates analysis (PCoA) plot conducted based on weighted Unifrac distance of bacterial community. ANOSIM value, R = 0.6543, $p = 0.005$. CON, no additives; CE, added cellulase (100 IU/g FM); LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); and LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM).

The bacterial community composition of *Pennisetum giganteum* z.x.lin silage at the phylum and genus levels is shown in Figure 2. In the silage, Firmicutes (CON, 57.6%; CE, 78.6%; LP, 73.6%; and LPCE, 88.1%) and Proteobacteria (CON, 46.4%; CE, 72.4%; CE, 63.1%; and LPCE, 79.3%) were the major phyla, with a total relative abundance of >98.8% in all

treatments (Figure 2a). As expected, *Lactobacillus* (CON, 3.86%; CE, 5.08%; LP, 1.78%; and LPCE, 85.16%) became the dominant genus after ensiling (Figure 2b). Compared with CON, CE, LP, and LPCE all exhibited a decreased relative abundance of *Serratia* (CON, 20.9%; CE, 7.8%; LP, 9.9%; and LPCE, 4.8%), *Pantoea* (CON, 5.7%; CE, 2.7%; LP, 3.9%; and LPCE, 1.1%), *Klebsiella* (CON, 3.9%; CE, 2.8%; LP, 2.9%; and LPCE, 2.0%), and *Pediococcus* (CON, 5.2%; CE, 0.9%; LP, 2.6%; and LPCE, 1.4%), while exhibiting an increased relative abundance of *Lactococcus* (CON, 2.4%; CE, 2.4%; LP, 4.3%; and LPCE, 3.3%). In addition, LPCE had the highest abundance of *Weissella* (CON, 1.7%; CE, 1.5%; LP, 1.9%; and LPCE, 2.3%) and *Leuconostoc* (CON, 0.9%; CE, 1.0%; LP, 0.9%; and LPCE, 1.2%), as well as the lowest relative abundance of *unclassified_f__Enterobacteriaceae* (CON, 2.3%; CE, 2.2%; LP, 2.3%; and LPCE, 0.7%) and *Enterobacter* (CON, 2.1%; CE, 2.3%; LP, 1.7%; and LPCE, 0.7%).

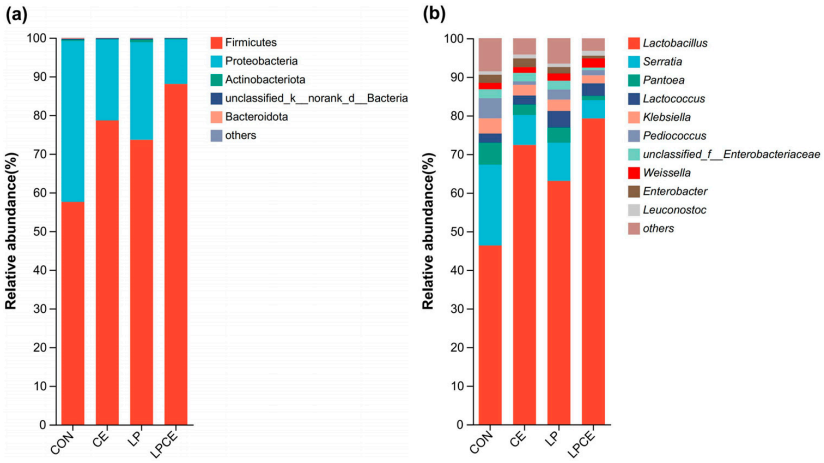

Figure 2. Relative abundance of top 5 bacterial phyla (**a**) and top 10 bacterial genera (**b**) of *Pennisetum giganteum* z.x.lin silage. CON, no additives; CE, added cellulase (100 IU/g FM); LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); and LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM).

The LEfSe showed that multiple bacterial taxa were potential biomarkers (LDA score od > 4.0, Figure 3). Enterobacterales, Proteobacteria, Gammaproteobacteria, Erwiniaceae, *Pantoea*, and *Pediococcu* were abundant in CON. Rikenellaceae was abundant in LP. *Lactobacillus*, Firmicutes, Lactobacillaceae, Bacilli, Lactobacillales, Deinococci, and *Deinococcus* were abundant in LPCE.

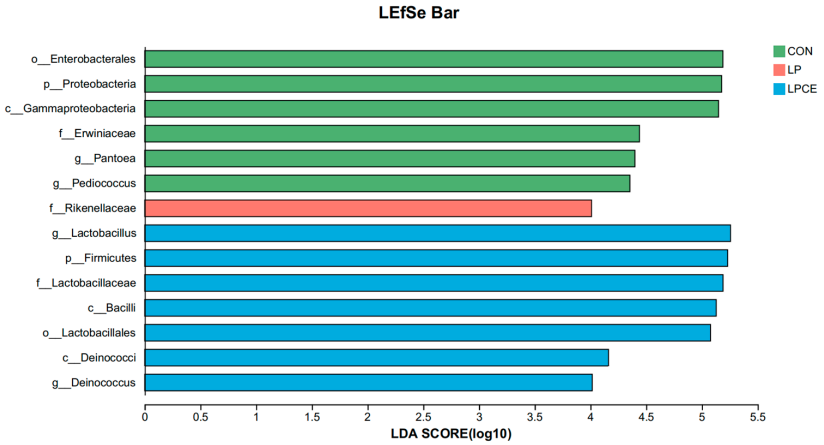

Figure 3. Comparison of the bacterial variations in *Pennisetum giganteum* z.x.lin silage using LEfSe. CON, no additives; LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); and LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM).

3.3. Correlation Analysis Between Silage Quality Characters and Bacterial Community

The correlation between silage quality characteristics and bacterial community at the genus level is given in Figure 4. CP exhibited a negative correlation ($p < 0.05$) with *Klebsiella*. NDF showed a negative correlation ($p < 0.05$) with *Lactobacillus* and positive association ($p < 0.05$) with *Serratia*, *Pantoea*, *Klebsiella*, and *Pediococcus*. ADF exhibited a negative correlation ($p < 0.05$) with *Lactobacillus* and a positive association ($p < 0.05$) with *Serratia*, *Pantoea*, *Klebsiella*, *Pediococcus*, *unclassified_f__Enterobacteriaceae*, and *Enterobacter*. WSC showed a positive correlation ($p < 0.05$) with *Lactobacillus* and a negative correlation ($p < 0.05$) with *Serratia*, *Pantoea*, *Klebsiella*, *Pediococcus*, *unclassified_f__Enterobacteriaceae*, and *Enterobacter*. AA was inversely related ($p < 0.05$) to *Lactobacillus* and positively correlated with *Pantoea* and *unclassified_f__Enterobacteriaceae*. NH_3-N was positively associated ($p < 0.05$) with *Lactobacillus* and inversely related ($p < 0.05$) to *Serratia*, *Pantoea*, and *Klebsiella.*

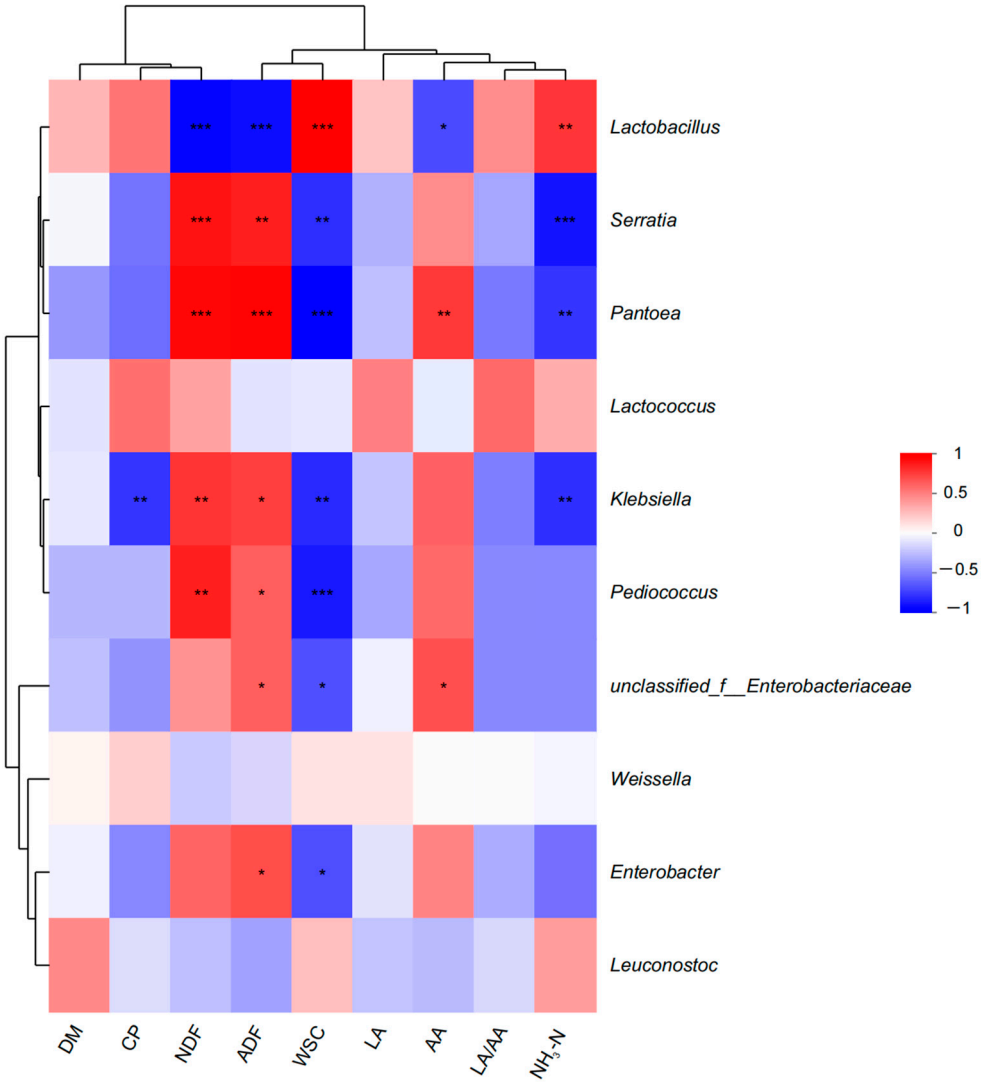

Figure 4. Heatmap of Spearman correlation analysis of bacterial community and silage quality characters at genus level. *, $0.01 \leq p < 0.05$; **, $0.001 \leq p < 0.01$; and ***, $p < 0.001$. AA, acetic acid; ADF, acid detergent fiber; CP, crude protein; DM, dry matter; LA, lactic acid; NDF, neutral detergent fiber; NH_3-N, ammonia nitrogen; and WSC, water-soluble carbohydrate.

3.4. 16S rRNA Gene-Predicted Functional Profiles of Pennisetum giganteum z.x.lin Silage

The major KEGG metabolic pathways in the silage are shown in Figure 5. The dominant microbial metabolic pathways consisted of global and overview maps, carbohydrate metabolism, amino acid metabolism, and membrane transport, with their proportions

all > 5%. Compared with CON, the other treatments significantly upregulated ($p < 0.05$) the global and overview maps. Meanwhile, CON was less abundant ($p < 0.05$) than CE and LPCE in the carbohydrate metabolism and more abundant ($p < 0.05$) than LP and LPCE in membrane transport. Additionally, the addition of additives also decreased ($p < 0.05$) the abundance of the energy metabolism and the metabolism of cofactors and vitamins, while simultaneously increasing ($p < 0.05$) the abundance of the nucleotide metabolism, translation, signal transduction, and replication and repair to varying degrees.

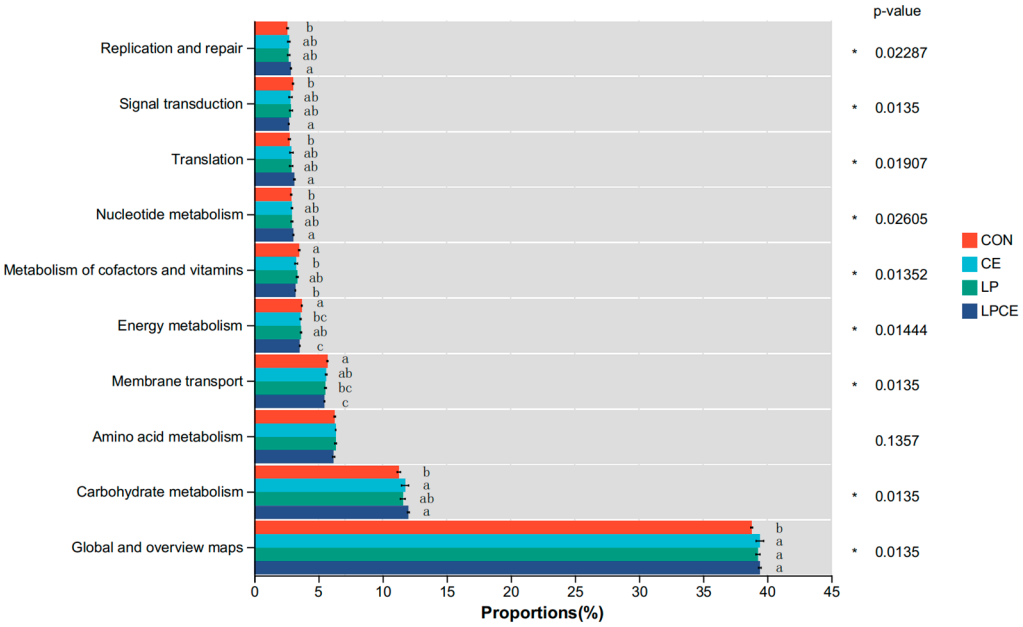

Figure 5. Level 2 Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog functional predictions of the top 10 metabolic functions using PICRUSt2 (v2.2.0). [a–c] Different letters indicate significantly difference ($p < 0.05$) between values for a given metabolic category. CON, no additives; CE, added cellulase (100 IU/g FM); LP, added *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM); and LPCE, added cellulase (100 IU/g FM) and *Lactiplantibacillus plantarum* (1×10^6 CFU/g FM). *, $0.01 \leq p < 0.05$.

4. Discussion

4.1. Effects of Additives on Silage Quality of Pennisetum giganteum z.x.lin

Due to its excellent biological characteristics, *Pennisetum giganteum* z.x.lin is expected to alleviate the forage shortage in northwest China as a potential feed resource for ruminants, such as goats [22]. With a good harvest speed and low weather damage, ensiling may be a practical strategy for preserving *Pennisetum giganteum* z.x.lin [23]. For a suitable anaerobic fermentation condition, silage raw material should contain WSC content of >6% [9]. Therefore, the low WSC content (38.0 g/kg DM) of *Pennisetum giganteum* z.x.lin may be insufficient to ensure successful LA fermentation. Generally, ruminants prefer diets with low fiber [9]. Meanwhile, fiber is one of the most challenging nutrients to digest in feed. A previous study reported that ADF content impacts feed digestibility and has a negative correlation with DM digestibility [24]. In this study, *Pennisetum giganteum* z.x.lin contained high levels of NDF (608.4 g/kg DM) and ADF (343.9 g/kg DM), which may have a negative impact on its digestibility. Therefore, adding cellulase and *Lactiplantibacillus plantarum* to *Pennisetum giganteum* z.x.lin may help to improve its silage quality and feeding value by degrading fiber into WSC.

Previous studies have showed that cellulase could reduce the DM content of silage due to its function of degrading structural carbohydrates [13,25]. However, LP had the lowest DM content in the present study, which is consistent with the study of Xiao et al. [26], who reported that *Lactiplantibacillus plantarum* could decrease the DM content of forage oat silage

because of increased LA fermentation. Indeed, LP showed a higher LA content than CON, but the reason for its reduced DM content needs further investigation. In this study, a lower CP content was found in CON, which may be attributed to the degradation of proteins by microorganisms during the fermentation process. Interestingly, the CP content in the additive treatment groups increased compared to the pre-ensiled forage. This was similar to the study of Zhang et al. [12], who reported that the LAB and cellulase inoculations resulted in a higher CP content in the silage compared to the fresh *Caragana korshinskii*, but the underlying reasons for this require further investigation. As expected, the silage inoculated with cellulase (CE and LPCE) showed lower contents of NDF and ADF than CON, indicating that cellulase promoted the decomposition of the fiber components of *Pennisetum giganteum* z.x.lin [9]. This may also help to improve the ruminal digestibility of *Pennisetum giganteum* z.x.lin silage [11]. However, the effect of cellulase on decomposing hemicellulose was limited in this study, which is inconsistent with the findings from Si and Iannaccone et al. [13,27], who reported that cellulase could effectively degrade the hemicellulose of mixed alfalfa and *Leymus chinensis* silage or industrial feeds. This may be due to the different degradation abilities of cellulases from different sources on hemicellulose. A suitable WSC content is important for LA fermentation. Compared to CON, the WSC content of CE, LP, and LPCE grew by 26.3%, 44.9%, and 2.5%, respectively. Therefore, cellulase and *Lactiplantibacillus plantarum* may help to preserve more WSC during ensiling, which agrees with the findings from Guo et al. [28], who reported that the addition these two additives increased the WSC content in mixed silage of corn and hulless barely straw compared with the control silage. On the one hand, cellulase could degrade some structural carbohydrates into WSC; on the other hand, *Lactiplantibacillus plantarum* could promote LA fermentation and suppress the utilization of WSC by undesirable microorganisms [13,28].

pH is a key parameter for assessing silage quality, and a pH of < 4.2 is suitable for conventional silage [1]. In the fermentation process, LA is a key beneficial organic acid, while AA is the product of hetero-fermentation [29]. In this study. LP exhibited the highest LA content and ratio of LA/AA, suggesting that *Lactiplantibacillus plantarum* could promote homo-fermentation and increase LA production during ensiling [30]. Previous studies have showed that cellulase can also inhibit hetero-fermentation and reduce AA production by increasing the WSC content of silage [13,31]. In this study, no significant difference was observed in the LA content or ratio of LA/AA among CON, CE, and LPCE. Nevertheless, compared to CON, the ratio of LA/AA in CE and LPCE climbed by 90.48% and 133.33%, respectively, suggesting that cellulase could enhance the fermentation quality of silage to some extent. PA is generated from the secondary fermentation of LA by *clostridium* [32]. Previous studies have showed that cellulase and LAB can reduce the PA content of silage [12,33]. In this study, all treatments had a low PA content, and even the PA content in LPCE was undetected, suggesting that the secondary fermentation of LA by *clostridium* was inhibited in the silage. Generally, the levels of NH_3-N and BA in high-quality silage are <100 g/kg TN and 2.0 g/kg DM, respectively [34]. Interesting, all treatments had a BA content below 2.0 g/kg DM, but only CON had an NH_3-N content below 100 g/kg TN. NH_3-N is generally considered as an important indication of the protein breakdown caused by microorganisms [35]. In this study, although there was no significant difference in the CP content between LPCE and CON, and the NH_3-N level in LPCE was significantly higher than that in CON, suggesting that the combination of cellulase and *Lactiplantibacillus plantarum* may promote NH_3-N production in a way independent of protein breakdown. Considering that LPCE exhibited the highest abundance in nucleotide metabolism, NH_3-N may be generated through nucleotide decomposition, but this hypothesis requires further investigation.

4.2. Effects of Additives on Bacterial Community of Pennisetum giganteum z.x.lin Silage

The bacterial community of silage plays a critical role in the anaerobic fermentation process. In the present study, all treatments had a coverage of > 99.9%, indicating that the sequencing was adequate to accurately represent the status of the bacterial community. Chao and Ace were employed to measure the bacterial community richness, while Sannon and Simpson to were used to assess the bacterial community diversity. Previous studies have indicated that cellulase and *Lactiplantibacillus plantarum* can reduce the richness and diversity of bacterial communities [9,36]. In the present study, the addition of additives did not significantly change the bacterial community richness of the silage, but both CE and LPCE had a lower bacterial community diversity than CON, suggesting that the addition of additives may promote LA fermentation and then suppress the growth of undesirable microorganisms during ensiling. In addition, PCoA showed that the bacterial community structures of LP, CE, and LPCE were obviously separated from those of CON through PCoA1. Therefore, cellulase and *Lactiplantibacillus plantarum* can decrease the bacterial community diversity and change the bacterial community structure of silage.

In terms of bacterial community composition, Firmicutes was the most abundant phylum, and its relative abundance was upregulated to varying degrees due to the inoculation of additives, which agrees with the study reported by Si et al. [13], who found that cellulase, *Lactiplantibacillus plantarum*, and their combination could all increase the relative abundance of Firmicutes in mixed silage of alfalfa and *Leymus chinensis*. Firmicutes is an important acid-producing hydrolytic phylum that is capable of growing rapidly under acidic conditions [37,38]. In the course of ensiling, LAB use WSC to generate LA, reduce the pH, and prevent the proliferation of undesirable microorganisms, so that the nutrients of silage can be preserved for an extended period [39]. Moreover, LAB are an important component of Firmicutes. Therefore, the addition of additives may promote the proliferation of LAB and increase the relative abundance of Firmicutes.

As an acid-tolerant LAB, *Lactobacillus* plays an essential role in enhancing LA accumulation and improving silage quality. Previous studies have showed that cellulase and *Lactiplantibacillus plantarum* can increase the relative abundance of *Lactobacillus* in silage [13,25,40]. Expectedly, *Lactobacillus* became the major genus in all four treatments, with its relative abundance in CE, LP, and LPCE being higher than that in CON. Members of *Enterobacteriaceae*, *Serratia*, *Pantoea*, *Klebsiella*, *unclassified_f__Enterobacteriaceae*, and *Enterobacter* were found in the silage, which was harmful to LA fermentation and nutrient preservation. Interestingly, most of them were more abundant in CON than the other treatments, suggesting that the additive treatments promoted LA fermentation and inhibited the activity of these harmful bacteria. Meanwhile, *Serratia* was the main harmful bacteria, and its relative abundance was surpassed only by that of *Lactobacillus*, which was similar to the findings from Li et al. [35], who reported that the relative abundance of *Serratia* was high in mixed silage of faba bean with forage wheat. It is noteworthy that LPCE had the highest relative abundance of *Lactobacillus*, but the lowest relative abundances of *Serratia*, *Pantoea*, *Klebsiella*, *unclassified_f__Enterobacteriaceae*, and *Enterobacter*, suggesting that the combination of cellulase and *Lactiplantibacillus plantarum* could more effectively improving the bacterial community composition of *Pennisetum giganteum* z.x.lin silage. Other LAB, including *Lactococcus*, *Pediococcus*, *Weissella*, and *Leuconostoc*, were also present in the silage. Although their relative abundance was much lower than that of *Lactobacillus*, they may be significant in the initiation of LA fermentation [41,42]. Due to their poor acid resistance, these LAB were gradually replaced by *Lactobacillus* during ensiling [41,43]. LEfSe was widely applied to further explore the differences in the bacterial communities of different treatments [12]. In this study, some undesirable microorganisms such as Enterobacterales were abundant in CON, while *Lactobacillus* was abundant in LPCE, suggesting that the

combination of cellulase and *Lactiplantibacillus plantarum* can synergistically enhance LA fermentation and inhibit the growth of undesirable microorganisms. Interestingly, *Pediococcus* was abundant in CON, which was likely due to the fact that the pH of CON decreased at a lower rate compared with other treatments; thus, *Pediococcus* had a better growing environment in CON.

4.3. Effect of Bacterial Community on Silage Quality of Pennisetum giganteum z.x.lin

The bacterial community is closely involved in the fermentation process and significantly affects silage quality [29]. As a major beneficial bacterium, *Lactobacillus* played an indispensable role in suppressing the activity of harmful bacterial and improving the silage quality of *Pennisetum giganteum* z.x.lin. In this study, cellulase promoted fiber decomposition and WSC preservation, thus providing more fermentation substrates for LAB. Meanwhile, the addition of *Lactiplantibacillus plantarum* promoted homo-fermentation and reduced AA production. As expected, *Lactobacillus* was inversely related to NDF, ADF, and AA and positively associated with WSC, which was similar to the study of Bao et al. [41], who reported that *Lactobacillus* had a negative correlation with AA and a positive correlation with WSC in alfalfa silage. Generally, the NH_3-N content is low om high-quality silage, but there are also reports that LAB can increase the NH_3-N content [44–46]. Interestingly, a similar situation was observed in this study, as *Lactobacillus* showed a significantly positive correlation with NH_3-N. As members of *Enterobacteriaceae*, *Serratia*, *Pantoea*, *Klebsiella*, *unclassified_f__Enterobacteriaceae*, and *Enterobacter* had a similar effect on the silage quality, as they accelerated WSC consumption and AA production. In this study, *Klebsiella* was negatively correlated with CP, suggesting that it was probably the main bacteria that degraded the proteins [47]. Additionally, the role of *Pediococcus* on the silage quality seemed to be opposite to that of *Lactobacillus*, which was possibly due to the fact that the rapid growth of *Lactobacillus* had an inhibitory effect on *Pediococcus* in the late stage of ensiling [43,46].

4.4. Effect of Additives on Function Shifts of Bacterial Community

During ensiling, metabolic gene clusters have a profound impact on the metabolism and metabolites of microorganisms by participating in a variety of secondary metabolic pathways, thereby affecting the silage quality [48]. In this study, global and overview maps were the most common metabolic category and were upregulated by additive treatments. This agreed with the study of Du et al. [9], who found that global and overview maps were the main metabolic category of microorganisms in high-cellulose silage, and their abundance could be upregulated by the combination of cellulase and *Lactiplantibacillus plantarum.* In the global and overview maps, there was a series of metabolic pathways, such as the nucleotide metabolism and biosynthesis of cofactors [49], but the reasons for the upregulation of global and overview maps caused by additive treatments need to be further studied. In this study, carbohydrate metabolism, amino acid metabolism, and membrane transport were also important metabolic categories. Bai et al. [50] reported that the amino acid metabolism should be suppressed in high-quality silage. Nevertheless, the amino acid metabolism did not differ significantly among the four treatments. This could be attributed to the fact that all treatments had a pH of <4.2 [51], which effectively inhibited the protein breakdown and amino acid utilization caused by undesirable microorganisms. Previous studies have showed that the relative abundance of total LAB is positively associated with the abundance of carbohydrate metabolism [12,52]. As expected, compared with CON, both LPCE and CE upregulated the carbohydrate metabolism, which was likely due to the fact that the fiber degradation caused by cellulase could provide LAB with more WSC for LA fermentation, thus enhancing their carbohydrate metabolic activity. However, compared with CON, both LP and LPCE downregulated

membrane transport, which may have been due to the fact that the silage without additives had more abundant transporters [53]. Interestingly, compared with CE and LP, LPCE exerted a more profound impact on function shifts in the bacterial community, suggesting that cellulase and *Lactiplantibacillus plantarum* probably exerted synergistic effects on accelerating bacterial community succession and metabolic function transformation in high-fiber silage.

5. Conclusions

This study was conducted to investigate the roles of cellulase and *Lactiplantibacillus plantarum* in the silage fermentation process of *Pennisetum giganteum* z.x.lin. Cellulase inoculation significantly decreased the contents of NDF and ADF. *Lactiplantibacillus plantarum* inoculation effectively increased the ratio of LA/AA and LA content, which benefited the preservation of CP and WSC. The combination of cellulase and *Lactiplantibacillus plantarum* showed the highest abundance of *Lactobacillus* and accelerated function shifts in the bacterial community, leading to the lowest membrane transport and the highest carbohydrate metabolism. In summary, the cellulase or *Lactiplantibacillus plantarum* inoculation could improve the silage quality of *Pennisetum giganteum* z.x.lin and their combination showed a greater ability to reshape bacterial community.

Author Contributions: Conceptualization, L.D., I.K., J.Y. and X.L.; methodology, X.Z., R.L. and J.H.; software, R.L. and J.H.; validation, R.L. and J.H.; formal analysis, Z.W. and X.Z.; investigation, Z.W. and X.Z.; resources, J.W., J.Y. and X.L.; data curation, Z.W., X.Z., R.L. and J.H.; writing—original draft preparation, Z.W. and X.Z.; writing—review and editing, Z.W., X.Z., R.L., J.H., L.D., I.K., J.W., J.Y. and X.L.; visualization, Z.W. and X.Z.; supervision, X.L.; project administration, X.L.; funding acquisition, X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Shaanxi Livestock and Poultry Breeding Double-chain Fusion Key Project (2022GD-TSLD-46-0501), the Science & Technological Project of Shaanxi Province, China (2022ZDLNY01-09; 2023KJXX-132; 2024NC-YBXM-125), and the Science & Technological Project of Xianyang, Shaanxi Province, China (L2022-QCYZX-NY-004).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are available upon request to the corresponding author.

Acknowledgments: The authors thank the Key Laboratory of Livestock Biology, Northwest A&F University, Yangling, China for the technical assistance and use of the research facilities.

Conflicts of Interest: Author Jie Wei was employed by the company Xi'an Architectural Design and Research Institute Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Liu, X.; Li, D.; Ge, Q.; Yang, B.; Li, S. Effects of Harvest Period and Mixed Ratio on the Characteristic and Quality of Mixed Silage of Alfalfa and Maize. *Anim. Sci. Technol.* **2023**, *306*, 115796. [CrossRef]
2. Reed, M.S.; Stringer, L.C.; Dougill, A.J.; Perkins, J.S.; Atlhopheng, J.R.; Mulale, K.; Favretto, N. Reorienting Land Degradation towards Sustainable Land Management: Linking Sustainable Livelihoods with Ecosystem Services in Rangeland Systems. *J. Environ. Manag.* **2015**, *151*, 472–485. [CrossRef]
3. Cui, Y.; Ouyang, S.; Zhao, Y.; Tie, L.; Shao, C.; Duan, H. Plant Responses to High Temperature and Drought: A Bibliometrics Analysis. *Front. Plant Sci.* **2022**, *13*, 1052660. [CrossRef] [PubMed]
4. Zhou, J.; Chen, S.; Shi, W.; David-Schwartz, R.; Li, S.; Yang, F.; Lin, Z. Transcriptome Profiling Reveals the Effects of Drought Tolerance in Giant Juncao. *BMC Plant Biol.* **2021**, *21*, 2. [CrossRef] [PubMed]

5. Jia, Y.; Liao, Z.; Chew, H.; Wang, L.; Lin, B.; Chen, C.; Lu, G.; Lin, Z. Effect of *Pennisetum giganteum* z.x.lin Mixed Nitrogen-Fixing Bacterial Fertilizer on the Growth, Quality, Soil Fertility and Bacterial Community of Pakchoi (*Brassica chinensis* L.). *PLoS ONE* **2020**, *15*, e0228709. [CrossRef]
6. Qiu, Y.; Lei, Y.; Zhao, H.; He, X.; Liu, B.; Huang, Y. Mesophilic Anaerobic Digestion of *Arundo donax* Cv. Lvzhou No. 1 and *Pennisetum giganteum* for Biogas Production: Structure and Functional Analysis of Microbial Communities. *Bioenerg. Res.* **2023**, *16*, 1205–1216. [CrossRef]
7. Yang, F.; Wang, Y.; Zhao, S.; Wang, Y. *Lactobacillus plantarum* Inoculants Delay Spoilage of High Moisture Alfalfa Silages by Regulating Bacterial Community Composition. *Front. Microbiol.* **2020**, *11*, 1989. [CrossRef] [PubMed]
8. Dong, J.; Li, S.; Chen, X.; Sun, Z.; Sun, Y.; Zhen, Y.; Qin, G.; Wang, T.; Demelash, N.; Zhang, X. Effects of *Lactiplantibacillus plantarum* Inoculation on the Quality and Bacterial Community of Whole-Crop Corn Silage at Different Harvest Stages. *Chem. Biol. Technol. Agric.* **2022**, *9*, 57. [CrossRef]
9. Du, Z.; Yamasaki, S.; Oya, T.; Cai, Y. Cellulase-Lactic Acid Bacteria Synergy Action Regulates Silage Fermentation of Woody Plant. *Biotechnol. Biofuels Bioprod.* **2023**, *16*, 125. [CrossRef] [PubMed]
10. Zhong, C.; Wang, R.; Zhou, Z.; Jia, S.-R.; Tan, Z.-L.; Han, P.-P. Functional Properties of Protein Isolates from *Caragana korshinskii* Kom. Extracted by Three Different Methods. *J. Agric. Food Chem.* **2012**, *60*, 10337–10342. [CrossRef] [PubMed]
11. Ju, J.; Zhang, G.; Xiao, M.; Dong, C.; Zhang, R.; Du, L.; Zheng, Y.; Wei, M.; Wei, M.; Wu, B. Effects of Cellulase and *Lactiplantibacillus Plantarum* on the Fermentation Quality, Microbial Diversity, Gene Function Prediction, and in Vitro Rumen Fermentation Parameters of *Caragana korshinskii* Silage. *Front. Food. Sci. Technol.* **2023**, *2*, 1108043. [CrossRef]
12. Zhang, Y.; Wang, M.; Usman, S.; Li, F.; Bai, J.; Zhang, J.; Guo, X. Lignocellulose Conversion of Ensiled *Caragana Korshinskii* Kom. Facilitated by *Pediococcus Acidilactici* and Cellulases. *Microb. Biotechnol.* **2023**, *16*, 432–447. [CrossRef]
13. Si, Q.; Wang, Z.; Liu, W.; Liu, M.; Ge, G.; Jia, Y.; Du, S. Influence of Cellulase or *Lactiplantibacillus plantarum* on the Ensiling Performance and Bacterial Community in Mixed Silage of Alfalfa and *Leymus chinensis*. *Microorganisms* **2023**, *11*, 426. [CrossRef]
14. Hu, Z.; Niu, H.; Tong, Q.; Chang, J.; Yu, J.; Li, S.; Zhang, S.; Ma, D. The Microbiota Dynamics of Alfalfa Silage During Ensiling and After Air Exposure, and the Metabolomics After Air Exposure Are Affected by *Lactobacillus casei* and Cellulase Addition. *Front. Microbiol.* **2020**, *11*, 519121. [CrossRef] [PubMed]
15. AOAC. *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2000.
16. Van Soest, P.J.; 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. [CrossRef]
17. Xu, J.; Zhang, K.; Lin, Y.; Li, M.; Wang, X.; Yu, Q.; Sun, H.; Cheng, Q.; Xie, Y.; Wang, C.; et al. Effect of Cellulase and Lactic Acid Bacteria on the Fermentation Quality, Carbohydrate Conversion, and Microbial Community of Ensiling Oat with Different Moisture Contents. *Front. Microbiol.* **2022**, *13*, 1013258. [CrossRef]
18. Wang, W.; Ungerfeld, E.M.; Degen, A.A.; Jing, X.; Guo, W.; Zhou, J.; Huang, X.; Mudassar, S.; Shi, F.; Bi, S.; et al. Ratios of Rumen Inoculum from Tibetan and Small-Tailed Han Sheep Influenced in Vitro Fermentation and Digestibility. *Anim. Feed Sci. Technol.* **2020**, *267*, 114562. [CrossRef]
19. 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. [CrossRef] [PubMed]
20. Xu, F.; Liao, H.; Yang, J.; Zhang, Y.; Yu, P.; Cao, Y.; Fang, J.; Chen, S.; Li, L.; Sun, L.; et al. Auxin-Producing Bacteria Promote Barley Rhizosheath Formation. *Nat. Commun.* **2023**, *14*, 5800. [CrossRef]
21. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
22. Qiu, Y.; Zhao, H.; He, X.; Zhu, F.; Zhang, F.; Liu, B.; Liu, Q. Effects of Fermented Feed of *Pennisetum giganteum* on Growth Performance, Oxidative Stress, Immunity and Gastrointestinal Microflora of Boer Goats under Thermal Stress. *Front. Microbiol.* **2022**, *13*, 1030262. [CrossRef] [PubMed]
23. Zhao, J.; Liu, H.-P.; Yin, X.-J.; Dong, Z.-H.; Wang, S.-R.; Li, J.-F.; Shao, T. Dynamics of Phyllosphere Microbiota and Chemical Parameters at Various Growth Stages and Their Contribution to Anaerobic Fermentation of *Pennisetum giganteum*. *Microbiol. Spectr.* **2023**, *11*, e02288-22. [CrossRef] [PubMed]
24. Lai, A.; Huang, Y.; Luo, H.; Jin, Y.; Wang, L.; Chen, B.; Deng, K.; Huang, W.; Zhang, Y. Ruminal Degradation Characteristics of Bagasse with Different Fermentation Treatments in the Rumen of Beef Cattle. *Anim. Sci. J.* **2024**, *95*, e13937. [CrossRef] [PubMed]
25. Wang, Q.; Wang, R.; Wang, C.; Dong, W.; Zhang, Z.; Zhao, L.; Zhang, X. Effects of Cellulase and *Lactobacillus plantarum* on Fermentation Quality, Chemical Composition, and Microbial Community of Mixed Silage of Whole-Plant Corn and Peanut Vines. *Appl. Biochem. Biotechnol.* **2022**, *194*, 2465–2480. [CrossRef]
26. 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. [CrossRef]

27. Iannaccone, F.; Alborino, V.; Dini, I.; Balestrieri, A.; Marra, R.; Davino, R.; Di Francia, A.; Masucci, F.; Serrapica, F.; Vinale, F. In Vitro Application of Exogenous Fibrolytic Enzymes from *Trichoderma* spp. to Improve Feed Utilization by Ruminants. *Agriculture* **2022**, *12*, 573. [CrossRef]
28. Guo, G.; Yuan, X.; Li, L.; Wen, A.; Shao, T. Effects of Fibrolytic Enzymes, Molasses and Lactic Acid Bacteria on Fermentation Quality of Mixed Silage of Corn and Hulless–Barely Straw in the Tibetan Plateau. *Grassl. Sci.* **2014**, *60*, 240–246. [CrossRef]
29. Kung, L.; Shaver, R.D.; Grant, R.J.; Schmidt, R.J. Silage Review: Interpretation of Chemical, Microbial, and Organoleptic Components of Silages. *J. Dairy Sci.* **2018**, *101*, 4020–4033. [CrossRef]
30. Li, F.; Ding, Z.; Ke, W.; Xu, D.; Zhang, P.; Bai, J.; Mudassar, S.; Muhammad, I.; Guo, X. Ferulic Acid Esterase-Producing Lactic Acid Bacteria and Cellulase Pretreatments of Corn Stalk Silage at Two Different Temperatures: Ensiling Characteristics, Carbohydrates Composition and Enzymatic Saccharification. *Bioresour. Technol.* **2019**, *282*, 211–221. [CrossRef]
31. Mu, L.; Xie, Z.; Hu, L.; Chen, G.; Zhang, Z. *Lactobacillus plantarum* and Molasses Alter Dynamic Chemical Composition, Microbial Community, and Aerobic Stability of Mixed (Amaranth and Rice Straw) Silage. *J. Sci. Food Agric.* **2021**, *101*, 5225–5235. [CrossRef] [PubMed]
32. Kung, J.R.L. Silage Fermentation End Products and Microbial Populations: Their Relationships to Silage Quality and Animal Productivity. In Proceedings of the Annual Conference of the American Association of Bovine Practitioners, Charlotte, NC, USA, 25–27 September 2008; Volume 25, p. 27.
33. Li, F.; Ke, W.; Ding, Z.; Bai, J.; Zhang, Y.; Xu, D.; Li, Z.; Guo, X. Pretreatment of *Pennisetum sinese* Silages with Ferulic Acid Esterase-Producing Lactic Acid Bacteria and Cellulase at Two Dry Matter Contents: Fermentation Characteristics, Carbohydrates Composition and Enzymatic Saccharification. *Bioresour. Technol.* **2020**, *295*, 122261. [CrossRef] [PubMed]
34. Guo, G.; Shen, C.; Liu, Q.; Zhang, S.L.; Wang, C.; Chen, L.; Xu, Q.F.; Wang, Y.X.; Huo, W.J. Fermentation Quality and in Vitro Digestibility of First and Second Cut Alfalfa (*Medicago sativa* L.) Silages Harvested at Three Stages of Maturity. *Anim. Feed Sci. Technol.* **2019**, *257*, 114274. [CrossRef]
35. Li, H.; Zeng, T.; Du, Z.; Dong, X.; Xin, Y.; Wu, Y.; Huang, L.; Liu, L.; Kang, B.; Jiang, D.; et al. Assessment on the Fermentation Quality and Bacterial Community of Mixed Silage of Faba Bean with Forage Wheat or Oat. *Front. Microbiol.* **2022**, *13*, 875819. [CrossRef] [PubMed]
36. 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. [CrossRef] [PubMed]
37. Chen, L.; Bai, S.; You, M.; Xiao, B.; Li, P.; Cai, Y. Effect of a Low Temperature Tolerant Lactic Acid Bacteria Inoculant on the Fermentation Quality and Bacterial Community of Oat Round Bale Silage. *Anim. Feed Sci. Technol.* **2020**, *269*, 114669. [CrossRef]
38. Romero, J.J.; Zhao, Y.; Balseca-Paredes, M.A.; Tiezzi, F.; Gutierrez-Rodriguez, E.; Castillo, M.S. Laboratory Silo Type and Inoculation Effects on Nutritional Composition, Fermentation, and Bacterial and Fungal Communities of Oat Silage. *J. Dairy Sci.* **2017**, *100*, 1812–1828. [CrossRef] [PubMed]
39. Cai, Y.; Benno, Y.; Ogawa, M.; Ohmomo, S.; Kumai, S.; Nakase, T. Influence of *Lactobacillus* spp. from an Inoculant and of *Weissella* and *Leuconostoc* spp. from Forage Crops on Silage Fermentation. *Appl. Environ. Microbiol.* **1998**, *64*, 2982–2987. [CrossRef]
40. Tian, J.; Xu, N.; Liu, B.; Huan, H.; Gu, H.; Dong, C.; Ding, C. Interaction Effect of Silo Density and Additives on the Fermentation Quality, Microbial Counts, Chemical Composition and in Vitro Degradability of Rice Straw Silage. *Bioresour. Technol.* **2020**, *297*, 122412. [CrossRef] [PubMed]
41. Bao, X.; Feng, H.; Guo, G.; Huo, W.; Li, Q.; Xu, Q.; Liu, Q.; Wang, C.; Chen, L. Effects of Laccase and Lactic Acid Bacteria on the Fermentation Quality, Nutrient Composition, Enzymatic Hydrolysis, and Bacterial Community of Alfalfa Silage. *Front. Microbiol.* **2022**, *13*, 1035942. [CrossRef] [PubMed]
42. Ren, H.; Wang, C.; Fan, W.; Zhang, B.; Li, Z.; Li, D. Effects of Formic or Acetic Acid on the Storage Quality of Mixed Air-Dried Corn Stover and Cabbage Waste, and Microbial Community Analysis. *Food Technol. Biotechnol.* **2018**, *56*, 71–82. [CrossRef] [PubMed]
43. Cai, Y.; Benno, Y.; Ogawa, M.; Kumai, S. Effect of Applying Lactic Acid Bacteria Isolated from Forage Crops on Fermentation Characteristics and Aerobic Deterioration of Silage. *J. Dairy Sci.* **1999**, *82*, 520–526. [CrossRef] [PubMed]
44. Dong, Z.; Shao, T.; Li, J.; Yang, L.; Yuan, X. Effect of Alfalfa Microbiota on Fermentation Quality and Bacterial Community Succession in Fresh or Sterile Napier Grass Silages. *J. Dairy Sci.* **2020**, *103*, 4288–4301. [CrossRef] [PubMed]
45. Khota, W.; Pholsen, S.; Higgs, D.; Cai, Y. Natural Lactic Acid Bacteria Population of Tropical Grasses and Their Fermentation Factor Analysis of Silage Prepared with Cellulase and Inoculant. *J. Dairy Sci.* **2016**, *99*, 9768–9781. [CrossRef]
46. Li, P.; Liao, C.; Yan, L.; Zhang, C.; Chen, L.; You, M.; Cheng, Q.; Chen, C. Effects of Small-Scale Silo Types and Additives on Silage Fermentation and Bacterial Community of High Moisture Alfalfa on the Qinghai-Tibetan Plateau. *Anim. Feed Sci. Technol.* **2023**, *299*, 115594. [CrossRef]

47. Zheng, Y.; Li, M.; Xu, J.; Sun, H.; Cheng, Q.; Xie, Y.; Wang, C.; Chen, C.; Li, P. Effects of Different Cutting Methods and Additives on the Fermentation Quality and Microbial Community of *Saccharum arundinaceum* Silage. *Front. Microbiol.* **2022**, *13*, 999881. [CrossRef]
48. Slot, J.C. Fungal Gene Cluster Diversity and Evolution. *Adv. Genet.* **2017**, *100*, 141–178. [CrossRef]
49. Kanehisa, M.; Goto, S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30. [CrossRef] [PubMed]
50. Bai, J.; Franco, M.; Ding, Z.; Hao, L.; Ke, W.; Wang, M.; Xie, D.; Li, Z.; Zhang, Y.; Ai, L.; et al. Effect of *Bacillus amyloliquefaciens* and *Bacillus Subtilis* on Fermentation, Dynamics of Bacterial Community and Their Functional Shifts of Whole-Plant Corn Silage. *J. Anim. Sci. Biotechnol.* **2022**, *13*, 7. [CrossRef]
51. Wang, H.; Hao, W.; Ning, T.; Zheng, M.; Xu, C. Characterization of Culturable Yeast Species Associating with Whole Crop Corn and Total Mixed Ration Silage. *Asian-Australas. J. Anim. Sci.* **2017**, *31*, 198–207. [CrossRef]
52. 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. [CrossRef] [PubMed]
53. Kilstrup, M.; Hammer, K.; Ruhdal Jensen, P.; Martinussen, J. Nucleotide Metabolism and Its Control in Lactic Acid Bacteria. *FEMS Microbiol. Rev.* **2005**, *29*, 555–590. [CrossRef] [PubMed]