frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 20 September 2023
DOI 10.3389/fsufs.2023.1255936

OPEN ACCESS

EDITED BY
Cristobal N. Aguilar,
Autonomous University of Coahuila, Mexico

REVIEWED BY
Guadalupe Virginia Nevárez-Moorillón,
Autonomous University of Chihuahua, Mexico
Liliana Londono Hernandez,
National Open and Distance University, Colombia

*CORRESPONDENCE
Chao Wang
✉ wangchao@imaaahs.ac.cn
Huaxin Niu
✉ niuhx@imun.edu.cn

RECEIVED 11 July 2023
ACCEPTED 31 August 2023
PUBLISHED 20 September 2023

CITATION
Wu B, Ai J, Li T, Qin W, Hu Z, Siqin T, Wu T, Wang C and Niu H (2023) Fermentation quality, aerobic stability, and microbiome structure and function of *Caragana korshinskii* silage inoculated with/without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. *Front. Sustain. Food Syst.* 7:1255936. doi: 10.3389/fsufs.2023.1255936

Fermentation quality, aerobic stability, and microbiome structure and function of *Caragana korshinskii* silage inoculated with/without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*

Baiyila Wu[1], Juanjuan Ai[2], Tiyu Li[1], Weize Qin[1], Zongfu Hu[1], Tuya Siqin[1], Tiemei Wu[1], Chao Wang[3]* and Huaxin Niu[1]*

[1]College of Animal Science and Technology, Inner Mongolia Minzu University, Tongliao, China, [2]Medical College, Inner Mongolia Minzu University, Tongliao, China, [3]Inner Mongolia Academy of Agriculture and Animal Husbandry Science, Hohhot, China

Caragana korshinskii is a forage shrub species with high-protein content that has been extensively used to alleviate feed shortages for ruminants in northern China. Herein, we investigated the effects of *Lactobacillus rhamnosus* and *Lactobacillus buchneri* on the fermentation quality, aerobic stability, and microbiome composition and the predicted functional characteristics of *C. korshinskii* silage. *C. korshinskii* silages were inoculated with and without *L. rhamnosus* or *L. buchneri*. After 14 and 56 days of ensiling, the aerobic stability was determined. The results revealed that after 14 and 56 days of ensiling, *L. rhamnosus*- and *L. buchneri*-inoculated silage exhibited increased acetic acid and lactic acid contents, whereas the pH and 2,3-butanediol and butyric acid contents were decreased compared with those of the control silage. The control silages that were opened at 14 and 56 d, deteriorated during the aerobic stability test, whereas silages inoculated with *L. rhamnosus* and *L. buchneri* did not exhibit any aerobic deterioration. The control silage showed an increased *Clostridium* and *Bacillus* abundance, whereas *Lactobacillus* abundance decreased compared with *L. rhamnosus*- and *L. buchneri*-inoculated silages, following the 7 days of aerobic exposure. The fermentation parameters were associated with microbial communities, including *Lactobacillus*, *Pedicoccus*, *Weissella*, *Clostridium*, and *Bacillus*. Carbohydrate and amino acid metabolisms in the control silage decreased after 7 days of aerobic exposure compared with lactic acid bacteria-inoculated silages. To conclude, next-generation sequencing combined with 16S ribosomal RNA gene-predicted functional analyses might provide new information about the silage quality during fermentation and the aerobic stability.

KEYWORDS

***Caragana korshinskii* silage, fermentation products, *Lactobacillus rhamnosus*, *Lactobacillus buchneri*, microbial community, microbial function characteristics**

1. Introduction

Caragana korshinskii is a type of leguminous shrub widely planted in the arid and semi-arid areas of China (Bai et al., 2023). According to a statistical report, >40 million tons of the existing leguminous *C. korshinskii* in China is produced *via* stumping and has a crude protein (CP) content of 15%, which is equivalent to 17 million tons of soybean CP content (Li et al., 2021). The untimely stump can drastically age and degrade *C. korshinskii*, resulting in increased resource wastage (Liu et al., 2023). Therefore, rational and effective utilization of *C. korshinskii* is important, which can effectively alleviate the present forage shortage and improve ecosystem stability.

The branches, leaves, flowers, and fruits of *C. korshinskii* are rich in nutrients, with high contents of protein, crude fat, and mineral elements, with a high essential amino acid content compared with those of the general straw feed, making it a good unconventional feed resource (Deng et al., 2017). The high degree of lignification accounts for a critical factor restricting *C. korshinskii* development as a feed resource, and suitable processing methods are conducive to reducing its fiber content. A previous study showed that *C. korshinskii* harvested at the flowering stage has a high nutritional value, and its palatability can be improved by the rubbing processing method (Zhong et al., 2014). Additionally, silage can effectively preserve the nutritional components of *C. korshinskii*, acting as a storage method to preserve the shrub feed and alleviate feed shortage in arid areas. The poor fermentation quality of leguminous plants is mainly due to characteristics such as low lactic acid bacteria (LAB) count and water-soluble carbohydrate (WSC) content, along with a high buffering capacity (You et al., 2022). Therefore, LAB addition is one of the effective ways to improve the poor fermentation ability of the *C. korshinskii* silage.

Usually, a LAB count of $\geq 10^5$ cfu/g in fresh material is required in high-quality silage. Exogenous LAB inoculation can meet the initial LAB count required for the fermentation of the leguminous silage (Zhang et al., 2023). We isolated several LAB strains with good fermentation parameters from *L. chinensis*, alfalfa, and corn silage. We found that the LAB additives in the *L. chinensis* silage promoted its fermentation quality while inhibiting the growth of harmful microorganisms (Wu et al., 2022). Nonetheless, those LAB strains do not affect the fermentation quality, bacterial community, or aerobic stability of the *C. korshinskii* silage. The information regarding the characteristics of *Lactobacillus rhamnosus* and *Lactobacillus buchneri*, isolated from the *C. korshinskii* silage, along with their effects on the fermentation product, microbial community, and aerobic stability of the *C. korshinskii* silage is lacking. Therefore, *L. rhamnosus* and *L. buchneri* strains were isolated from a high-quality *C. korshinskii* silage and identified using their phenotypes and 16S ribosomal RNA (rRNA) gene sequence and then were cultured. Investigating the microbial changes caused by LAB addition to the *C. korshinskii* silage during fermentation and after aerobic exposure is of great necessity. The high quality of silage depends on the succession of the microbial community and microbial metabolites during the fermentation process (Zou et al., 2023). Investigation of the microbial community during ensiling and after aerobic exposure can provide insights for improving the preservation of the *C. korshinskii* silage. During the past decade, culture-independent/dependent methods, including 16S rRNA gene sequencing, real-time polymerase chain reaction (PCR), and denaturing gradient gel electrophoresis, have been applied to investigate the epiphytic microbiota within fresh materials together with microbiota within the ensiled feed (Stevenson et al., 2006; Pang et al., 2011; Wu et al., 2014). Although studies have reported the microbiome composition during the fermentation stage, their results only identify various operational taxonomic units (OTUs), rather than providing the classification details of microorganisms in the silage. Next-generation sequencing (NGS) can be applied in evaluating LAB effectiveness in promoting the fermentation quality and aerobic stability of silage (Wang et al., 2020).

Herein, we investigated the effects of *L. rhamnosus* and *L. buchneri* inoculant on the fermentation product, microbial community, function prediction of 16 S rRNA gene, and the aerobic stability of the *C. korshinskii* silage during the fermentation process and after aerobic exposure through high throughput sequencing technology.

Materials and methods

Ensiling and bacterial inoculants

On June 10, 2022, boot-stage *C. korshinskii* was collected from an experimental field of the Jarud Banner Fengyi Agriculture and Animal Husbandry Company from the Inner Mongolia Autonomous Region, China. Following 3-h wilting, *C. korshinskii* was cut into 15–20 mm-long segments using the crop cutter for 326 g/kg dry matter (DM). *L. rhamnosus* (CGMCC No. 134426) and *L. buchneri* (CGMCC No. 187961) were isolated from *C. korshinskii* silage in Inner Mongolia, China. Cultures of *L. rhamnosus* and *L. buchneri* were made by anaerobic incubation in MRS broth at 37°C for 48 h. cultures of *L. rhamnosus* and *L. buchneri* were diluted with sterile physiological saline as additives. The *C. korshinskii* silage was inoculated using *L. rhamnosus* and *L. buchneri* at 1×10^6 cfu/g of fresh matter (FM). The control group was sprayed with an equivalent volume of sterile physiological saline. The inoculated and uninoculated samples (300 g each) were tightly packaged in plastic pouches (BN-10, 250 × 350 mm; Wangnuo, Beijing, China) using the commercially available vacuum sealer (ZK-320; Ouxin, Beijing, China). Every treatment was performed in triplicate. Additionally, the silos were preserved for 14 and 56 days under ambient temperature.

Aerobic stability test

After completely opening the silos stored for 14 and 56 days, silage (150 g) was placed into an uncompacted polyethylene bottle (500 mL) and exposed to air for 7 days under ambient temperature.

Chemical composition and quantitative analysis of the microbiome

The DM levels in the freshly collected samples and silages were determined through 48-h drying under 65°C. Following this, the samples were pulverized in a 1-mm-sieve Wiley mill (ZM200, Retsch GmbH). The CP level was analyzed according to the standard

procedures from the Association of Official Analytical Chemists (AOAC, 1990). Previously reported approaches were adopted for measuring the acid detergent fiber and neutral detergent fiber (Van Soest et al., 1991), and WSCs (Wu and Nishino, 2016).

The fresh samples (20 g each) were homogenized in a blender using sterile water (180 mL) for a 1-min period, and the homogenized mix was filtered with a 0.22-μm membrane to prepare the silage sample water extracts, used for determining the pH, alcohol, lactic acid, and short-chain fatty acid levels. The pH value of the extract was analyzed using the glass electrode pH meter (SX-620, Sanxin, Shanghai, China). Ion-exclusion polymer high-performance liquid chromatography with a refractive index detector was employed for detecting the fermentation products.

A serial dilution (range: 10^{-1} to 10^{-6}) was performed on a clean bench for quantifying different microbes. For quantifying enterobacteria, the violet red bile agar was used (CM0107B, Oxoid Ltd., United Kingdom), whereas LAB was quantified using the de Man, Rogosa, and Sharpe agar (CM0361B, Oxoid Ltd., UK). Mold and yeast were quantified on the potato dextrose agar (CM0139B, Oxoid Ltd., UK) spread plates with a 3.5 pH (maintained using sterile lactic acid). The number of colonies in FM (cfu/g) was used for calculating the number of living microorganisms.

Microbiome sequencing and analysis

The refrigerated silage (10 g) was blended with 40 mL of sterile phosphate-buffered saline (pH 7.4) using an electronic oscillator at 120 rpm for 2 h, and the sample was filtered through two layers of gauze. The filtrate was centrifuged for 10 min at 13,000 ×*g* and 4°C, and the supernatants were removed, whereas the pellets were maintained on the dry ice. PCR amplification, DNA extraction, and metagenomic sequencing were performed at the Majorbio Bio-Pharm Technology, Shanghai, China. Thereafter, the Illumina MiSeq sequencing and processing of sequenced data were also performed. OTUs were clustered using UPARSE version 7.1 (Edgar, 2013) at a similarity threshold of 97%. After identifying and eliminating the chimeric sequences, taxonomic analysis was conducted on the characteristic OTU sequences (0.7 confidence level), based on 16S rRNA databases (including Silva v138) using the Ribosomal Database Project Classifier (version 2.2) (Yang et al., 2019). Raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA990987.

Kyoto encyclopedia of genes and genomes (KEGG) enrichment

The KEGG database was employed for the functional enrichment of bacterial communities with Tax4Fun (version 0.3.1) (Aßhauer et al., 2015). The KEGG pathways were compared and displayed using bubble charts.

Statistical analyses

Data were statistically analyzed using the John's Macintosh Project software (version 13; SAS Institute, Tokyo, Japan). The effect of LAB was assessed using the one-way analysis of variance along with subsequent multiple comparisons based on Tukey's honestly significant difference test. The *value of p* of <0.05 was considered statistically significant for comparing means among the various treatments. Additionally, correlation analysis of the bacterial community with fermentation products and KEGG metabolic pathways was performed using the Majorbio Cloud Platform.

Results and discussion

Chemical and microbial components in pre-ensiling *Caragana korshinskii*

Table 1 presents the chemical and microbial components in pre-ensiling *C. korshinskii*. DM content of *C. korshinskii* was determined to be 326 g/kg of FM, which was relatively low compared with the large needlegrass and Chinese leymus silages (Zhang et al., 2016). CP level was 158.32 g/kg of DM, which was relatively high compared with the DM level (102.53 g/kg), as reported by Bai et al. (2023). WSC of fresh samples is an important silage fermentation substrate, and a WSC level of >6% of DM can ensure the expected fermentation. In this study, the WSC content in the pre-ensiling *C. korshinskii* was 51.85 g/kg of DM, suggesting it is a limitation in achieving high-quality silage with no additives. Epiphytic LAB, yeast, and enterobacteria counts in pre-ensiling *C. korshinskii* were 4.52, 5.74, and 5.46 log cfu/g of FM, respectively, based on the culturable method. Epiphytic LAB count in freshly harvested crops is often recognized as a critical factor for determining the requirement of LAB inoculation within the fresh silage material and estimating the silage fermentation quality. The LAB count of $<10^5$ cfu/g in the raw material can cause poor fermentation during the ensiling process (Cai et al., 1998). LAB addition is necessary to improve the silage fermentation quality because of the low LAB count and the presence of many harmful microorganisms in the raw materials of *C. korshinskii*.

TABLE 1 Chemical and microbial compositions of pre-ensiling *Caragana korshinskii*.

	Caragana korshinskii
Dry matter (g/kg)	326 ± 4.32
pH	5.63 ± 0.28
Crude protein (g/kg DM)	158.32 ± 1.37
Neutral detergent fiber (g/kg DM)	462.82 ± 13.52
Acid detergent fiber (g/kg DM)	387.78 ± 9.73
Water-soluble carbohydrate (g/kg DM)	51.85 ± 0.74
Lactic acid bacteria (log cfu/g)	4.52 ± 0.48
Yeasts (log cfu/g)	5.74 ± 0.15
Enterobacteria (log cfu/g)	5.46 ± 0.29

Data are the mean of duplicate analyses. Cfu, colony-forming unit; and DM, dry matter.

Roles of *Lactobacillus rhamnosus* and *Lactobacillus buchneri* in fermentation products, microbial counts, and aerobic stability

After 14 days of storage, lactic and acetic acid levels in the untreated silage were 9.60 and 4.77 g/kg of DM, respectively (Table 2). With prolonged storage time, lactic acid and acetic acid levels increased, whereas propionic acid was not detected in both 14- and 56-day silage. The ethanol and 1,2-propanediol contents in the untreated silage were 2.70 and 0.62 g/kg of DM, respectively. According to Muck (2010), ethanol and 1,2-propanediol can be produced from sugar by heterofermentative LAB species such as *Lactobacillus hilgardii*, *Lactobacillus brevis*, and *L. buchneri*. *L. rhamnosus*, a homofermentative LAB strain, have been used to improve the fermentation quality of the whole-crop corn silage (Li and Nishino, 2011b). In the *L. rhamnosus*-inoculated group, the LAB count and the lactic and acetic acid levels were increased, whereas the pH value and the butyric acid, ethanol, and 2,3-butanediol contents were reduced compared with those of the untreated group. In the *L. buchneri*-inoculated group, the 1,2-propanediol and acetic acid contents were increased after 56 and 120 days of storage (Li and Nishino, 2011a). Herein, *L. buchneri* addition increased the 1,2-propanediol, acetic acid, and lactic acid levels, whereas the pH value, butyric acid, and 2,3-butanediol levels decreased after 14 and 56 days. This result indicated that regardless of ensiling time, metabolism of *L. buchneri* is known to be more active in *C. korshinskii* silage. The enterobacteria and yeast counts in the untreated silage were high during the ensiling process. The *L. buchneri*- and *L. rhamnosus*-inoculated silage exhibited fast antagonistic and acidification functions, which inhibited the enterobacteria count, resulting in the ethanol content efficiency was limited.

The heating in the uninoculated silage was observed on day 2 following the aerobic exposure, regardless of the ensiling time. Yeast count and pH value in the uninoculated silage increased to $>10^7$ cfu/g and > 6.0, respectively (Table 3). At yeast count of $>10^5$ cfu/g, the silage shows reduced aerobic stability (Muck et al., 2018). The homofermentative/heterofermentative LAB-inoculated whole-crop corn silage caused aerobic deterioration during the 7 days of aerobic exposure due to yeast and mold count rapidly increased. However, no heating was observed in the LAB-inoculated silage during the 7 days of aerobic exposure. Although the *L. rhamnosus*-inoculated silage showed an improved fermentation quality after 56 d, Li and Nishino (2011a) found that *L. rhamnosus* inoculation had no significant effect on yeast inhibition, resulting in the aerobic deterioration of the Italian ryegrass silage. However, Zhao et al. (2020) reported that *L. rhamnosus* produced the bacteriocins, along with improving the fermented food quality, which inhibited the growth of harmful microorganisms, including mold, enterobacteria, and yeast, and increased the aerobic stability of the fermented food. These studies showed that different LAB species added silage had different effects on improving fermentation quality and inhibiting spoilage. The *L. buchneri*-inoculated silage exhibited increased 1,2-propanediol and acetic acid contents compared with the uninoculated silage, following the 7 days of aerobic exposure. Acetic acid improved the aerobic stability of silage by inhibiting the growth of undesirable microorganisms after air exposure. Hence, *L. buchneri*-inoculated silage had improved aerobic stability.

TABLE 2 Fermentation products and microbial counts of *Caragana korshinskii* silage stored with/without *Lactobacillus rhamnosus* or *Lactobacillus buchneri* for 14 and 56 days.

	14 days				56 days				2-way ANOVA		
	C	LR	LB	SE	C	LR	LB	SE	T	S	T × S
Dry matter (g/kg)	327a	332a	334a	3.14	337A	324B	325B	1.40	NS	NS	**
pH	5.49a	4.52c	4.80b	0.04	4.74A	4.07B	4.13B	0.07	**	**	**
Lactic acid (g/kg DM)	9.60b	15.73a	15.00a	0.21	12.70C	19.43A	14.68B	0.13	**	**	**
Acetic acid (g/kg DM)	4.77c	6.34b	8.29a	0.28	5.31B	4.20C	6.72A	0.09	**	**	**
Butyric acid (g/kg DM)	3.29a	0.45c	0.76b	0.03	2.56A	0.85B	1.02B	0.11	NS	**	**
Ethanol (g/kg DM)	2.70a	1.60b	1.58b	0.07	1.77A	0.63B	0.78B	0.05	**	**	NS
2,3-Butanediod (g/kg DM)	3.22a	0.86c	1.53b	0.05	4.88A	1.86C	2.50B	0.12	**	**	**
1,2-Propanediol (g/kg DM)	0.62c	1.34b	2.19a	0.06	1.74B	1.82B	2.77A	0.09	**	**	**
Lactic acid bacteria (log cfu/g)	6.35b	7.45a	7.69a	0.15	6.40B	7.46A	7.71A	0.16	NS	**	NS
Yeasts (log cfu/g)	6.29a	5.40b	4.58c	0.12	5.83A	4.32B	4.26B	0.06	**	**	**
Enterobacteria (log cfu/g)	6.45a	4.35b	4.43b	0.08	5.48A	4.61B	3.66C	0.12	**	**	**

Data are means from triplicate silages. Values for identical ensiling times with varying following letters (a–c, A–C) differ significantly ($p < 0.05$). **, $p < 0.01$; *, $p < 0.05$; and NS, $p \geq 0.05$. C, control group; LR, *Lactobacillus rhamnosus* treatment group; LB, *Lactobacillus buchneri* treatment group; ANOVA, analysis of variance; T, treatment effects; S, ensiling time; T × S, interaction of treatment effects and ensiling time; NS, not significant; DM, dry matter; and cfu, colony-forming unit.

10.3389/fsufs.2023.1255936

TABLE 3 Fermentation products and microbial counts of *Caragana korshinskii* silage subjected to aerobic stability test for 7 days.

	14 days + AS				56 days + AS				2-way ANOVA		
	C	LR	LB	SE	C	LR	LB	SE	T	S	T × S
Dry matter (g/kg)	329a	332a	326a	3.42	333A	327A	331A	4.20	NS	NS	NS
pH	6.42a	4.69c	4.88b	0.03	6.80A	4.24C	4.35B	0.04	**	**	**
Lactic acid (g/kg DM)	3.50b	14.67a	13.93a	0.30	3.50C	18.40A	13.65B	0.14	**	**	**
Acetic acid (g/kg DM)	2.63c	6.04b	7.67a	0.13	2.34C	3.83B	5.78A	0.07	**	**	**
Butyric acid (g/kg DM)	1.33a	0.29c	0.52b	0.06	2.39A	0.92B	1.16B	0.11	**	**	NS
Ethanol (g/kg DM)	1.43a	0.63b	0.56b	0.08	0.81B	0.36C	0.95A	0.03	**	**	**
2,3-Butanediod (g/kg DM)	0.62b	0.46c	1.33a	0.07	2.74B	0.86C	3.53A	0.07	**	**	**
1,2-Propanediol (g/kg DM)	0.45c	0.84b	1.26a	0.05	0.77B	0.92B	1.73A	0.06	**	**	*
Lactic acid bacteria (log cfu/g)	6.12b	7.65a	7.76a	0.12	6.16B	7.73A	7.51A	0.11	NS	**	NS
Yeasts (log cfu/g)	7.36a	5.50b	4.28c	0.10	7.10A	4.13B	3.83B	0.09	**	**	**
Enterobacteria (log cfu/g)	7.55a	4.25b	4.07b	0.07	6.55A	4.21B	3.29C	0.04	**	**	**

Data presented as means from triplicate silages. Values for identical aerobic stability test with varying following letters (a–c, A–C) differ significantly ($p < 0.05$). **, $p < 0.01$; *, $p < 0.05$; and NS, $p \geq 0.05$. AS, aerobic stability; C, control group; LR, *Lactobacillus rhamnosus* treatment group; LB, *Lactobacillus buchneri* treatment group; ANOVA, analysis of variance; T, treatment effects; S, ensiling time; T × S, interaction of treatment effects and ensiling time; NS, not significant; DM, dry matter; and cfu, colony-forming unit.

Roles of *Lactobacillus rhamnosus* and *Lactobacillus buchneri* in bacterial diversity during ensiling and after aerobic exposure

Through NGS, we sequenced the 16S rRNA gene of 39 samples (3 fresh materials and 36 silages). A total of 5,920,574 high-quality sequence readings were generated, with an average of 151,810 readings per sample (Table 4). All the readings were clustered in 7,234 OTUs at a sequence similarity of 97%. The coverage values in every sample were approximately 0.99, suggesting a high bacterial flora diversity. We also utilized Chao/Shannon index and OTUs for assessing the bacterial diversity and abundance within the feed. After 14 days of ensiling, the OTUs, abundance-based coverage estimator (ACE), and Chao 1 levels in *L. rhamnosus* and *L. buchneri* inoculation were higher than those in the untreated silage, indicating that LAB addition increased the bacterial abundance. The OTUs, ACE, and Chao 1 levels were lower in the *L. buchneri*- and *L. rhamnosus*-inoculated samples compared with those in non-inoculated samples after 56 days of ensiling. Consistent with the study by Guo et al. (2018), OTUs, ACE, and Chao1 levels increased in the *L. buchneri* and *L. rhamnosus*-treated silage compared with those in untreated silage. The Shannon index in LAB-inoculated *C. korshinskii* silage was the lowest on day 56 possibly because of the low pH value, which caused a rapid decrease in the bacterial diversity.

After the aerobic deterioration test, OTUs, ACE, and Chao 1 index in the spoiled silage were decreased, indicating the proliferation of low-acid-resistant aerobic microorganisms during the aerobic stability test (Wilkinson and Davies, 2013). However, as revealed by denaturing gradient gel electrophoresis analysis, frequent observation of predominant bacteria within alfalfa silage indicated a less diverse bacterial community (Wu and Nishino, 2016).

The Venn plot shows the different groups with common and unique OTUs (Figure 1A). All groups contained 65 common OTUs and 1,150 exclusive OTUs, which were distributed as follows: fresh material, 222; 14-day silos, 201; 56-day silos, 369; 7 days of air-exposed 14-day silos, 143; and 7 days of air-exposed 56-day silos, 215. Figure 1B illustrates the nonmetric multidimensional scaling analysis of the bacterial community in the *C. korshinskii* silage with/without *L. rhamnosus* or *L. buchneri* inoculation. There was an obvious separation of bacterial communities in the pre-ensiling crop compared to the silage sample. In a previous study, native grass silage after *Ephedra sinica* inoculation exhibited similar results (Du et al., 2022). Because aerobic spoilage was measured in the CAS and CAE groups, following 7 days of aerobic exposure, the alterations among the bacterial communities in the CAS and CAE groups cause aerobic spoilage, separating them from others.

Roles of *Lactobacillus rhamnosus* and *Lactobacillus buchneri* in the dynamics of bacterial communities during ensiling and after aerobic exposure

Figure 2 illustrates the bacterial communities in the fresh material and silage at the phylum level. In the fresh forage, *Firmicutes* were the bacteria with the highest phylum level, followed by *Proteobacteria*, *Cyanobacteria*, *Bacteroidota*, and *Actinobacteriota*. However, in every sample during ensiling process and after aerobic exposure, *Firmicutes* and *Proteobacteria* were the most dominant phyla, accounting for

TABLE 4 The bacterial alpha diversity of *Caragana korshinskii* silage with/without *Lactobacillus rhamnosus* or *Lactobacillus buchneri* during ensiling process and after aerobic exposure.

	Days	Sample ID	Read	OTU	Shannon	Chao1	ACE	Coverage
Bacterial alpha diversity	0 days	FM	84,020	543	2.61	645.61	667.70	0.99
	14 days	C	154,523b	452c	1.43c	602.29c	616.58c	0.99
		LR	242,932a	665a	2.01b	728.23a	759.74a	0.99
		LB	147,883b	515b	2.71a	687.89b	690.21b	0.99
	14 days+AS	C	91,489y	1,023x	3.34x	1163.47x	1172.85x	0.99
		LR	276,571x	441y	1.10z	528.50y	549.40y	0.99
		LB	205,354xy	384y	1.90y	478.06y	467.42y	0.99
	56 days	C	126,104B	543A	1.87A	648.23A	668.15A	0.99
		LR	170,062A	416B	1.43B	524.16B	531.50B	0.99
		LB	102,920C	358B	1.60B	492.72B	556.97B	0.99
	56 days+AS	C	70,340Y	842X	4.17X	930.66X	916.58X	0.99
		LR	205,630X	537Y	2.07Y	654.92Y	686.64Y	0.99
		LB	158,723X	511Y	2.02Y	655.97Y	669.16Y	0.99

C, control group; LR, *Lactobacillus rhamnosus* treatment group; LB, *Lactobacillus buchneri* treatment group; OTU, operational taxonomic unit; AS, aerobic stability; FM, fresh matter; and ACE, abundance-based coverage estimator.

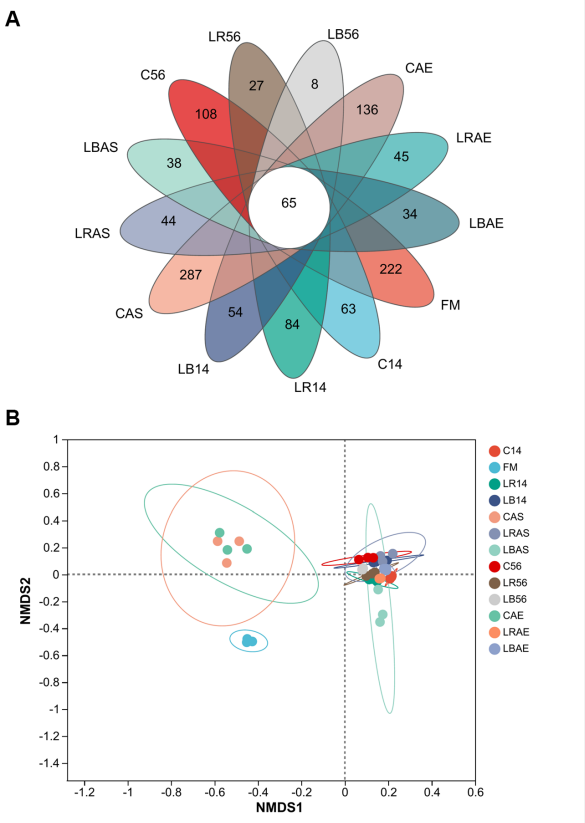

FIGURE 1
Venn diagram **(A)** and nonmetric multidimensional scaling **(B)** of the bacterial community at the operational taxonomic unit level. FM, fresh material; C, control group; LR, *Lactobacillus rhamnosus* treatment group; and LB, *Lactobacillus buchneri* treatment group. Numbers after C, LR, and LB denote storage periods. AS and AE indicates aerobic stability test parameters.

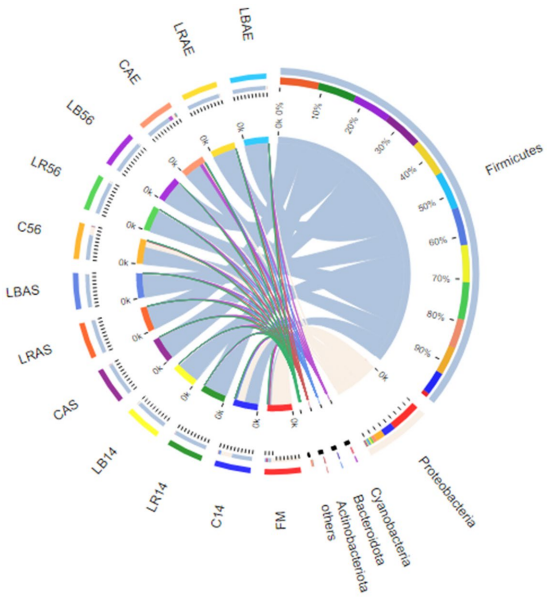

FIGURE 2
Bacterial community at the phylum level in silage with/without *Lactobacillus rhamnosus* and *Lactobacillus buchneri* inoculation. FM, fresh material; C, control group; LR, *Lactobacillus rhamnosus* treatment group; and LB, *Lactobacillus buchneri* treatment group. Numbers after C, LR, and LB denote storage periods. AS and AE indicates aerobic stability test parameters.

>83.3% of the analyzed sequences. *Pseudomonadaceae*, *Erwiniaceae*, and *Leuconostocaceae* were the predominant families and could be detected within pre-ensiling crops, including alfalfa, whole-crop corn, and *Leymus chinensis* silage (Figure 3A) (Ni et al., 2017; Wu et al., 2022). Though some microorganisms died during the fermentation of *C. korshinskii* silage with/without *L. rhamnosus* and *L. buchneri* inoculation, *Lactobacillacae* and *Leuconostocaceae* became the predominant families, regardless of short or long storage period. Li P. et al. (2022) and Li X. et al. (2022) found that the total proportion of *Lactobacillacae* and *Leuconostocaceae* reached 65% in the 30- and 60-day silos (Li P. et al., 2022). *Lactobacillacae* proportion was lower within the uninoculated silage compared with that in the

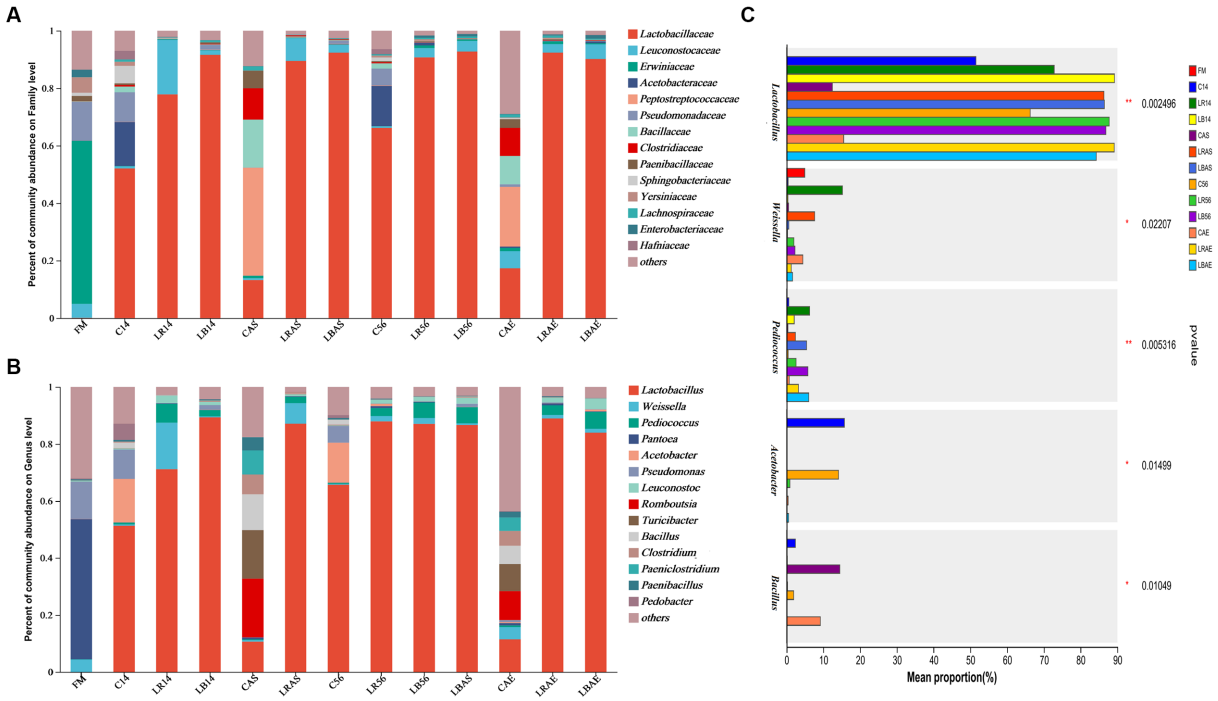

FIGURE 3
Bacterial community at the family level **(A)**, genus level **(B)**, and error bar plot **(C)** in silage with/without *Lactobacillus rhamnosus* and *Lactobacillus buchneri* inoculation. FM, fresh material; C, control group; LR, *Lactobacillus rhamnosus* treatment group; and LB, *Lactobacillus buchneri* treatment group. Numbers after C, LR, and LB denote storage periods. AS and AE indicates aerobic stability test parameters.

L. rhamnosus- and *L. buchneri*-inoculated silage after 14 and 56 days of ensiling. *L. plantarum*-inoculated oat silage has shown similar results (Li X. et al., 2022). However, after the 7 days of spoilage test, the microbiome structure of the LAB-treated silage showed considerable differences compared with that of the untreated silage. The control silage exhibited an increased abundance of *Peptostreptococcaceae*, *Clostridiaceae*, and *Bacillaceae*, whereas a decrease in *Lactobacillaceae* abundance compared with the *L. rhamnosus*- and *L. buchneri*-inoculated silage during the 7 days of spoilage test following 14 and 56 days of ensiling.

Figure 3B illustrates bacterial communities in the fresh material and silage at a genus level. The main genera in the fresh material included *Pseudomonas*, *Pantoea*, and *Weissella*. Our previous study showed similar results that *Pantoea* and *Pseudomonas* were the predominant genera in the pre-ensiling crop in *L. chinensis* (Wu et al., 2022). Although they are different raw materials, sometimes the microbes attached to the surface of raw materials are similar in Inner Mongolian Plateau. *Lactobacillus* had the highest abundance within the 14- and 56-day silage, which was consistent with the findings of Wang et al. (2020). After 14 and 56 days of ensiling, the control silage exhibited an increased *Acetobacter* and *Bacillus* abundance, whereas a decreased *Lactobacillus* abundance compared with that in the *L. rhamnosus*- and *L. buchneri*-treated silage (Figure 3C). *Acetobacter* species can induce aerobic deterioration of silage, which has been detected within the 14-, 56-, and 120-day whole-crop corn silage (Li and Nishino, 2011b). However, after the aerobic stability test of the 14- and 56-day silage, the control silage exhibited an increased *Clostridium* and *Bacillus* abundance, whereas a decreased *Lactobacillus* abundance compared with that in the *L. rhamnosus*- and *L. buchneri*-treated silage. Before aerobic deterioration, *Bacillus* count in the air-exposed silage increases (Okatsu et al., 2019). Liu et al. (2013) discovered that *Serratia nematodiphila*, *Lysinibacillus fusiformis*, *Myroides odoratimimus*, *Stenotrophomonas maltophilia*, *Acinetobacter soli*, and *Bacillus pumilus* present in the 65-day ensiled corn stalk silage, and the aerobic spoilage caused by them during the 7 days of aerobic exposure. *Clostridium* species are obligate anaerobic bacteria, and their presence in the whole-crop maize silage has been related to the anaerobic stability during the ensiling phase but not to aerobic spoilage in the feed-out phase (Driehuis and Oude Elferink, 2000). Jonsson (1991) reported that in the *Clostridium tyrobutyricum*-inoculated silage, the *Clostridium* count increased from 10^5 cfu/g at the silo opening to 10^7 cfu/g after the aerobic deterioration. Additionally, Vissers et al. (2007) reported that the *Clostridium* count in the grass and whole-crop corn silages must be above 10^5 cfu/g, especially on the surface of bunker silos and in the top of tower silos. Therefore, we speculated that deterioration of silage in control group may be related to the existence of *Clostridium* species.

Relation between the bacterial community and pH and fermentation products

Natural silage fermentation and aerobic exposure of silage are mostly dependent on the complex processes that involve interactions of main bacteria with various fermentation products. Pahlow et al. (2003) reported that LAB produced acetic acid, lactic acid, carbon dioxide, and ethanol by fermenting sugar. *Clostridium butyricum* and

Clostridium tyrobutyricum ferment WSCs and some sugars into acetic acid, butyric acid, and carbon dioxide, whereas *Bacillus* species ferment organic acids into water and carbon dioxide during aerobic exposure to silage (Muck, 2010). After 14- and 56-day ensiling, Spearman's correlations between the bacterial community and pH and fermentation products were predicted (Figure 4). According to Spearman's correlation results, *Lactobacillus*, *Pedicoccus*, and *Methylobacterium* genera were positively related ($p < 0.01$) to the lactic acid and acetic acid levels. However, *Weissella* and *Lactobacillus* richness was negatively correlated ($p < 0.01$) with the pH and the ethanol, 2,3-butanediol, and butyric acid levels. *Clostridium*, *Paenibacillus*, *Romboutsia*, *Bacillus*, and *Paeniclostridium* genera were negatively correlated ($p < 0.01$) with the 1,2-propanediol and lactic acid levels, whereas positively correlated to pH ($p < 0.01$), with the correlation values being 0.46, 0.40, 0.57, 0.65, and 0.73, respectively. The butyric acid content exhibited a positive relation ($p < 0.01$) to *Clostridium* (R = 0.48). These findings contradict a previous study (Wu et al., 2022) because different silages have different microbial compositions and produce different metabolites during the fermentation process and aerobic exposure.

KEGG functional annotation of bacterial communities

The KEGG function profiles on the first and second pathway levels are illustrated in Figures 5A,B. The predicted functions under the first pathway level in all silages were mainly related to metabolism. Following the 7 days of aerobic exposure, the untreated silage showed a lower abundance regarding metabolism compared with the *L. rhamnosus*- and *L. buchneri*-treated silage. The functional profiles at the second pathway level exhibited a primary association with the amino acid and carbohydrate metabolisms, followed by energy, cofactors, vitamins, and nucleotide metabolisms, and glycan biosynthesis and metabolism, which was similar to the findings of Zhao et al. (2022). The relative abundance of the common metabolism differed in all silage samples. *L. buchneri*-treated silage exhibited a lower abundance of amino acid and carbohydrate metabolisms compared with that in the control silage after 14 and 56 days of ensiling, whereas after the aerobic stability test, the control silage had a lower abundance of amino acid and carbohydrate metabolisms compared with that in the LAB-treated silage. Ensiling primarily ferments WSCs within the silage into organic acids, including acetic acid, butyric or lactic acid, *via* LAB in an anaerobic environment, which explains the cause of increased carbohydrate metabolism during the ensiling process. Amino acids are essential substances in crops and have a critical effect on increasing protein synthesis and primary metabolism, which accounts for the increased amino acid metabolism level within the control silage, whereas inhibition of amino acid metabolism within *L. buchneri*-inoculated silage may be due to a decrease in the pH value after 14 and 56 days of ensiling.

Figure 6 illustrates the functional profiles at the third pathway level. After 7 days of aerobic exposure, the carbohydrate metabolism, including glycolysis/gluconeogenesis, starch and sucrose metabolism, amino sugar and nucleotide sugar metabolism, pyruvate metabolism, fructose, and mannose metabolism, pentose phosphate pathway, propanoate metabolism, galactose metabolism, glyoxylate and dicarboxylate metabolism, butanoate metabolism, pentose and glucuronate interconversions, citrate cycle; and amino acid metabolism, including alanine, aspartate, and glutamate metabolism, glycine, serine, and threonine metabolism, cysteine and methionine metabolism, lysine biosynthesis, and tyrosine metabolism in the control silage decreased compared with those in the LAB-inoculated silage. Most amino acid and carbohydrate metabolisms in the control silage were considerably inhibited after 7 days of aerobic exposure, which was consistent with the aerobic spoilage findings in the control silage. This was probably because of the rapid increase in pH values after 7 days of air exposure, which restricted LAB-induced carbohydrate and protein catabolism (Mcdonald et al., 1991).

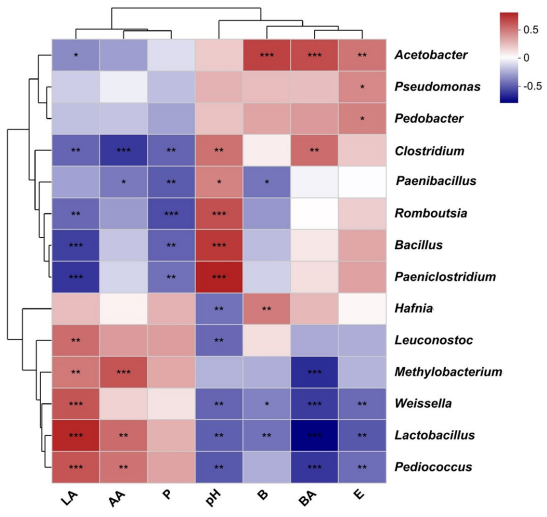

FIGURE 4
Correlation between the bacterial community and pH and fermentation products. Heatmap analysis was completed with Spearman's rho and *p*-values. *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; and ***, $p < 0.001$. BA, butyric acid; AA, acetic acid; LA lactic acid; E, ethanol; B, 2,3-butanediol; and P, 1,2-propanediol.

Conclusion

After 14 and 56 days of ensiling, *L. rhamnosus*- and *L. buchneri*-inoculated silage exhibited increased lactic and acetic acid contents, whereas a decrease in the pH and 2,3-butanediol and butyric acid contents compared with those in the control silage. Heating could be observed on day 2 of the aerobic stability test in 14- and 56-day uninoculated silages; however, heating was not observed during the 7 days of air exposure of *L. rhamnosus* and *L. buchneri*-treated silages. The control silage presented with increased *Clostridium* and *Bacillus* abundance, whereas a decreased *Lactobacillus* abundance compared with that in *L. rhamnosus*- and *L. buchneri*-treated silages subjected to 7 days of air exposure. The fermentation parameters were associated with the microbial communities, especially for *Lactobacillus*, *Pedicoccus*, *Weissella*, *Clostridium*, and *Bacillus*. Following 7 days of aerobic exposure, carbohydrate and amino acid metabolisms decreased within the control silage compared with that in the LAB-inoculated silage. In conclusion, *L. rhamnosus* or *L. buchneri* inoculation improved the fermentation quality, aerobic stability, and microbiome structure and function of the *C. korshinskii* silage.

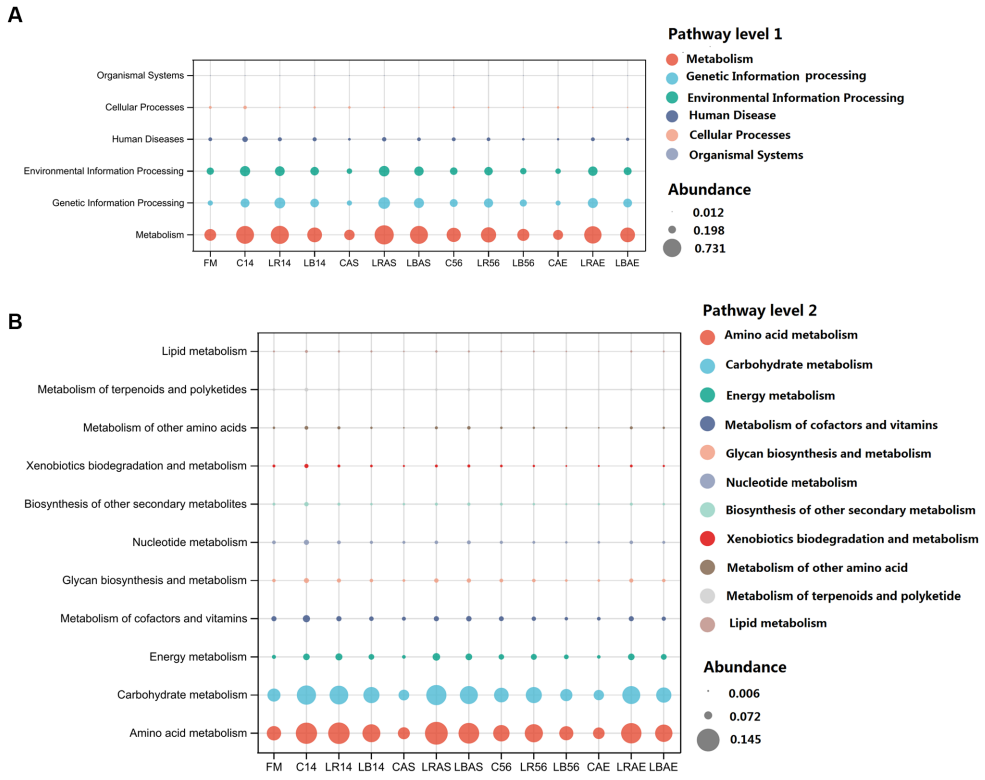

FIGURE 5
16S ribosomal RNA gene estimated Kyoto encyclopedia of genes and genomes functional profiles at first **(A)** and second **(B)** pathway levels. FM, fresh material; C, control group; LR, *Lactobacillus rhamnosus* treatment group; and LB, *Lactobacillus buchneri* treatment group. Numbers after C, LR, and LB denote storage periods. AS and AE indicates aerobic stability test parameters.

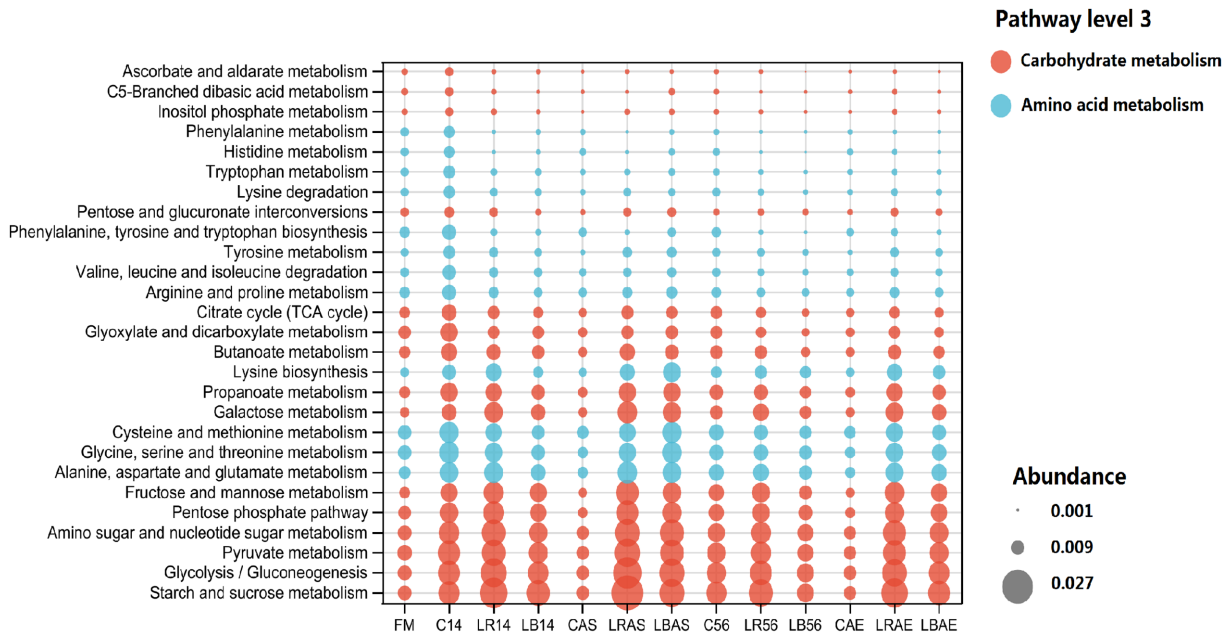

FIGURE 6
16S ribosomal RNA gene estimated Kyoto encyclopedia of genes and genomes functional profiles at the third pathway level. FM, fresh material; C, control group; LR, *Lactobacillus rhamnosus* treatment group; and LB, *Lactobacillus buchneri* treatment group. Numbers after C, LR, and LB denote storage periods. AS and AE indicates aerobic stability test parameters.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA990987.

Author contributions

BW: Conceptualization, Writing – original draft, Funding acquisition, Supervision, Writing – review & editing. JA: Data curation, Formal analysis, Writing – original draft. TL: Conceptualization, Data curation, Writing – original draft. WQ: Conceptualization, Data curation, Writing – original draft. ZH: Data curation, Formal analysis, Writing – original draft. TS: Conceptualization, Data curation, Writing – original draft. TW: Conceptualization, Data curation, Writing – original draft. CW: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. HN: Funding acquisition, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by the Program for Young Talents of Science and Technology of the Universities of Inner Mongolia Autonomous Region [grant number NJYT22054]; Scientific and Technological Innovation Guidance Project of Inner Mongolia [grant number KCJL-202211]; Scientific Research Project of Inner Mongolia University Minzu University [grant number NMDYB20031]; Doctoral Funding of the Inner Mongolia Minzu University [grant number BS610]; Natural Science Foundation of Inner Mongolian [grant number 2022QN03027, 2020BS03025, 2021BS03016].

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

AOAC. (1990). *Methods of analysis*. Arlington, TX: Association of Official Analytical Chemists.

Aßhauer, K. P., Bernd, W., Rolf, D., and Peter, M. (2015). Tax4fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31, 2882–2884. doi: 10.1093/bioinformatics/btv287

Bai, B., Qiu, R., Wang, Z., Liu, Y., Bao, J., Sun, L., et al. (2023). Effects of Cellulase and lactic acid Bacteria on ensiling performance and bacterial community of *Caragana korshinskii* silage. *Microorganisms* 11:337. doi: 10.3390/microorganisms11020337

Cai, Y., Benno, Y., Ogawa, M., Ohmomo, S., and Nakase, T. (1998). Influence of *Lactobacillus* spp. from an inoculant and of *Weissella* and *Leuconostoc* spp. from forage crops on silage fermentation. *Appl. Environ. Microbiol.* 64, 2982–2987. doi: 10.1128/AEM.64.8.2982-2987.1998

Deng, L., Han, Q. S., Zhang, C., Tang, Z. S., and Shangguan, Z. P. (2017). Above-ground and below-ground ecosystem biomass accumulation and carbon sequestration with *Caragana korshinskii* Kom plantation development. *Land Degrad. Dev.* 28, 906–917. doi: 10.1002/ldr.2642

Driehuis, F., and Oude Elferink, S. J. W. H. (2000). The impact of the quality of silage on animal health and food safety: a review. *Vet. Q.* 22, 212–216. doi: 10.1080/01652176.2000.9695061

Du, S., You, S., Jiang, X., Li, Y., and Jia, Y. (2022). Dynamics of the fermentation quality and microbiotsa in *Ephedra sinica* treated native grass silage. *J. Appl. Microbiol.* 133, 3465–3475. doi: 10.1111/jam.15779

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998. doi: 10.1038/nmeth.2604

Guo, X. S., Ke, W. C., Ding, W. R., Ding, L. M., Xu, D. M., Wang, W. W., et al. (2018). Profiling of metabolome and bacterial community dynamics in ensiled *Medicago sativa* inoculated without or with *Lactobacillus plantarum* or *Lactobacillus buchneri*. *Sci. Rep.* 8:357. doi: 10.1038/s41598-017-18348-0

Jonsson, A. (1991). Growth of *Clostridium tyrobutyricum* during fermentation and aerobic deterioration of grass silage. *J. Sci. Food Agric.* 54, 557–568. doi: 10.1002/jsfa.2740540407

Li, X., Chen, F., Xiong, Y., Guo, L., Xu, J., Lin, Y., et al. (2022). *Perilla frutescens* as potential antimicrobial modifier to against forage oat silage spoilage. *Front. Microbiol.* 13:1053933. doi: 10.3389/fmicb.2022.1053933

Li, Y., and Nishino, N. (2011a). Bacterial and fungal communities of wilted Italian ryegrass silage inoculated with and without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. *Lett. Appl. Microbiol.* 52, 314–321. doi: 10.1111/j.1472-765X.2010.03000.x

Li, Y., and Nishino, N. (2011b). Effects of inoculation of *Lactobacillus rhamnosus* and *Lactobacillus buchneri* on fermentation, aerobic stability and microbial communities in whole crop corn silage. *Grassl. Sci.* 57, 184–191. doi: 10.1111/j.1744-697X.2011.00226.x

Li, W., Zhang, S., Zhang, T., Shen, Y., Han, L., Peng, Z., et al. (2021). Bacterial cellulose production from ethylenediamine pretreated *Caragana korshinskii* Kom. *Ind. Crop. Prod.* 164:113340. doi: 10.1016/j.indcrop.2021.113340

Li, P., Zhao, W., Yan, L., Chen, L., Chen, Y., Gou, W., et al. (2022). Inclusion of abandoned rhubarb stalk enhanced anaerobic fermentation of alfalfa on the Qinghai Tibetan plateau. *Bioresour. Technol.* 347:126347. doi: 10.1016/j.biortech.2021.126347

Liu, L., Guo, Y., Liu, X., Yao, Y., and Qi, W. (2023). Relationship between the roots of *Hippophae rhamnoides* at different stump heights and the root microenvironment in feldspathic sandstone areas. *PeerJ* 11:e14819. doi: 10.7717/peerj.14819

Liu, Q. H., Shao, T., and Zhang, J. G. (2013). Determination of aerobic deterioration of corn stalk silage caused by aerobic bacteria. *Anim. Feed Sci. Technol.* 183, 124–131. doi: 10.1016/j.anifeedsci.2013.05.012

Mcdonald, P., Henderson, A. R., and Heron, S. J. E. (1991). *The biochemistry of silage*, *2nd*. Lincon: Chalcombe Publications.

Muck, R. E. (2010). Silage microbiology and its control through additives. *Rev. Bras. Zootec.* 39, 183–191. doi: 10.1590/S1516-35982010001300021

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., and Kung, L. Jr. (2018). Silage review: recent advances and future uses of silage additives. *J. Dairy Sci.* 101, 3980–4000. doi: 10.3168/jds.2017-13839

Ni, K., Minh, T. T., Tu, T. T., Tsuruta, T., Pang, H., and Nishino, N. (2017). Comparative microbiota assessment of wilted Italian ryegrass, whole crop corn, and wilted alfalfa silage using denaturing gradient gel electrophoresis and next-generation sequencing. *Appl. Microbiol. Biotechnol.* 101, 1385–1394. doi: 10.1007/s00253-016-7900-2

Okatsu, Y., Swanepoel, N., Maga, E. A., and Robinson, P. H. (2019). Impacts of some factors that effect spoilage of silage at the periphery of the exposed face of corn silage piles. *Anim. Feed Sci. Technol.* 247, 234–247. doi: 10.1016/j.anifeedsci2018.11.018

Pahlow, G., Muck, R. E., Driehuis, F., Elferink, S. J. W. H. O., and Spoelstra, S. F. (2003) in *Microbiology of ensiling*. eds. D. R. Buxton, R. E. Muck and R. E. Harrison, Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am (Madison, WI: Silage Science and Technology), 31–93.

Pang, H., Qin, G., Tan, Z., Li, Z., Wang, Y., and Cai, Y. (2011). Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S

ribosomal RNA and recA gene analysis. *Syst. Appl. Microbiol.* 34, 235–241. doi: 10.1016/j.syapm.2010.10.003

Stevenson, D. M., Muck, R. E., Shinners, K. J., and Weimer, P. J. (2006). Use of real time PCR to determine population profiles of individual species of lactic acid bacteria in alfalfa silage and stored corn Stover. *Appl. Microbiol. Biotechnol.* 71, 329–338. doi: 10.1007/s00253-005-0170-z

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597. doi: 10.3168/jds.S0022–0302(91)78551–2

Vissers, M. M. M., Driehuis, F., Giffel, M. C. T., Jong, P. D., and Lankveld, J. M. G. (2007). Concentrations of butyric acid Bacteria spores in silage and relationships with aerobic deterioration. *J. Dairy Sci.* 90, 928–936. doi: 10.3168/jds.S0022-0302(07)71576-X

Wang, T., Teng, K., Cao, Y., Shi, W., Xuan, Z., Zhou, J., et al. (2020). Effects of *Lactobacillus hilgardii* 60TS-2, with or without homofermentative *Lactobacillus plantarum* B90, on the aerobic stability, fermentation quality and microbial community dynamics in sugarcane top silage. *Bioresour. Technol.* 312:123600. doi: 10.1016/j.biortech.2020.123600

Wilkinson, J. M., and Davies, D. R. (2013). The aerobic stability of silage: key findings and recent developments. *Grass Forage Sci.* 68, 1–19. doi: 10.1111/j.1365-2494.2012.00891.x

Wu, B., Hu, Z., Wei, M., Yong, M., and Niu, H. (2022). Effects of inoculation of *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* on fermentation quality, aerobic stability, and microbial community dynamics of wilted *Leymus* chinensis silage. *Front. Microbiol.* 13:928731. doi: 10.3389/fmicb.2022.928731

Wu, B., and Nishino, N. (2016). Identification and isolation of *Lactobacillus fructivorans* from wilted alfalfa silage with and without molasses. *J. Appl. Microbiol.* 120, 543–551. doi: 10.1111/jam.13031

Wu, B., Zhang, Q., Liu, Z., Yu, Z., and Nishino, N. (2014). Bacterial communities in alfalfa and corn silages produced in large-scale stack and bunker silos in China. *Grassl. Sci.* 60, 247–251. doi: 10.1111/grs.12063

Yang, L., Yuan, X., Li, J., Dong, Z., and Shao, T. (2019). Dynamics of microbial community and fermentation quality during ensiling of sterile and nonsterile alfalfa with or without *Lactobacillus plantarum* inoculant. *Bioresour. Technol.* 275, 280–287. doi: 10.1016/j.biortech.2018.12.067

You, J., Zhang, H., Zhu, H., Xue, Y., Cai, Y., and Zhang, G. (2022). Microbial community, fermentation quality, and in vitro degradability of ensiling Caragana with lactic acid Bacteria and Rice bran. *Front. Microbiol.* 13:804429. doi: 10.3389/fmicb.2022.804429

Zhang, Y., Wang, M., Usman, S., Li, F., Bai, J., Zhang, J., et al. (2023). Lignocellulose conversion of ensiled *Caragana korshinskii* Kom. Facilitated by *Pediococcus acidilactici* and cellulases. *Microb. Biotechnol.* 16, 432–447. doi: 10.1111/1751-7915.14130

Zhang, Q., Wu, B., Nishino, N., Wang, X., and Yu, Z. (2016). Fermentation and microbial population dynamics during the ensiling of native grass and subsequent exposure to air. *Anim. Sci. J.* 87, 389–397. doi: 10.1111/asj.12427

Zhao, R., Lu, Y., Ran, J., Li, G., Lei, S., Zhu, Y., et al. (2020). Purification and characterization of bacteriocin produced by *Lactobacillus rhamnosus* zrx01. *Food Biosci.* 38:100754. doi: 10.1016/j.fbio.2020.100754

Zhao, J., Yin, X. J., Wang, S. R., Li, J. F., Dong, Z. H., and Shao, T. (2022). Changes in the fermentation products, taxonomic and functional profiles of microbiota during high-moisture sweet sorghum silage fermentation. *Front. Microbiol.* 13:967624. doi: 10.3389/fmicb.2022.967624

Zhong, C., Sun, Z., Zhou, Z., Jin, M. J., Tan, Z. L., and Jia, S. R. (2014). Chemical characterization and nutritional analysis of protein isolates from *Caragana korshinskii* Kom. *J. Agric. Food Chem.* 62, 3217–3222. doi: 10.1021/jf500349s

Zou, X., Zhou, W., Wu, S., Chen, X., and Zhang, Q. (2023). Dynamic changes in the bacterial communities and metabolites of *Moringa oleifera* leaves during fermentation with or without pyroligneous acid. *LWT* 177:114593. doi: 10.1016/j.lwt.2023.114593