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Effects of Two Bacterial Exopolysaccharides on Microbial Community, Fermentation Characteristics and Aerobic Stability in Oat Silage

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ABSTRACT

This study investigated whether two exopolysaccharides could serve as exogenous carbon sources to enhance fermentation quality in oat silage, providing a theoretical foundation for their future application in silage. The oats were harvested at the heading stage and, following a period of wilting, were chopped into 2–3 cm lengths for the ensiling experiment. The treatments applied were as follows: (1) a control group (CK), which received only sterile water; (2) a group with added dextran (D); and (3) a group with added levan (L). The fermentation process was monitored at various intervals: 3, 7, 14, 30 and 60 days (d), respectively. Following 60 days of ensiling, the silage was subjected to a 5-day period of aerobic exposure (AE). EPS changed the fermentation quality of silage, altered the composition of the bacterial community, and had an impact on the feature dissimilarity between sample groups. Meanwhile, EPS showed different regulatory effects on carbohydrate metabolism at different fermentation times. EPS treatment increased the lactic acid content and decreased the pH of silage. After 60 days of fermentation, the treatment also increased the relative abundance of *Lactobacillus*. Dextran and levan increased the relative abundance of *Hafnia–Obesumbacterium* and *Sediminibacterium*, respectively. Under the treatment of dextran, silage retained more WSC content and achieved higher aerobic stability. Upon comparing the bacterial correlation networks, it became evident that the fermentation time altered the composition of inter-bacterial correlations. In conclusion, EPS can effectively enhance the fermentation quality of oat silage, with dextran yielding the most pronounced positive effects.

1 | Introduction

By 2050, the global population is expected to exceed 9 billion, with an increase of nearly 2 billion over the next 25 years (Smith et al. 2024). This population growth means that global food, feed, energy and resources will face unprecedented challenges. Although many problems have been alleviated through technical innovation, serious challenges remain (Taylor et al. 2023). Forage crops are primarily grown as food for ruminants and play a crucial role in increasing the output value of livestock products (Fernandes et al. 2024). The storage of forage is one of the key aspects to ensure its quality. Silage is an effective measure for the long-term preservation of forage, which improves the acidity of the environment through

Abbreviations: AA, acetic acid; CP, crude protein; EPS, exopolysaccharides; LA, lactic acid; LAB, lactic acid bacteria; NDF, neutral detergent fibre; NH^{4+}-N, ammonia nitrogen; SCPs, single-cell proteins; WSC, water-soluble carbohydrates.

microbial fermentation to achieve long-term preservation (Wu et al. 2020). Silage can improve the palatability and dry matter (DM) digestibility of feed, and also reduce methane emissions from ruminants (Bica et al. 2022).

Oat (*Avena sativa* L.) is an important economically significant Poaceae plant that can be used for both food and feed purposes. Oat is a global crop whose production can rank seventh in the world compared with other cereals (Kamal et al. 2022). Oat forage can generally be made into hay or silage for feed Unlike hay, silage involves a complex fermentation process that necessitates multiple factors to reach completion. During ensiling, there is a loss of approximately 70 g/kg of water-soluble carbohydrates (WSC) compared to hay forage (Klevenhusen and Zebeli 2021). The varying cultivation environments and harvest periods of oats significantly impact the process, often limiting the efficacy of silage fermentation. LAB, metabolizable sugars, temperature and other factors are crucial for successful silage fermentation. Consequently, the use of additives becomes an important strategy to facilitate and enhance the fermentation process. Currently, many additives have been used in oat silage, including sodium benzoate, propionic acid, glyphosate, cellulase, xylanase and *plant lactobacillus* (Bueno et al. 2020; Jia et al. 2021; Xiong et al. 2022; Liu et al. 2023), all of which have improved the fermentation quality of oat silage.

EPS belongs to a type of macromolecule polymer, synthesised through microbial metabolism, which is a recognised as a safe ingredient, making it an acceptable additive (Zhao et al. 2018). Recently, EPS has garnered widespread attention owing to its unique biological properties and functions. Consequently, it has been utilised across various sectors, including the food industry, medical applications and the chemical industry, among others (Sun and Zhang 2021). In addition, EPS has prebiotic properties and can stay in the gastrointestinal tract to support the growth of *Bifidobacterium* and *Lactobacilli*, so it is greatly useful to combine EPS with fermented foods (Patel et al. 2011). EPS has the potential to support the growth of different LAB such as *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus rhamnosus* (İspirli et al. 2018). According to the monosaccharide composition of EPS, it can be divided into homopolysaccharides and heteropolysaccharides (Sun and Zhang 2021). Dextran is a type of homopolysaccharide, which is a (1 → 6)-α-D-glucan with side chains linked by α-(1 → 2)/α-(1 → 3)/α-(1 → 4) glycosidic bonds (Mohd Nadzir et al. 2021). Dextran is soluble in ethylene glycol, water and formamide. Based on their characteristics, dextran is usually used in food and biomedical industries (Ye et al. 2019). Levan is also a type of homopolysaccharide, composed of fructose units, which are connected by β-2, 6-glycosidic bonds in the main chain and β-2, 1 in the branches (Mohd Nadzir et al. 2021). In the field of biomedicine, levan has gained countless applications due to its antibacterial properties, biocompatibility and antioxidant activity (Domżał-Kędzia et al. 2019; Koşarsoy Ağçeli and Cihangir 2020). During the silage process, microorganisms mainly rely on glucose and 5-C carbohydrates (such as fructose) for fermentation (Papadimitriou et al. 2016). Numerous studies have focused on adding monosaccharides to silage; yet, the potential of EPS to promote silage fermentation remains unexplored. Consequently, this study selected two types of EPS—dextran and levan—to investigate whether EPS can support fermentation and enhance the quality of oat silage.

2 | Materials and Methods

2.1 | Raw Materials and EPS

This experiment was conducted on 20 June 2022, in ArKhorchin Banner (43°77′N, 120°78′E), Chifeng, China. Oat was harvested at the heading stage using a harvester, and we randomly selected 1 ha as the experimental area. Oats were wilted in the field for 4 h, after which we randomly collected sufficient experimental samples and brought them to the laboratory for subsequent operations. At this time, the dry matter (DM) content was 281.35 g/kg fresh wight (FW). A forage chopper (model: DD-zcd, Weifang DeDong E-Commerce Co. Ltd. Linyi, China) was used to cut oat materials to obtain a length of 2–3 cm of silage raw materials. Before ensiling, the chemical composition of oat was as follows: DM = 290.24 g/kg FW, NDF = 663.04 g/kg DM, ADF = 378.19 g/kg DM, CP = 123.47 g/kg DM, WSC = 62.13 g/kg DM. The lactic acid bacteria (LAB) count was 1.51 log CFU/g FW, and the yeast count was 2.01 log CFU/g FW. The dextran and levan were purchased from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China); both are bacterial extracellular polysaccharides and homopolysaccharides. Levan, isolated from *Erwinia herbicola*, is presented as a white powder with a purity of at least 98%. Similarly, dextran, derived from *Leuconostoc mesenteroides*, is also in the form of a white powder and has a purity of exactly 99%.

2.2 | Silage Preparation and Treatments

Before conducting the silage experiment, we disinfected the experimental environment with 75% ethanol to prevent impurities from affecting the fermentation of the silage. The main steps of oat ensiling are represented in Figure 1. Chopped oat raw materials are processed as follows:(1) control (CK, distributed water); (2) added dextran of silage (D); and (3) added levan of silage (L). Fermentation times were 3, 7, 14, 30 and 60 days (d), respectively. The concentrations of dextran and levan added in this study were both 100 mg/kg FW. The dextran and levan were dissolved in sterile water and thoroughly mixed in an ultrasonic machine separately; the final concentration was 25 mg/mL. A volume of 4 mL/kg FW was added for silage to ensure a final concentration of 100 mg/kg FW; the control was treated with sterile water of the same content. Before ensiling, oat raw materials were randomly selected for chemical composition and marker gene sequencing analysis (0 d). Then, the oat raw materials were divided into three parts according to calculations. The prepared additive solutions (dextran, levan and sterile water) were evenly sprayed on the surface of the oat raw materials and mixed thoroughly. Approximately 400 g of oat raw materials were packed into polyethylene bags (25 × 35 cm) and vacuum sealed using a vacuum sealing machine. Each group of treatments was repeated 4 times. All ensiling samples were stored at room temperature (25°C ± 2°C) in the dark. All samples were opened at corresponding times, and sufficient samples were selected randomly for chemical composition, fermentation quality and bacterial community analysis.

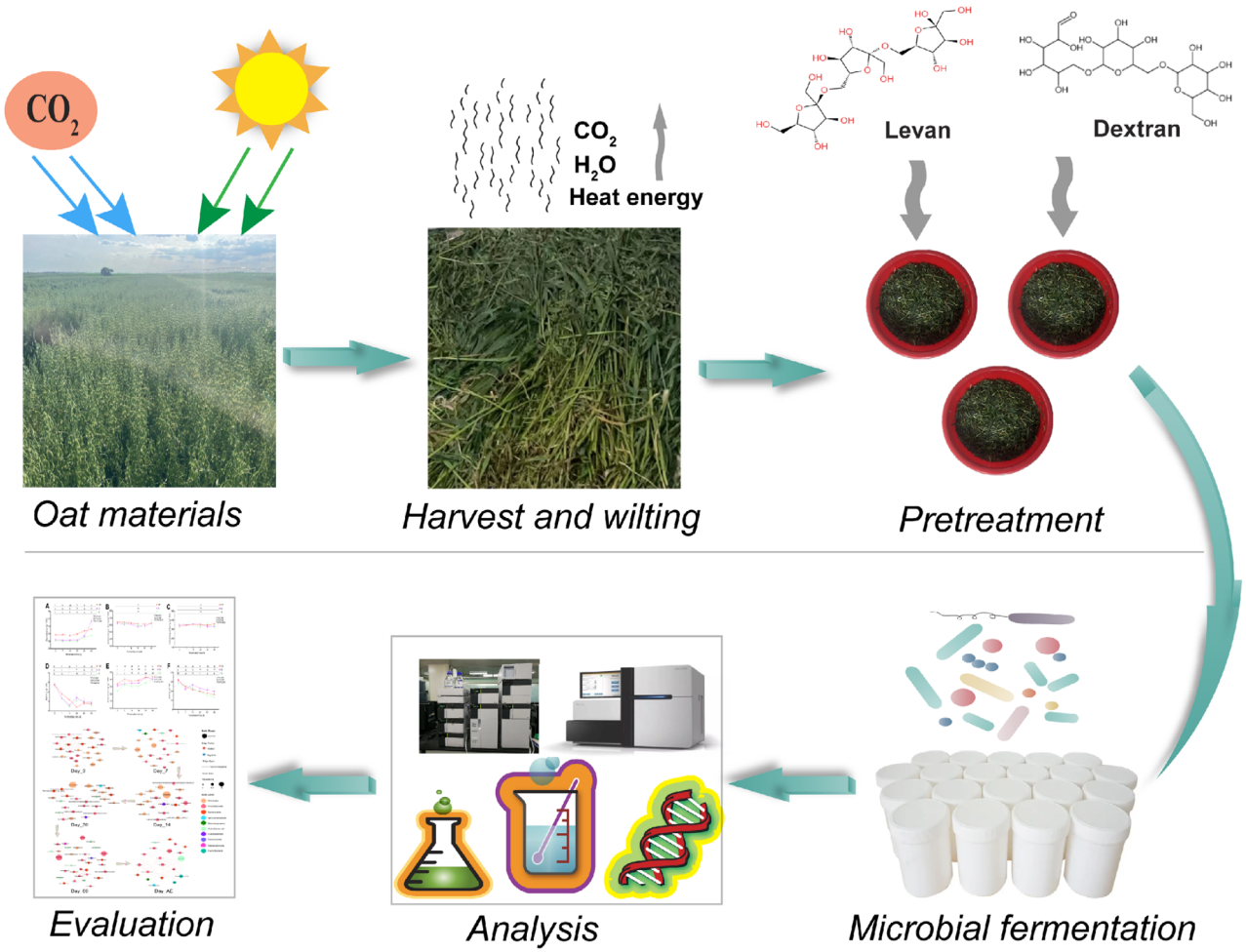

FIGURE 1 | Graphical abstract (major steps involved in oat ensiling).

2.3 | Chemical Composition, Fermentation Quality and Bacterial Community Analyses

To ascertain the DM content, subsamples of fresh material or silage were subjected to drying for 72 h at 65°C. The dry matter recovery (DMR) in the mixed silages was assessed by determining the variation in DM before and after the ensiling process. To conduct the next chemical composition analysis, 40 mesh samples were obtained by grinding the dry samples into small pieces using a cutting grinder (SM 200). The full-automatic Kjeltec (Model: 8400, FOSS, Hilleroed, Denmark) was used to measure the CP content of the samples; the neutral detergent fibre insoluble protein (NDFIP) was obtained by measuring the protein content in NDF (Li et al. 2018). The WSC content of samples was determined by the anthrone colorimetry method (Cai et al. 1998). The ANKOM fibre analyser (Model: A2000i, Beijing Anke Borui Technology Co. Ltd., Beijing, China) was used to determine the ADF and NDF contents of samples by the method of Van Soest et al. (1991). The 10 g of oat silage sample was mixed with 90 mL of sterile water in a sterile bag (25 × 30 cm), tapping for 2 min using a sterile homogeniser, then filtered through four layers of cheesecloth to obtain the extract of silage. The liquid extract pH was determined immediately by a pH meter (model: LEICI pH S-3 C, Shanghai Yitian Scientific Instrument Co. Ltd., Shanghai, China). The ammonia-N content was determined according to the method of Broderick and Kang (Reich and Kung 2010). The liquid extract of silage organic acid was determined by high-performance liquid chromatography (model: Waters E2695, Milford, MA, USA) under the settings of Liu et al. (Liu et al. 2023).

2.4 | Bacterial Community Diversity Analysis

After opening the bag, enough samples of oat silage were placed in a 50 mL sterilised centrifuge tube and frozen and sealed at −80°C. To obtain the biological information of oat silage, microbial DNA from all test samples was extracted using the E.Z.N.A. stool DNA Kit (Omega Biotek, Georgia, USA). The 16S rDNA V3–V4 region of the eukaryotic ribosomal RNA gene was amplified via PCR with the primers 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT), where each sample had a unique eight-base barcode. The PCR protocol involved an initial denaturation at 95°C for 2 min, followed by 27 cycles of 98°C for 10 s, 62°C for 30 s and 68°C for 30 s, with a final extension at 68°C for 10 min. The amplicons were retrieved from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, California, USA), following the manufacturer's guidelines. Their concentration was determined using QuantiFluor-ST (Promega, Wisconsin, USA). After purification, the amplicons were combined in equal proportions, and paired-end sequencing (2 × 250) was conducted on

an Illumina HiSeq 2500 platform (Illumina, California, USA) by Gene Denovo Biological Technology Co. Ltd. (Guangzhou, China) (Liu et al. 2023).

2.5 | Aerobic Stability

The aerobic stability was performed after 60 d of ensiling, and all samples were opened and loaded into a 1 L sterile plastic bottle. The multichannel temperature recorder (model: MDL-1048 A; Shanghai Tianhe Automation Instrument Co. Ltd., Shanghai, China) was used to monitor temperature variations at 10 min intervals. This method allowed for the determination of aerobic stability by analysing the differences in temperature changes over time. The time required for an environmental increase of 2°C is defined as the aerobic stability (Yin et al. 2023); after exposure to air for 5 d, each sample has been taken out for chemical and microbial analyses randomly.

2.6 | Statistical Analyses

Two-way ANOVA was performed using the Statistical Package for the Social Sciences (SPSS, Version 26.0) to analyse the chemical composition and fermentation quality data. Tukey's multiple comparisons were employed to assess significant differences between treatments within the same ensiling duration and between different fermentation durations under the same treatment. This analysis also examined the interactions between the two factors. Duncan's multiple comparison test was employed to identify significant differences between mean values, with statistical significance considered at $p < 0.05$. The results were presented in graphical form using GraphPad Prism 8. Bacterial sequencing data were analysed using the Omicromart platform (http://www.omicsmart.com, accessed on May 1, 2024), including species abundance composition, correlation networks and differential abundance analysis.

3 | Results

3.1 | The Effects of Fermentation Time and EPS on the Chemical Composition and Fermentation Quality of Oat Silage

The chemical composition of oat silage after being treated with fermentation times and EPS is shown in Figure 2. As fermentation time progressed, the DM content significantly increased in all treatment groups. EPS treatment notably reduced the DM content in the silage from day 3 to day 60 of fermentation. However, the DM content significantly increased in the dextran-treated group during the aerobic exposure (AE) period (Figure 2A). Compared to the other treatments, the dextran-treated group significantly reduced the NDF content in the silage from day 3 to day 14 of fermentation. In the EPS-treated groups, there were no significant changes in the NDF and ADF content as fermentation time progressed (Figure 2B,C). The NDFIP content in the silage initially declined and then increased, showing a fluctuating trend. Both the presence of EPS and the duration of fermentation play significant roles in influencing the NDFIP levels within oat silage (Figure 2D). Compared with fermentation for 3 d, the NDFIP content significantly decreased after 7 d ($p < 0.05$), but was not affected by EPS at this time. After 14 d of fermentation, both types of EPS significantly reduced the NDFIP content ($p < 0.05$) compared to the control. However, after 30 d of fermentation, both types of

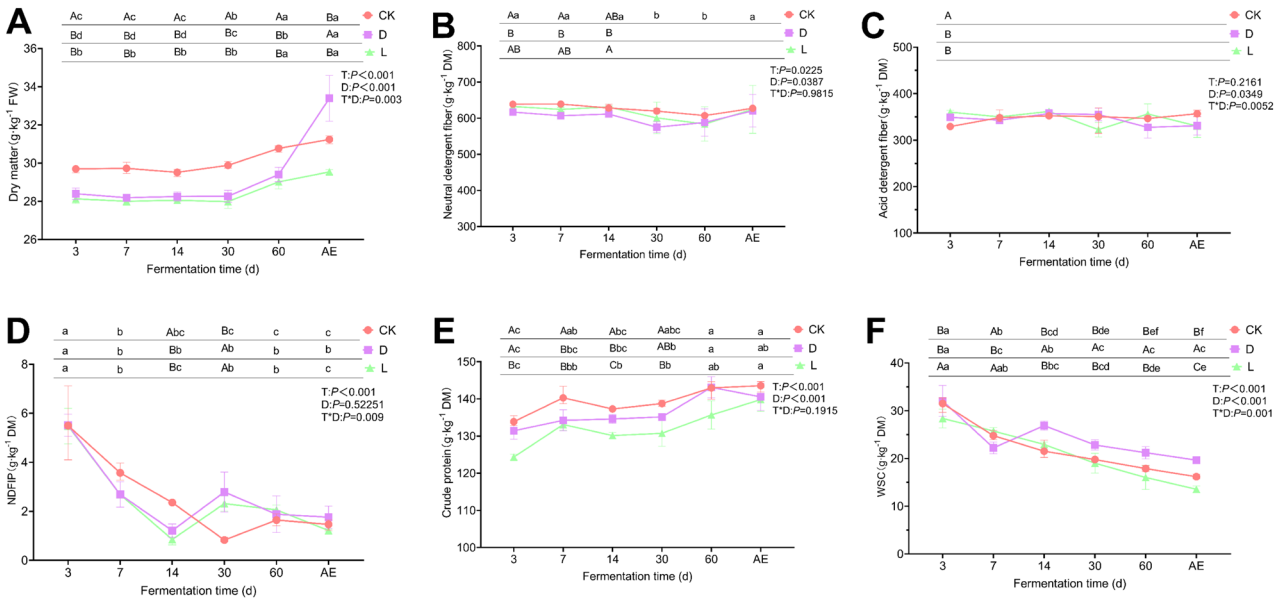

FIGURE 2 | Chemical composition of silage under the treatment of fermentation time and EPS. CK, distributed water; D, added dextran of silage; L, added levan of silage. Day 3, 7, 14, 30 and 60, respectively, represent fermentation of silage for 3, 7, 14, 30 and 60 days. AE represents the aerobic exposure stage of silage. NDF, neutral detergent fibre; ADF, acid detergent fibre; NDFIP, neutral detergent fibre insoluble protein; WSC, water-soluble carbohydrates. T, fermentation times; D, inoculated with EPS; T × D, the interaction between fermentation times and EPS. Values with different lowercase letters show significant differences among ensiling days in the same treatment; values with different capital letters show significant differences among treatments on the same ensiling day ($p < 0.05$).

EPS significantly increased the NDFIP content ($p < 0.05$). The EPS did not significantly affect the NDFIP content after 60 d of ensiling ($p > 0.05$). With the extension of fermentation time, the CP content of oat silage showed an upward trend (Figure 2E). After 60 d of fermentation, the CP content significantly increased compared to 3 d of fermentation ($p < 0.05$). The EPS treatment significantly influenced the CP content in the silage, resulting in a decrease in CP content across various fermentation durations. Fermentation time had a significant impact on the WSC content of oat silage (Figure 2F); as fermentation time extended, the WSC content continued to decrease significantly ($p < 0.05$). The application of dextran in the treatment effectively preserved a higher WSC content for oat fermentation ($p < 0.05$). After 14 d of fermentation, the WSC levels in the dextran-treated samples were significantly elevated compared to those in the control group ($p < 0.05$).

The fermentation quality dominated by fermentation time and EPS is shown in Figure 3. During the fermentation process, the pH levels exhibited a distinct pattern, initially rising before gradually decreasing. The duration of fermentation had a significant impact on the pH of the oat silage ($p < 0.05$). The EPS treatment notably decreased the pH of the silage following 14 d of fermentation (Figure 3A). After 60 d of fermentation, the treatment with levan resulted in the lowest pH of 4.45 in oat silage. Both the fermentation times and EPS had a significant impact on the lactic acid (LA) content in oat silage (Figure 3B). As the silage time extended, the LA content within the silage continued to rise. However, during the AE stage, there was a significant drop in LA content ($p < 0.05$). Compared to the control, EPS markedly elevated the LA content of oat silage after 14, 30 and 60 d of fermentation. The highest LA content, reaching 74.83 g/kg DM, was observed under levan treatment after 60 d of fermentation. The trend in acetic acid (AA) content following oat fermentation mirrored that of LA. Both fermentation time and EPS significantly influenced the AA content in oat silage ($p < 0.05$). The EPS treatment significantly increased the AA content after 7 d of fermentation ($p < 0.05$), while dextran significantly increased the AA content after 30 and 60 d of fermentation ($p < 0.05$).

Unlike LA, the content of AA continued to increase during the AE stage, with the highest content reaching 30.71 g/kg DM in the dextran-treated group (Figure 3C). Both fermentation time and EPS treatment significantly altered the ammonia nitrogen content in the silage (Figure 3D). The ammonia nitrogen content in the silage significantly increased with the prolongation of fermentation time. The EPS treatment reduced the ammonia nitrogen content in oat after 14 and 30 d of fermentation significantly ($p < 0.05$). During the AE stage, the treatment with dextran significantly increased the ammonia nitrogen content ($p < 0.05$), which was as high as 32.06 g/kg DM. The combination of different fermentation

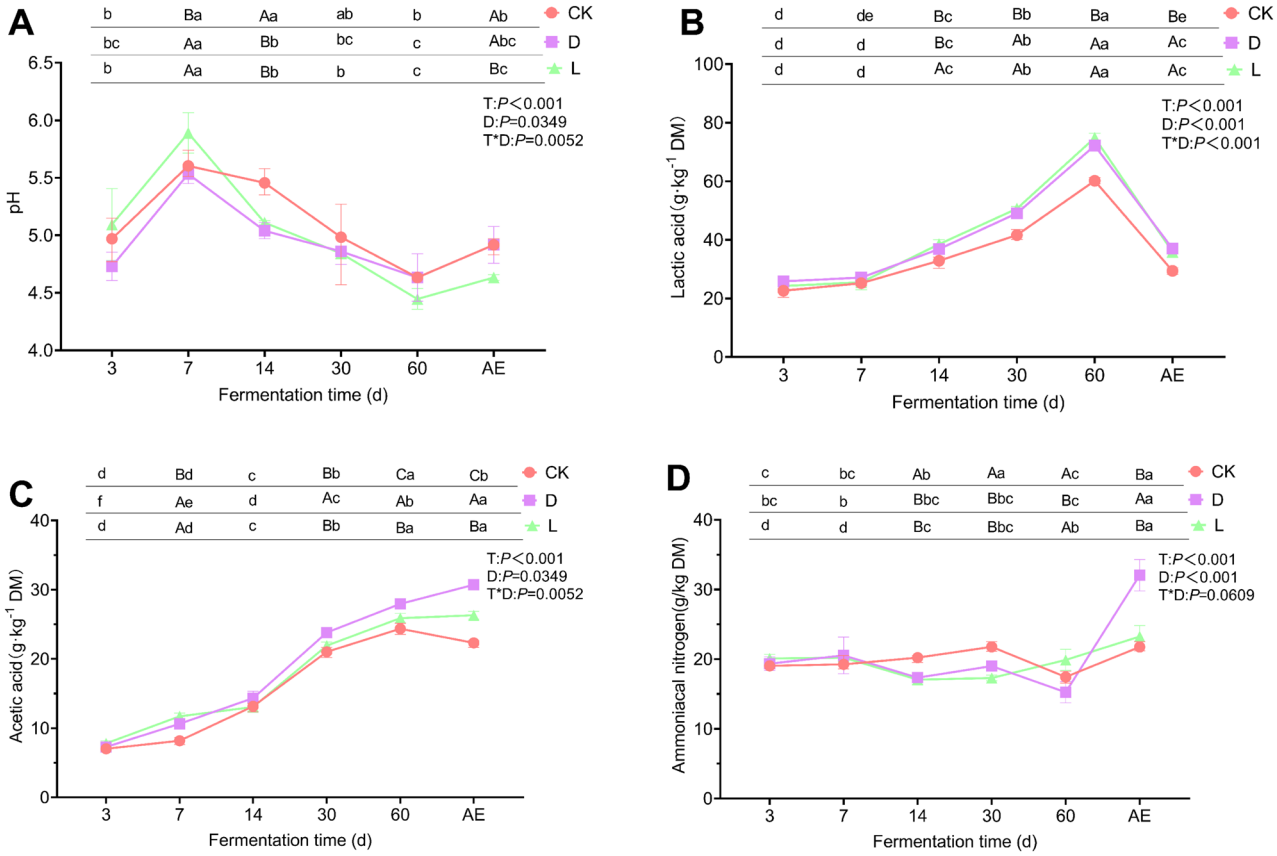

FIGURE 3 | The fermentation quality of oat silage under the treatment of fermentation time and EPS. CK, distributed water; D, added dextran of silage; L, added levan of silage. Day 3, 7, 14, 30 and 60, respectively, represent fermentation of silage for 3, 7, 14, 30 and 60 days. T, fermentation times; D, inoculated with EPS; T × D, the interaction between fermentation times and EPS. Values with different lowercase letters show significant differences among ensiling days in the same treatment; values with different capital letters show significant differences among treatments in the same ensiling day ($p < 0.05$).

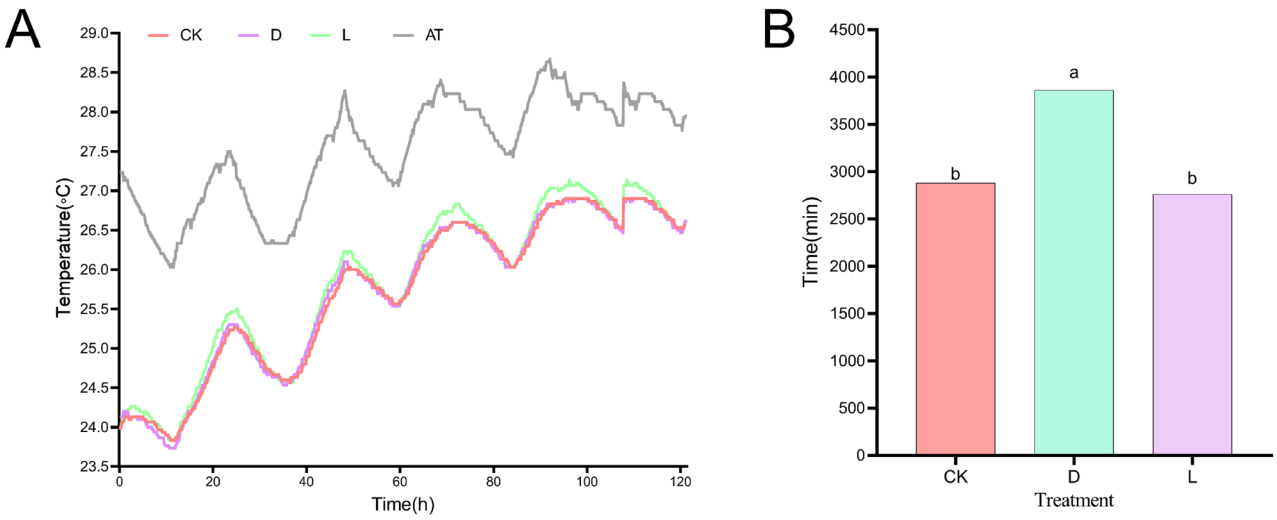

FIGURE 4 | Dynamics of temperature (°C) during aerobic exposure of ensiling (A) and the time when the temperature of silage is 2°C higher than the initial temperature (B). Means with different lowercase letters indicate significant differences in aerobic stability time ($p < 0.05$). CK, distributed water; D, added dextran of silage; L, added levan of silage.

times and EPS treatment significantly affected the pH, LA and AA content in the silage ($p < 0.05$). As the duration of AE increases, the internal temperature of the silage gradually rises. Compared to the control group, the temperature in the dextran-treated group takes longer to increase by 2°C (Figure 4A,B), resulting in a significant improvement in aerobic stability ($p < 0.05$).

3.2 | The Effect of Fermentation Time and EPS on the Composition of Oat Bacterial Community

Before fermentation, the bacteria relative abundance was mainly distributed in *Weissela*, *Leuconostoc* and *Enterooacter*; the abundance of oat bacteria was 52%, 18% and 13%, respectively (Figure 5A). Both EPS and fermentation duration influenced the relative abundance composition of bacterial communities in oats. In comparison to the control group, EPS diminished the relative abundance of *Weissela* after 3 and 7 d of fermentation, while enhancing the relative abundance of *Lactobacillus* after 30 and 60 d of fermentation (Figure 5A). It is noteworthy that dextran enhanced the relative abundance of *Leuconostoc* in oat at various fermentation times when compared to the control group. Moreover, during the AE stage, both dextran and levan treatments increased the *Lactobacillus* relative abundance, which was 24% and 8% higher than the control treatment, respectively. From the analysis of the river chart (Figure 5B–D), it is evident that as fermentation time extends, the relative abundance of *Weissela* gradually diminishes, whereas the relative abundance of *Lactobacillus* and *Pediococcus* progressively increases. The change in the *Enterooacter* relative abundance is not obvious due to the influence of EPS treatment.

With the advancement of various fermentation durations, the relative abundance of *Lactobacillus* in dextran and levan-treated groups displayed a gradual broadening on the river map. Conversely, the relative fractionation of *Weissella* and *Pediococcus* showed a progressive narrowing. The EPS modified the composition of the river map, with dextran and levan specifically enhancing the relative abundance of *Hafnia–Obesumbacterium* and *Sediminibacterium*, respectively. Statistical analysis revealed that different fermentation times significantly affected the relative abundance of *Weissella*, *Pediococcus*, *Lactobacillus* and *Enterococcus* in the silage ($p < 0.05$) (Table 1). Dextran treatment notably enhanced the relative abundance of *Lactobacillus* throughout the 30-day fermentation period and during the AE stage (Table 1). Simultaneously, dextran treatment significantly increased the relative abundance of *Leuconostoc* across different fermentation stages compared to other treatments ($p < 0.05$). However, EPS treatment did not alter the relative abundance of *Pediococcus* throughout the entire fermentation process significantly (Table 1). Concurrently, EPS treatment significantly reduced the relative abundance of *Lactococcus* during the 30, 60 d of fermentation, and the AE period ($p < 0.05$).

3.3 | The Effect of Different Fermentation Times and EPS on Bacterial Diversity in Silage

The results of the effects of fermentation time and EPS on alpha diversity are shown in Figure 6. Prior to fermentation, the Sob, Chao, Shannon and Simpson indices of oat were all at their lowest levels (Figure 6A–D). Conversely, with the extension of silage time, all four indices show an upward trend. EPS and fermentation time did not significantly alter the alpha diversity of oat after fermentation ($p > 0.05$). Irrespective of EPS addition, oat displayed elevated levels of Sob, Chao, Shannon and Simpson indices during the AE stage. Notably, the Chao index of untreated oat was significantly higher than that observed at other fermentation times. The trend of alpha diversity in oat treated with dextran was similar to that of the control group, while the levan-treated group generally reduced alpha diversity. The distribution of beta diversity distance calculated based on Bray-Curtis is shown in Figure 6E. Under the different treatment of fermentation times and EPS, beta diversity exhibits significant feature dissimilarity, with the impact of fermentation time being more pronounced. As the fermentation time changes, the feature dissimilarity of beta diversity mainly aggregates three parts: fermented for 3, 7 d; fermented for 14, 30 d; and fermented for 60 d, AE (Figure 6E). During 3

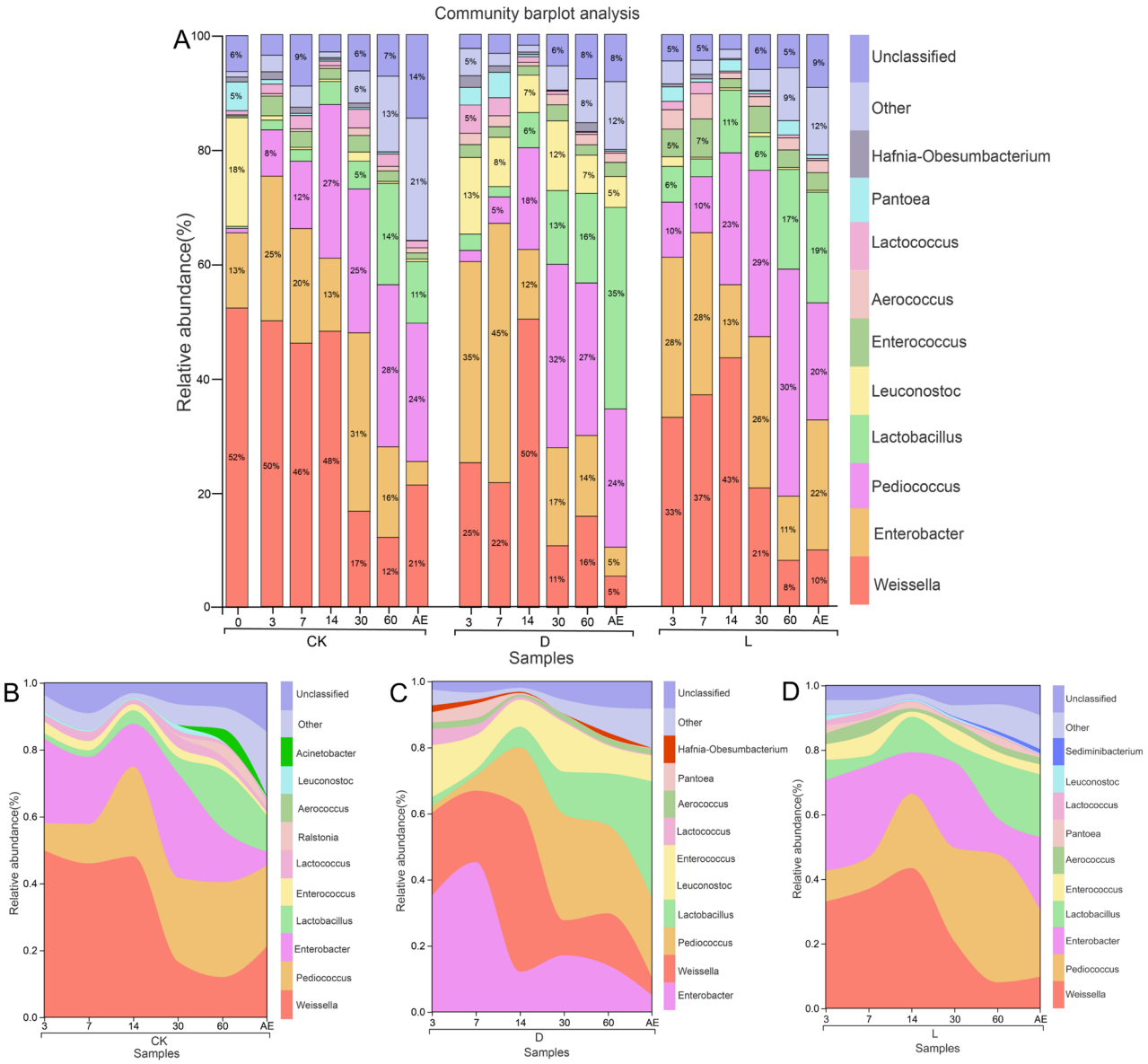

FIGURE 5 | Changes in relative abundance composition of bacterial communities under the treatment of fermentation time and EPS. CK, distributed water; D, added dextran of silage; L, added levan of silage. Day 3, 7, 14, 30 and 60, respectively, represent fermentation of silage for 3, 7, 14, 30 and 60 days.

d of fermentation and the AE stage, a significant distribution in the distance matrix was observed in the dextran-treated group, suggesting that dextran influenced the feature dissimilarity among sample groups.

The correlation networks of bacteria with different fermentation times are shown in Figure 7. Significant variations in species abundance, composition and edge number were observed across different fermentation times. After fermentation for 3–30 d, the correlation networks of oat bacteria mainly consist of Firmicutes and Proteobacteria. However, after 60 d of fermentation and during the AE stage, the correlation networks of significantly correlated bacteria are primarily composed of Proteobacteria (Figure 7E,F). After 7, 14 d of fermentation, there was a notable reduction in the number of edge numbers, and the significant positive correlation with bacteria diminished, while the significant negative correlation experienced an increase (Figure 7B,C). After 14 d of fermentation, *Weissella* showed a strong effect with high relative abundance and edge number. However, after 30 d of fermentation, *Lactobacillus* exhibited the highest relative abundance, which only showed a significant positive correlation with *Leuconostoc*. It is noteworthy that during fermentation for 30, 60 d and AE stage, bacterial correlation networks did not exhibit significant negative correlations, showing only significant positive correlations. Interestingly, at this time, the three fermentation stages had the same number of edges. *Ralstonia* assumes particular importance after 30 d of fermentation, distinguished by its high relative abundance and edge number (Figure 7D). It is evident that Proteobacteria constitutes a significant proportion. During the AE stage, *Nocardioides* exhibited a marked positive correlation with Proteobacteria, characterised by a high relative abundance and edge number.

TABLE 1 | Differences in bacterial genus levels under different fermentation times and EPS treatment.

Genus	Treatment	Ensilage period					
		3	7	14	30	60	AE
Weissella	CK	49.96Aa	46.02Aa	48.12a	16.66b	12.06Ab	21.24Ab
	D	25.11Cb	21.61Bb	50.20a	10.57b	15.72Ab	5.26Bb
	L	33.07Ba	37.00ABa	43.46a	20.72b	8.06Bb	9.89Bb
Enterobacter	CK	25.28ab	20.03ab	12.78ab	31.18a	15.87ab	4.10Bb
	D	35.14a	45.33a	12.17b	17.14b	14.13b	5.08Bc
	L	28.00	28.36	12.81	26.48	11.23	22.73A
Pediococcus	CK	8.09b	11.79b	26.87a	25.16a	28.34a	24.20a
	D	1.95b	4.64b	17.79ab	32.10a	26.67a	24.17a
	L	9.62d	9.80d	23.04c	29.05ab	39.68a	20.48bc
Lactobacillus	CK	1.71b	2.01b	4.03b	4.87Bb	17.70a	10.75Bab
	D	2.87b	1.79b	6.16b	12.88Ab	15.68b	35.22Aa
	L	6.26b	3.09b	10.95b	5.90Bb	17.43a	19.32Ba
Leuconostoc	CK	0.76B	0.42B	0.41B	1.58B	0.35B	0.45B
	D	13.40Aa	8.61Aab	6.55Aab	12.19Aab	6.68Aab	5.40Aa
	L	1.69B	0.32B	0.42B	0.67B	0.32B	0.33B
Enterococcus	CK	3.45	2.77	1.85	2.90	1.83	1.07
	D	2.26	1.86	1.61	2.80	1.81	2.47
	L	4.82ab	6.70a	1.62b	4.63ab	3.07ab	3.10ab
Aerococcus	CK	0.42b	0.44b	0.49b	1.32a	0.79ab	0.89ab
	D	1.98	1.93	0.65	1.84	1.81	1.62
	L	3.39	4.39	1.03	1.67	2.15	2.13
Lactococcus	CK	1.65B	2.36	0.81	3.25A	2.18A	1.24A
	D	4.94Aa	3.16a	0.96b	0.57Bb	0.38Bb	0.34Bb
	L	1.48Bab	2.04a	0.29b	0.50Bab	0.50Bab	0.35Bb
Pantoea	CK	0.80	0.36	0.27	0.31	0.33	0.04
	D	3.08	4.43	0.41	0.10	0.12	0.28
	L	2.52	0.57	1.97	0.47	2.49	0.60
Hafnia–Obesumbacterium	CK	1.36	1.09	0.32	0.79	0.03	0.02
	D	2.05	1.12	0.41	0.06	1.55	0.01
	L	0.52	0.78	0.26	0.16	0.01	0.03

Note: CK, distributed water; D, added dextran of silage; L, added levan of silage. Day 3, 7, 14, 30 and 60, respectively, represent fermentation of silage for 3, 7, 14, 30 and 60 days. Values with different lowercase letters show significant differences among ensiling days in the same treatment; values with different capital letters show significant differences among treatments in the same ensiling day ($p < 0.05$).

3.4 | The Effects of Different Fermentation Times and EPS on Carbohydrate Metabolism

Both fermentation duration and EPS influence the carbohydrate metabolism (level_3) in oat silage (Figure 8). Before fermentation, oats exhibit the lowest level of carbohydrate metabolism. However, as fermentation progresses, carbohydrate metabolism becomes increasingly active. After fermentation for 3, 7, 14 and 60 d, the overall carbohydrate metabolism level under EPS treatment was upregulated. However, during fermentation for 30 d and AE stage, the overall carbohydrate metabolism level under the EPS treatment was downregulated. The pentose phosphate pathway, along with the metabolism of fructose and mannose, glycolysis/gluconeogenesis and pyruvate metabolism, is among the most vigorous carbohydrate metabolic processes during oat fermentation. Among these, the pentose phosphate pathway

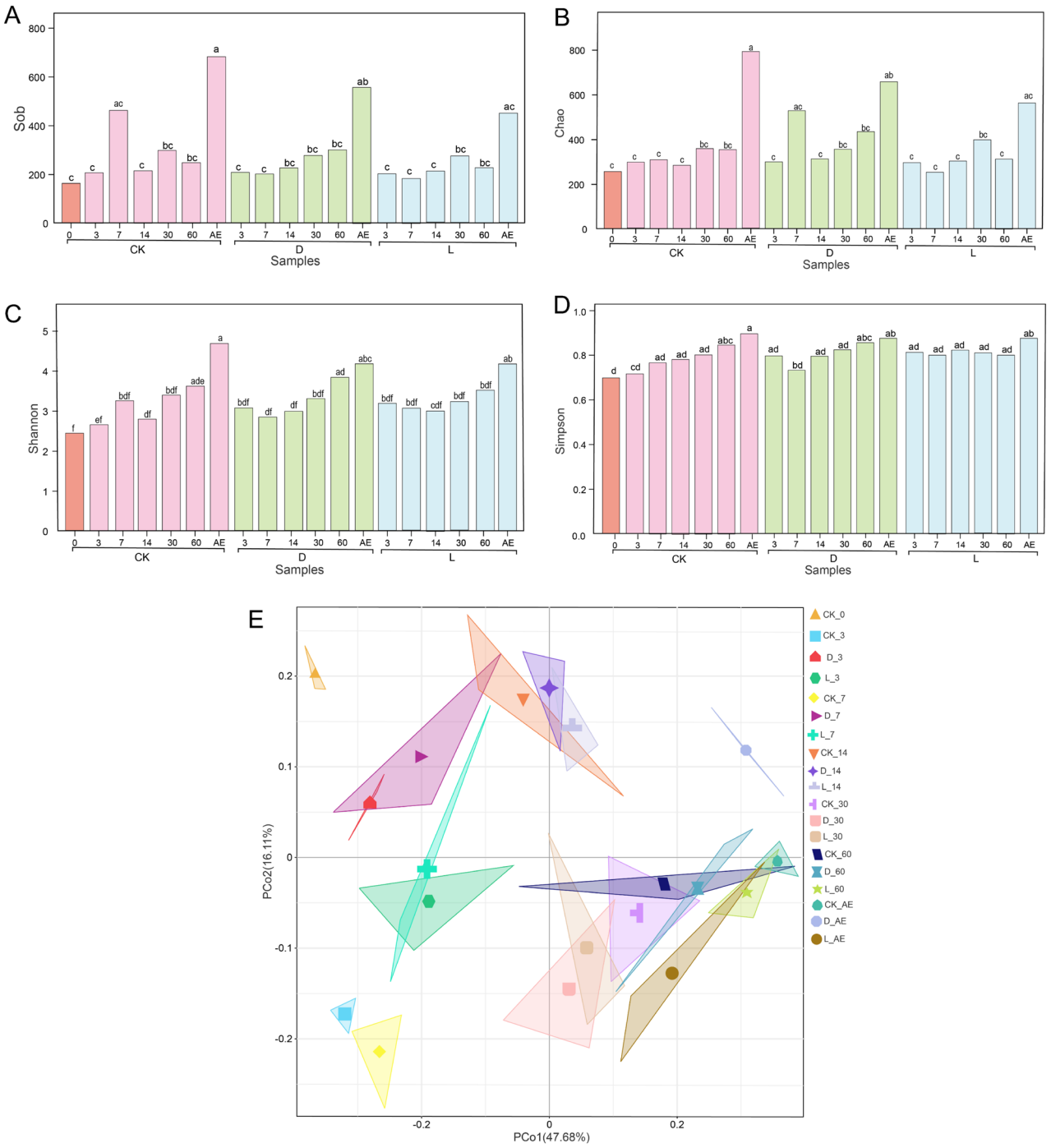

FIGURE 6 | The feature dissimilarity alpha diversity (A–D) and beta diversity (E) under the treatment of fermentation time and EPS. The distribution of beta diversity distance is calculated based on Bray-Curtis. CK, distributed water; D, added dextran of silage; L, added levan of silage. Day 3, 7, 14, 30 and 60, respectively, represent fermentation of silage for 3, 7, 14, 30 and 60 days. Different lowercase letters indicate significant differences under different treatments (fermentation time and EPS) ($p < 0.05$).

exhibits the highest level of metabolic activity. After 60 d of fermentation, the overall carbohydrate metabolism level of oat fermented with levan treatment was upregulated, indicating a higher metabolic level. The citrate cycle was significantly upregulated with the change of fermentation time, and at the same time, after 60 d of fermentation, the EPS all upregulated the citrate cycle.

4 | Discussion

4.1 | Chemical Composition and Fermentation Quality of Oat Ensiling

The ultimate goal of oat fermentation is to obtain high-quality silage products. Recently, research interest in EPS within

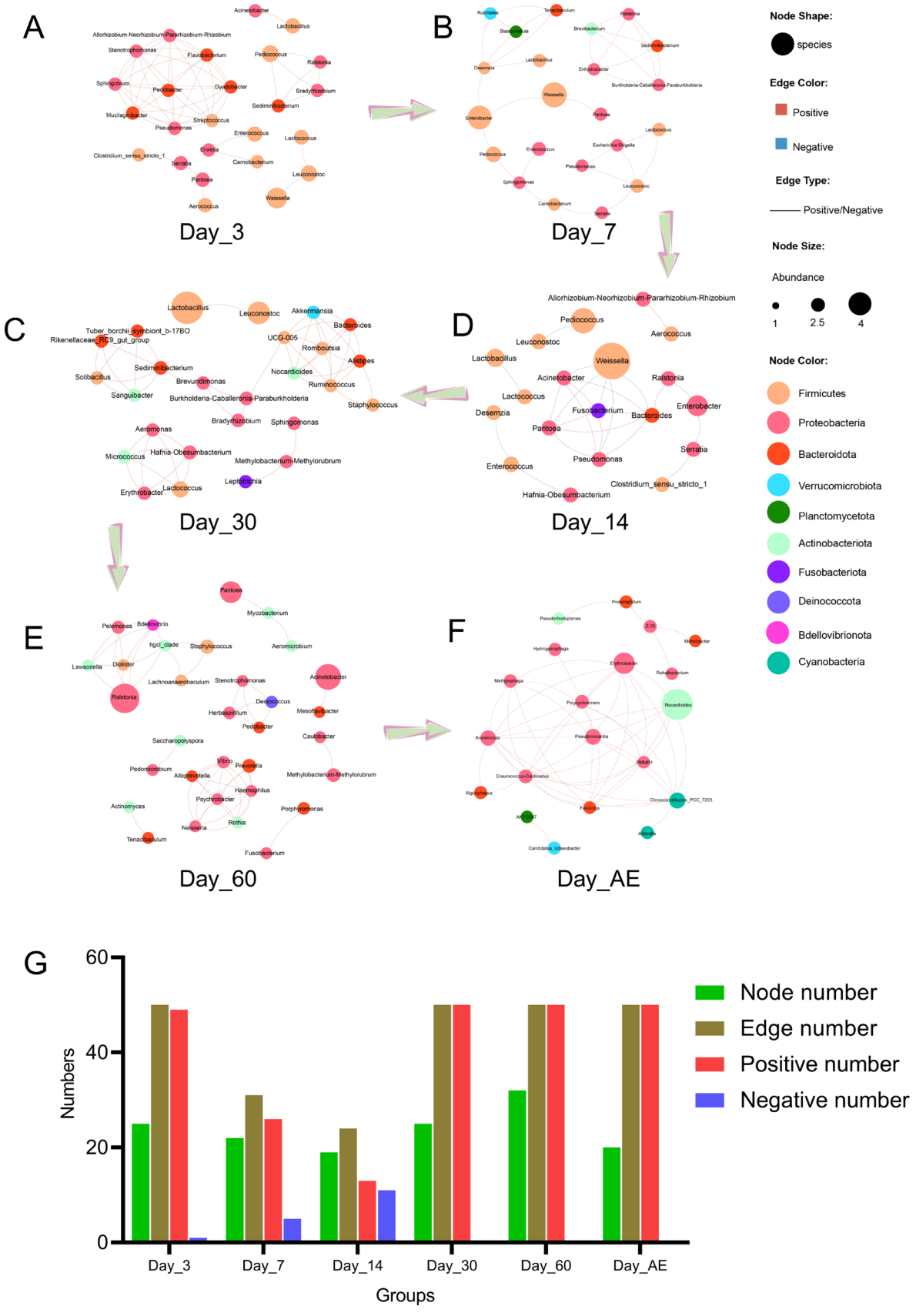

FIGURE 7 | Dynamic changes in bacterial correlation networks (A–F) under different fermentation times. We chose to plot the significant correlation between bacterial communities ($p < 0.05$). The different node numbers, edge numbers, positive numbers and negative numbers (G) were treated with different fermentation times. Day_3, Day_7, Day_14, Day_30, and Day_60, representing fermentation for 3, 7, 14, 30 and 60 days, respectively. Day_AE, representing the aerobic exposure stage.

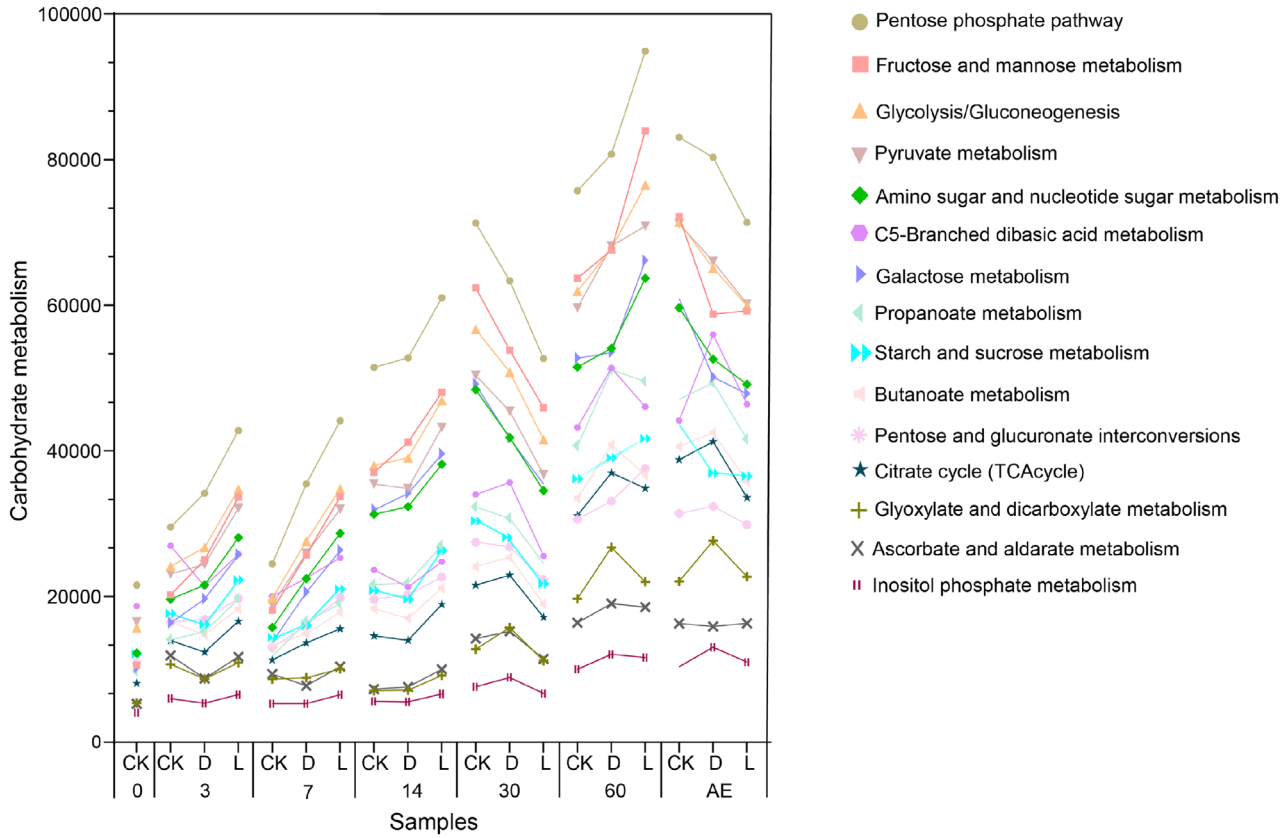

FIGURE 8 | Level_3 carbohydrate metabolism analysis was based on the KEGG database under the treatment of fermentation time and EPS. CK, distributed water; D, added dextran of silage; L, added levan of silage. Day 3, 7, 14, 30 and 60, respectively, represent fermentation of silage for 3, 7, 14, 30 and 60 days.

fermented foods has been on the rise, with their distinctive physicochemical properties being extensively utilised in both yogurt and broader food fermentation processes (Tiwari et al. 2021). The impact of two types of EPS on the chemical composition of oat silage was not substantial, with no alterations observed in the content of NDF and ADF post-ensiling. This outcome might be attributed to the absence of specific strains or enzymes capable of degrading structural carbohydrates during the silage fermentation process (Li et al. 2018). The overall content in silage exhibits an initial decline followed by an increase, potentially attributed to shifts in microbial composition during the later stages of fermentation. This variation could stem from the distinct nucleotide compositions of different microorganisms (Glencross et al. 2024). Under dextran treatment, oat silage preserved a higher level of WSC, suggesting that microorganisms might have exploited dextran as a substrate for fermentation during the microbial fermentation process. This observation corroborates previous findings that EPS can sustain microbial growth (Hassan 2008). It is noteworthy that the CP content exhibited a pronounced increase during the later stages of silage production. This could be attributed to the single-cell proteins (SCPs) generated by microbial activity (Glencross et al. 2024). Furthermore, the efficiency of protein synthesis appears to surpass that of protein hydrolysis. According to reports, the nucleotide content of SCP resources in bacteria and yeast is almost 16% of their biomass, and some bacteria have obvious advantages as they can consume CO_2 or CH_4 as part of the SCP production process (Øverland et al. 2010). The dextran bolsters the aerobic stability post-fermentation, potentially due to a substantial rise in the relative abundance of *Lactobacillus*, which suppresses other bacterial populations during the AE stage (İspirli et al. 2018).

The fermentation time and EPS significantly affect the fermentation quality of oat silage. In this study, two types of EPS notably elevated the LA and AA content, while concurrently lowering the pH of the silage. This phenomenon could be attributed to both EPS enhancing the relative abundance of *Lactobacillus* in oat silage. This finding aligns with previous research indicating that EPS can facilitate the growth of *Lactobacillus plantarum* and *L. fermentum* (Tiwari et al. 2021). In earlier investigations, the in vitro fermentation of EPS over a 48-h period markedly enhanced the proliferation of *Lactobacillus* sp. and *Bifidobacterium* sp., concurrently boosting the production of LAB (Tiwari et al. 2021). Therefore, in this study, EPS was used as another microbial fermentation carbon source to enhance fermentation quality by boosting the prevalence of *Lactobacillus*.

4.2 | Bacterial Community Composition, Diversity and Correlation Network of Oat Ensiling

Ensiling is a process that yields a product fermented by a consortium of microorganisms, utilising carbon sources such as glucose and pentose under anaerobic conditions. The quality of the silage is significantly determined by the composition of

its bacterial community (Ni et al. 2017). Initially, the *Weissella* occupied a certain ecological niche. However, as time passed, other species more adapted to the silage environment, such as *Lactobacillus* and *Pediococcus*, took over the dominant position. Consequently, this led to a decrease in the relative abundance of the *Weissella* (Wang et al. 2021). The relative abundance of *Pediococcus* significantly increased with the prolongation of fermentation time. This is primarily because the anaerobic environment formed by the silage is conducive to its reproduction, thereby producing lactic acid and lowering the pH of the silage environment (Zhang et al. 2021). However, The EPS treatment did not significantly affect the relative abundance of *Pediococcus*, which may be due to its selective preference for fermentation substrates (Jiang et al. 2021). *Enterobacter* significantly decreases with the extension of fermentation time, mainly due to the anaerobic and acidic environment in silage, which is unfavourable for its proliferation (Zhao et al. 2022). Different fermentation times and EPS changed the abundance composition of oat bacteria, and the relative abundance of *Leuconostoc* increased under dextran treatment. This could be because *Leuconostoc* prefers to use dextran as a carbon source for fermentation. Both types of EPS enhanced the relative abundance of *Lactobacillus* following fermentation periods of 30 and 60 d. This suggests that these EPS variants can effectively function as fermentation substrates to facilitate the proliferation of *Lactobacillus*, aligning with findings from previous studies (Tiwari et al. 2021). The longer time required for the increase in relative abundance of *Lactobacillus* may be due to the low attachment rate of LAB to the raw materials (Liu et al. 2023). Intriguingly, during the AE stage, the administration of dextran and levan resulted in a 24% and 8% increase, respectively, in the relative abundance of *Lactobacillus*. This observation hints that microaerophilic LAB may more efficiently exploit the EPS. An alternative explanation is that, at this juncture, the WSC content is reduced, prompting *Lactobacillus* to preferentially utilise EPS for proliferation under conditions of nutrient scarcity (Patel and Prajapati 2013). The addition of dextran and levan also enhanced the relative abundance of *Hafnia–Obesumbacterium* and *Sediminibacterium*, respectively. This suggests that distinct bacterial genera exhibit differential responses to the supplemented substrates (Wu et al. 2020).

Alpha diversity is an important criterion for measuring intra-sample diversity and can be compared between sample groups (Knight et al. 2018). The two types of EPS did not modify the alpha diversity indices during the fermentation process. However, compared to before ensiling, these indices increased after ensiling, likely due to changes in the nutritional composition and microbial population of the oat silage feed during the ensiling process (Wang et al. 2020). The fermentation time changed the alpha diversity of silage, indicating that with the extension of fermentation time, the richness and evenness of bacterial species were improved (Zi et al. 2021). In this investigation, the peak alpha diversity was noted during the AE phase, indicating the widespread proliferation of aerobic microorganisms in oat silage subsequent to air exposure (Ferrero et al. 2021). The increase in the relative abundance of *Lactobacillus* under the treatment of EPS was an important reason for the decrease in Sob and Chao indices. Beta diversity serves as a crucial approach for assessing the similarity between samples. It produces a distance matrix of the beta diversity distances among all sample pairs by examining the feature disparities between each set of samples (Knight et al. 2018). Varying fermentation durations markedly modified the distance distribution among oat silage samples, suggesting substantial feature dissimilarity between samples as fermentation times extended (He et al. 2020). Compared to the control group, the dextran-treated group showed notable differences in features during fermentation on day 3 and at the AE stage. This discrepancy could be attributed to the enhanced utilisation of dextran by aerobic microorganisms.

At varying stages of silage, intricate interrelationships among the internal bacteria emerge, characterised by competition, promotion and coexistence. Comprehending these relationships can offer a more intuitive gauge of the fermentation extent in silage. Through screening significantly correlated bacterial genera for plotting, we discovered that the proportion of bacterial genera to abundance varied markedly under different fermentation durations. This indicates that the strains exerting dominant influence at different fermentation stages were distinct (Yin et al. 2023). After 14 d of ensiling, *Weissella* exhibited complex edges and a high relative abundance, suggesting that oat ensiling was actively undergoing fermentation at this stage but had not yet concluded the fermentation process (Wang et al. 2022). As the silage time prolongs, the significant negative correlation between bacteria first increases and then disappears, indicating that bacterial activity has been active within the silage. However, this is not conducive to silage fermentation as it may cause more nutritional loss. Following 30 and 60 d of fermentation, a significant positive correlation was noted among bacterial genera. This could be attributed to the pH value in the silage not achieving the optimal fermentation state at this juncture, resulting in the sustained growth of other microorganisms (Khota et al. 2016).

4.3 | Carbohydrate Metabolism of Oat Ensiling

Carbohydrate metabolism represents a principal active metabolic component in the silage fermentation process, where microorganisms utilise carbon sources for their proliferation, thereby contributing to fermentation (Zhou et al. 2024). In this study, EPS showed different effects on regulating carbohydrates at different silage times. Following fermentation periods of 3, 7 and 14 d, the overall carbohydrate metabolism level was enhanced. This might be due to EPS facilitating the better survival of producer strains in acidic conditions, thereby stimulating microbial activity (Kim et al. 2000). After 30 d of silage, EPS led to a general decline in carbohydrate metabolism. This could be attributed to an uptick in the relative abundance of *Lactobacillus* and a reduction in pH, resulting in a deceleration of the growth of other microorganisms (Okoye et al. 2023). The pentose phosphate pathway is one of the most active metabolic pathways, indicating that microorganisms in silage mainly rely on glucose for fermentation, which is consistent with previous views (Li et al. 2020). The active metabolism of fructose and mannose indicates that pentose is also the main carbon source utilised by microbial fermentation in silage (Zhao et al. 2019). Following fermentation periods of 3, 7, 14 and 60 d, glycolysis is enhanced under EPS treatment. This is attributed to the fermentation environment becoming anaerobic due to microbial activity, which favours LAB in converting glucose into pyruvate. Subsequently,

pyruvate can be transformed into lactic acid with the assistance of lactate dehydrogenase, a process advantageous for silage fermentation (Papadimitriou et al. 2016).

5 | Conclusion

Dextran and levan as exogenous carbon source additives have shown positive effects on oat fermentation. EPS elevated the relative abundance of *Lactobacillus* following 60 d of fermentation in silage, augmented the LA content and diminished the pH of the silage. The treatment with dextran preserved more WSC during the silage process and improved the aerobic stability. The dominant roles of bacterial genera varied at different silage durations, with Firmicutes emerging as the pivotal factor governing fermentation. The EPS regulated carbohydrate metabolism levels and altered the formation of final metabolites. In short, the EPS can improve the quality of oat ensiling; the best ensiling effect was achieved by dextran.

Author Contributions

Wei Liu: writing – original draft, formal analysis, data curation, investigation, visualization. **Zhijun Wang:** formal analysis, visualization. **Lin Sun:** writing – review and editing, visualization. **Shuai Du:** writing – review and editing. **Gentu Ge:** project administration, funding acquisition. **Yushan Jia:** funding acquisition, project administration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in OAT Raw sequence reads at https://www.ncbi.nlm.nih.gov/search/all/?term=PRJNA1129694, reference number PRJNA1129694.

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