

Article

Functional Prediction of Bacteria–Enzyme Co-Regulation on Rapeseed Straw Silage: Fermentation Quality and Fiber Degradation

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Abstract

This study utilized rapeseed straw as the raw material and employed a completely randomized design with four treatments: a distilled water control (CK), individual supplementation of *Lactiplantibacillus plantarum* (1.0×10^6 CFU/g fresh weight) (Lp), individual supplementation of xylanase (50,000 U/g fresh weight) (XY), and a combined bacterium–enzyme treatment (XYLp). Each treatment was replicated five times, vacuum-sealed, and fermented at 25 °C for 60 days to systematically evaluate the effects of different treatments on the fermentation quality, nutritional composition, and microbial community structure of rapeseed straw silage. The results demonstrated that, compared with the CK group, all additive treatments significantly decreased pH and increased lactic acid (LA) content ($p < 0.05$). Among them, the Lp group exhibited the lowest pH value (4.27), which was significantly lower than all other treatments except XYLp ($p < 0.05$). Both the Lp and XYLp groups showed significantly higher LA content than the other groups ($p < 0.05$). Crude protein (CP) content was significantly higher in all additive treatments than in the CK group ($p < 0.05$). The XYLp group exhibited the most substantial fiber degradation, with acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents being significantly lower than CK and reaching the lowest values among all treatments ($p < 0.05$). Both the XY and XYLp groups showed significantly lower hemicellulose and holocellulose contents compared to the CK and Lp groups ($p < 0.05$). Microbial community analysis revealed that the synergistic bacterium–enzyme treatment significantly enriched fibrolytic genera, including *Kosakonia* and *Pediococcus*, and upregulated the expression of key fibrolytic enzymes such as cellulase (EC: 3.2.1.4), β -glucosidase (EC: 3.2.1.21), and endo-1,4- β -xylanase (EC: 3.2.1.8). Functional prediction further indicated that the bacterial–enzyme synergy enhanced fibrous structure degradation and fermentable substrate release by activating carbohydrate metabolism pathways and bacterial secretion systems. These findings suggest that the combined application of *Lactiplantibacillus plantarum* and xylanase has the potential to be a promising strategy for enhancing fiber degradation and overall fermentation quality in rapeseed straw silage.

Keywords: fiber degradation; xylanase; microbial-enzyme synergy; fermentation quality



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1. Introduction

As a crucial oil crop, rapeseed produces straw that has significant value for agricultural resource recycling. This straw, abundant in nitrogen, phosphorus, potassium, cellulose, hemicellulose, and protein, can be utilized to improve soil fertility through direct incorporation into fields or processed into high-quality roughage for herbivores by means of enzymatic hydrolysis and fermentation [1].

The application of rapeseed straw in animal feed is constrained by several inherent characteristics, notably a highly lignified epidermis, a rigid texture, and anti-nutritional factors like erucic acid and glucosinolates. These attributes collectively result in low metabolizable energy, poor palatability, and reduced digestibility [2]. Studies have shown that different rapeseed straw varieties treated by physical methods did not significantly improve their rumen degradation rate and structure [3].

Therefore, appropriate processing and conditioning are necessary in practical production to disrupt its fibrous structure and mitigate the content of these anti-nutritional factors [2,4]. To conserve the nutritional value of forage and enhance its digestibility, ensiling is frequently employed as an effective preservation technique. The critical factor governing silage quality is lactic acid bacteria (LAB) activity. Through the fermentation of the water-soluble carbohydrate (WSC) into lactic acid, a low pH environment is established, which effectively suppresses spoilage microorganisms [5,6]. The cell walls of rapeseed straw are rich in structural polysaccharides, including cellulose, hemicellulose (with xylan as the primary component), and pectin [7]. The application of xylanase, a key fibrolytic enzyme, in both silage and ruminant diets has been shown to enhance fiber degradation and elevate feed nutritional value [7]. Du et al. found that supplementing wheat straw with *Lactiplantibacillus plantarum* and cellulase significantly elevated lactic acid concentration, degraded fibrous components, and modified the microbial community structure, leading to the dominance of *Lactobacillus* [8]. A synergistic effect was observed by Bao et al. when laccase was combined with *Pediococcus pentosaceus* for silage fermentation. This combined approach resulted in more pronounced improvements, including higher lactic acid production, reduced pH and dry matter loss, as well as superior lignin and fiber degradation relative to individual treatments [9]. Although extant literature has documented bacterial–enzyme synergies in silage production, the primary focus has remained on macro-level alterations in fermentation parameters and microbial community structure. The underlying mechanisms, particularly the regulatory effects on the expression profiles of key fibrolytic enzymes in rapeseed straw silage co-treated with *L. plantarum* and xylanase, have remained largely unexplored.

This study aims to elucidate the synergistic mechanism between *Lactiplantibacillus plantarum* and xylanase in rapeseed straw silage. We seek to define their specific roles in improving fermentation quality, enhancing fiber degradation, and modulating microbial function. These insights aim to establish a theoretical basis for the creation of effective bacterium–enzyme additives, paving the way for the valorization and high-value utilization of rapeseed straw.

2. Materials and Methods

2.1. Preparation of Rapeseed Straw Raw Material

The rapeseed straw used in this study was sourced from the Hulunbuir Grassland Ecosystem National Field Scientific Observation and Research Station of the Chinese Academy of Agricultural Sciences (latitude 49°23′13″ N, longitude 120°02′47″ E; altitude 627–635 m; mean annual temperature −2.4 °C). The forage rapeseed was planted on 22 May 2023, in a plain terrain characterized by chernozem soil with light loamy texture, located on a mid-slope sunny aspect at an elevation of approximately 627–680 m. The entire

rapeseed plant was harvested at pod maturity on 20 August 2023. After threshing to separate the seeds, the remaining straw was collected using machinery (Model: HFD50 × 80) supplied by Linyi Fuda Tools Co., Ltd., Beijing, China. Subsequently, the harvested straw underwent a 3 h wilting treatment in the field before being mechanically chopped into 3 cm segments. Fresh samples (400 g) were immediately frozen and transported to the laboratory for compositional analysis (Table 1).

Table 1. Chemical and microbial composition of rapeseed straw raw materials.

Items	Content	SD
Dry matter (% of FM)	55.26	1.53
Crude protein (% of DM)	3.26	0.22
Acid detergent fiber (% of DM)	32.40	1.32
Neutral detergent fiber (% of DM)	48.33	1.94
Water-soluble carbohydrates (% of DM)	4.64	0.15
Lactic acid bacteria (log ₁₀ CFU/g FM)	4.46	0.16
Aerobic bacteria (log ₁₀ CFU/g FM)	5.38	0.24
Yeasts (log ₁₀ CFU/g FM)	5.86	0.16
Coliform bacteria (log ₁₀ CFU/g FM)	6.63	0.26
Mold (log ₁₀ CFU/g FM)	6.75	0.22

Note: DM, dry matter; FM, fresh matter; CFU, colony-forming units.

Two additives were utilized: *Lactiplantibacillus plantarum* MTD/1 (provided by Luke Biological Technology Co., Ltd., Nanjing, Jiangsu, China, viable count: 1×10^{10} CFU/g) and xylanase (supplied by Anhui Bomei Biotechnology Co., Ltd., Hefei, China; total activity: 50,000 U/g). Four treatments were established: a distilled water control (CK), *L. plantarum* alone (Lp, 1.0×10^6 CFU/g fresh weight), xylanase alone (XY, 50,000 U/g fresh weight), and their combination (XYLp). Chopped rapeseed straw (approximately 250 g) was aseptically packed into polyethylene bags (32 × 26 cm). The bags were subsequently vacuum-sealed and fermented at 25 °C for 60 days. The resulting silage density in the bags was approximately 332 kg of dry matter per cubic meter (kg DM/m³), which is within the recommended range for laboratory-scale silage studies to ensure anaerobic conditions. The 60-day fermentation period was selected based on established practices in silage research to ensure the stabilization of the fermentation process and microbial community [10,11]. This experiment was conducted following a completely randomized design with five replicates per treatment, and all samples were uniformly arranged to maintain consistent fermentation conditions.

2.2. Analytical Methods for Silage Quality Assessment

After 60 days of fermentation, fresh rapeseed straw and silage samples were dried in a blast drying oven at 65 °C for 48 h to determine dry matter (DM) content. The samples were then ground through a 1 mm sieve (Model FW100, Teste Instrument, Cangzhou, China) for subsequent chemical composition analysis [12]. Measurement of the crude protein (CP) content was performed employing the Kjeldahl method [13]. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the procedure described by Van Soest [14]. The proportions of cell wall components, including cellulose, hemicellulose, and holocellulose, were calculated using the method proposed by Yang et al. [15]. The concentration of organic acids in the filtrate was analyzed by high-performance liquid chromatography (HPLC; 1260 Infinity II, Waters, Santa Clara, CA, USA). The HPLC system was equipped with a UV detector set at 210 nm, using 3 mmol/L HClO₄ as the mobile phase at a flow rate of 1.0 mL/min and a column temperature of 50 °C [16]. WSC content was quantified utilizing the anthrone method [17].

Microbial enumeration in the fresh material (FM) was conducted using the plate count method, with results expressed as colony-forming units per gram of fresh weight (CFU/g FW). Following incubation at 30 °C for 48 h, counts of lactic acid bacteria and *E. coli* were obtained on De Man, Rogosa, and Sharpe (MRS) agar (Difco Laboratories, Detroit, MI, USA) and Brilliant Green Bile Broth agar (Nissui Ltd., Tokyo, Japan), respectively. Molds and yeasts were separately counted on Potato Dextrose Agar (Nissui Ltd., Tokyo, Japan) and Nutrient Agar (Nissui Ltd., Tokyo, Japan) following 24 h of incubation at 30 °C [16].

2.3. Bacterial Community Analysis

Ten-gram silage samples were homogenized in 90 mL of sterile water using a shaker (160 rpm, 2 h, 4 °C) to collect microorganisms. Prior to centrifugation (10,000× g, 15 min, 4 °C), the suspension was filtered through two layers of sterile gauze and subjected to repeated washing with sterile water to recover residual microbial cells. The resulting pellet was then stored at −80 °C.

Total microbial DNA was extracted using the TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using universal primers (forward: 5'-ACTCCTACGGGAGGCAGCA-3'; reverse: 5'-GGACTACHVGGGTWTCTAAT-3'), following established PCR protocols [18]. Sequencing data were analyzed for diversity indices and microbial community composition using a bioinformatics cloud platform (<https://www.omicsmart.com>, accessed on 30 May 2024).

2.4. Statistical Analysis

All data are presented as the mean of five replicates. The nutritional and fermentation parameters were evaluated by one-way ANOVA in SPSS (Version 27.0, IBM Corp., Armonk, NY, USA), with statistical significance determined at $p < 0.05$. Where significant differences were detected by ANOVA, Duncan's multiple range test was applied for post hoc comparisons under the same alpha level of 0.05. To visualize beta-diversity, principal coordinate analysis (PCoA) was performed based on a Bray–Curtis distance matrix, with statistical significance of intergroup differences assessed by permutational multivariate analysis of variance (PERMANOVA). Microbial raw data were downloaded from the dior platform, and Venn diagrams were subsequently generated using the online analysis platform (<https://www.omicshare.com/tools/home/report/reportpca.html>, accessed on 19 November 2025). Upset plots and Mantel test analyses were performed via the online tool (https://chiplot.online/upset_plot.html, accessed on 19 November 2025). Bar charts and functional prediction histograms representing bacterial species relative abundance percentages were created using Origin 2024. LDA plots were generated with the online analysis website (<https://www.bioincloud.tech/>, accessed on 19 November 2025).

3. Results and Discussion

3.1. The Impact of Additives on the Quality of Silage from Rapeseed Straw

As detailed in Table 2, the effects of various additives on rapeseed straw silage quality indicate that successful fermentation is critically dependent on precise pH control, a core indicator for which a pH below 4.2 is generally required for high-quality silage [19]. All additive treatments in this study significantly reduced the pH value ($p < 0.05$), with the Lp treatment showing the lowest pH. This can be attributed to the rapid proliferation of lactic acid bacteria from *L. plantarum* during the early fermentation stage, which converted water-soluble carbohydrates into short-chain organic acids (e.g., lactic, acetic, and propionic acids). The accumulation of these acids subsequently led to a decrease in environmental pH. Lactic acid and acetic acid, as the two predominant organic acids, exert decisive influences on

the fermentation quality and aerobic stability of silage through their production ratios and absolute concentrations [20]. In this experiment, the combined LP and XYLP treatments yielded significantly higher lactic acid content compared to all other groups ($p < 0.05$). Furthermore, the XYLP group exhibited a more pronounced accumulation of lactic acid than the Lp group. This result can be explained by the fact that lactic acid is the primary end product of water-soluble carbohydrate degradation by homofermentative lactic acid bacteria. Although the Lp treatment promoted substantial lactic acid accumulation in the early fermentation stage, outperforming the CK and XY groups, it was ultimately limited by the high fiber content (cellulose, hemicellulose, and lignin) of rapeseed straw. This structural barrier hindered efficient carbohydrate utilization, consequently resulting in lower final lactic acid production in the Lp group compared to the XYLP group. Although high concentrations of lactic acid contribute to effective preservation of silage, they may negatively affect aerobic stability. In contrast, acetic acid and its derivatives (e.g., ethyl acetate) not only enhance the aerobic stability of silage but have also been shown to exhibit strong inhibitory effects on yeasts and molds [21,22].

Table 2. Fermentation quality and chemical composition in rapeseed straw silage.

Items	CK	Lp	XY	XYLP
Fermentation quality				
pH	5.40 ± 0.17 a	4.27 ± 0.04 c	4.79 ± 0.08 b	4.52 ± 0.11 bc
Lactic acid (% of DM)	1.35 ± 0.06 c	1.84 ± 0.07 a	1.59 ± 0.03 b	1.77 ± 0.08 a
Acetic acid (% of DM)	1.06 ± 0.02 a	0.76 ± 0.05 b	1.09 ± 0.11 a	1.01 ± 0.05 a
Chemical compositions				
Dry matter (%)	40.22 ± 0.69	41.78 ± 1.17	41.84 ± 0.94	41.44 ± 0.61
Crude protein (% of DM)	5.02 ± 0.10 b	5.65 ± 0.44 a	5.30 ± 0.12 a	5.42 ± 0.34 a
ADF (% of DM)	15.25 ± 0.60 a	13.02 ± 0.83 ab	12.56 ± 1.63 ab	10.31 ± 0.28 b
NDF (% of DM)	45.27 ± 1.28 a	45.06 ± 0.87 a	44.50 ± 2.79 a	42.18 ± 0.94 b
Cellulose (% of DM)	16.49 ± 1.85 b	17.75 ± 2.16 a	12.20 ± 0.57 b	13.78 ± 0.70 b
Hemicellulose (% of DM)	16.00 ± 1.50 a	8.63 ± 1.03 a	10.22 ± 1.26 b	9.20 ± 2.28 b
Holocellulose (% of DM)	32.49 ± 1.81 b	33.37 ± 2.29 a	21.42 ± 1.28 c	23.99 ± 1.63 c

Note: DM, dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber; Propionic acid and butyric acid were not detected in all rapeseed straw silages. CK, control treatment; XY, rape straw inoculated with Xylanase treatment; Lp, rape straw was treated by inoculating *Lactiplantibacillus plantarum*; XYLP: Rapeseed straw was inoculated with Xylanase and *Lactiplantibacillus plantarum* for treatment; Means with different letters differ significantly from each other ($p < 0.05$).

Dry matter content serves as a key indicator of the physical environment in silage and shows a positive correlation with nutritional composition. Both plant cell respiration and microbial fermentation contribute to dry matter losses during the ensiling process [23]. In practical silage production, excessively low DM content leads to effluent loss, removing substantial soluble nutrients and promoting clostridial growth. Conversely, excessively high dry matter content hinders adequate compaction, resulting in excessive residual oxygen that stimulates the activity of aerobic microorganisms (e.g., yeasts and molds) and triggers aerobic spoilage. The dry matter content across all treatment groups showed no significant differences in this experiment, suggesting that the addition of enzyme preparations and lactic acid bacteria had a minimal impact on this silage parameter.

As a key indicator of silage nutritional value, the feeding value of the final product is directly determined by both the absolute content of crude protein and its preservation rate during fermentation [24]. Numerous studies have demonstrated that treatments with lactic acid bacteria and enzyme preparations can effectively increase the crude protein content of feeds. In the present study, the crude protein content in all additive-treated groups was significantly higher than in the CK group ($p < 0.05$). First, the rapid acidification driven by lactic acid production effectively suppressed the activity of proteolytic clostridia

and bacilli, which are responsible for decomposing true plant proteins into ammonia and non-protein nitrogen (NPN). This led to superior preservation of the original plant protein. Second, the proliferation of the inoculated *Lactiplantibacillus plantarum* and the activity of xylanase (which released additional fermentable substrates) contributed to a net increase in microbial crude protein (MCP). The combined effect of reduced proteolytic loss and enhanced MCP synthesis resulted in a higher measured CP content in the silage [8,25,26].

NDF and ADF are widely recognized as the most representative indicators for assessing the fiber components in feeds at the current stage [27]. ADF content shows a significant negative correlation with animal digestibility. Lower ADF values generally indicate higher feed digestibility and consequently improved nutritional value [28]. NDF reflects the total cell wall fiber content, and its level directly affects feed intake and digestive efficiency in animals. Cellulose, as the primary component of acid detergent fiber, is a structural carbohydrate that is difficult to degrade [29]. Hemicellulose content is represented by the difference between neutral detergent fiber and acid detergent fiber, and it exhibits significantly higher degradability than cellulose [30]. Holocellulose (defined as the sum of cellulose and hemicellulose) represents the total structural carbohydrate content in a sample, and together they determine the overall digestion behavior of the fiber components [31]. Compared to the CK group, all additive treatments, particularly the XYLp group, led to reduced levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF), with the XYLp group achieving significantly lower values. This phenomenon may be attributed to xylanase-mediated disruption of the fibrous structure in rapeseed straw, which provided lactic acid bacteria with more abundant substrates compared to other groups. The enhanced bacterial proliferation and metabolic activity led to increased carbon dioxide production, consequently reducing fiber content. These findings are consistent with the results reported by He et al. [32].

In the experiment, the contents of hemicellulose and holocellulose were the lowest in the XY and XYLp groups, indicating the ability of xylanase to degrade fibrous components. It is plausible that the metabolic activity of *L. plantarum* preferentially targeted readily decomposable components like hemicellulose and pectin, leading to the observed relative enrichment of cellulose in the Lp group. As cellulose remains structurally stable and resistant to degradation, it became relatively enriched, leading to a significant increase in its proportional content relative to dry matter [33,34].

3.2. The Influence of Additives on the Microbial Consortia in Rapeseed Straw

The microbial community in silage originates from the epiphytic microbiota and the ensiling environment, wherein bacterial communities play critical roles throughout the entire fermentation process [35,36]. *Lactobacillus*, as the dominant bacterial genus in anaerobic silage environments, significantly influences the ensiling process. Dynamic changes in its population abundance and composition are closely associated with the final quality of the silage product [37]. As shown in Figure 1A, the Good's coverage index of each treatment group was infinitely close to 1, indicating that the sequencing depth sufficiently captured the majority of bacterial species in the samples and supporting the validity of subsequent analyses. Alpha diversity, reflecting species richness, evenness, and overall diversity, was highest in the CK group as indicated by the Chao1 index (Figure 1B), which suggests greater species richness after 60 days of ensiling. The lowest Chao1 index was recorded in the XYLp group, indicating its minimal species richness. This result may be attributed to the establishment of strongly dominant bacterial species under anaerobic conditions, which, combined with nutritional and fermentation profiles, likely suppressed the proliferation of other microorganisms detrimental to the silage fermentation process. The Shannon index primarily reflects species richness and evenness (Figure 1C). The

higher Shannon index in the XY group, compared to the Lp and XYLp groups, likely stems from the strong competitive pressure exerted by *Lactiplantibacillus plantarum* in the latter, which reduced diversity. In contrast, the XY group, less subjected to such pressure, maintained higher diversity. Beta diversity, on the other hand, characterizes the differences in bacterial community structure among individuals or treatment groups [38]. The impact of xylanase and *Lactiplantibacillus plantarum* inoculants on the bacterial community of rapeseed straw silage was investigated. The bacterial composition was characterized using Upset plots (Figure 1E) and principal coordinate analysis (PCoA) (Figure 1D). The PCoA plot revealed clear separation among all groups except between XY and XYLp, indicating distinct bacterial compositions between the XY/XYLp groups and the other treatments. According to the Upset and Venn diagrams, 127 OTUs were common to all groups, while the numbers of unique OTUs were 514, 105, 224, 106, and 64 in the FM, CK, Lp, XY, and XYLp groups, respectively (Figure 1E). These results demonstrate that the different treatments strongly shaped distinct microbial communities, leading to clear bacterial differentiation.

Bacterial community structure and dynamics are closely associated with the fermentation quality and nutritional characteristics of silage [38]. At the phylum level (Figure 2A), Cyanobacteria and Proteobacteria dominated the FM group. After ensiling, Cyanobacteria were gradually replaced by Firmicutes. All additive-treated groups showed higher relative abundances of Firmicutes than the CK group, with the Lp group exhibiting the highest relative abundance of Firmicutes and the lowest of Proteobacteria. In addition, Proteobacteria displayed higher relative abundances in the CK, XY, and XYLp groups. Xiong (2022) [38] reported that Firmicutes are key microorganisms in silage fermentation, as most lactic acid-producing bacteria belong to this phylum. A significantly higher average relative abundance of Firmicutes and Proteobacteria was observed in the Lp group compared to other groups in this study. Although other phyla were less abundant, they still played roles in the ensiling process, which aligns with previous findings [38,39]. The microbial composition at the genus level (Figure 2B) underwent a successional shift: the FM group was initially dominated by the plant pathogenic genera *Xanthomonas* and *Pseudomonas*, which were subsequently replaced by *Lactobacillus* after ensiling. The Lp group showed the highest relative abundance of *Lactobacillus* along with a low abundance of *Xanthomonas*, while the XY and XYLp groups were dominated by both *Lactobacillus* and *Xanthomonas*. Notably, compared to the Lp group, the relative abundance of *Xanthomonas* increased in the XYLp group. Yang (2024) [40] reported that *Xanthomonas* was initially low in fresh material but increased rapidly under additive treatments, which is consistent with the present findings. A possible explanation is that *L. plantarum* suppressed a large number of aerobic bacteria in the early fermentation stage, leading to a rapid decline of *Xanthomonas* in the Lp group. In contrast, in the xylanase-supplemented groups, the substantial release of sugars during early fermentation intensified microbial competition, which may have unexpectedly eliminated some key competitors of *Xanthomonas*, thereby facilitating its proliferation [40]. Furthermore, *Xanthomonas* pathogens possess an extensive genetic repertoire (at least 160 genes) associated with plant cell wall degradation and modification, comparable to that observed in other dedicated biomass-degrading bacteria such as *Ruminococcus albus* and *Clostridium cellulolyticum*. This genetic capacity explains the notable abundance of *Xanthomonas* in the XY and XYLp groups, as their inherent fibrolytic ability provides a competitive advantage under conditions of enhanced fiber substrate availability [41].

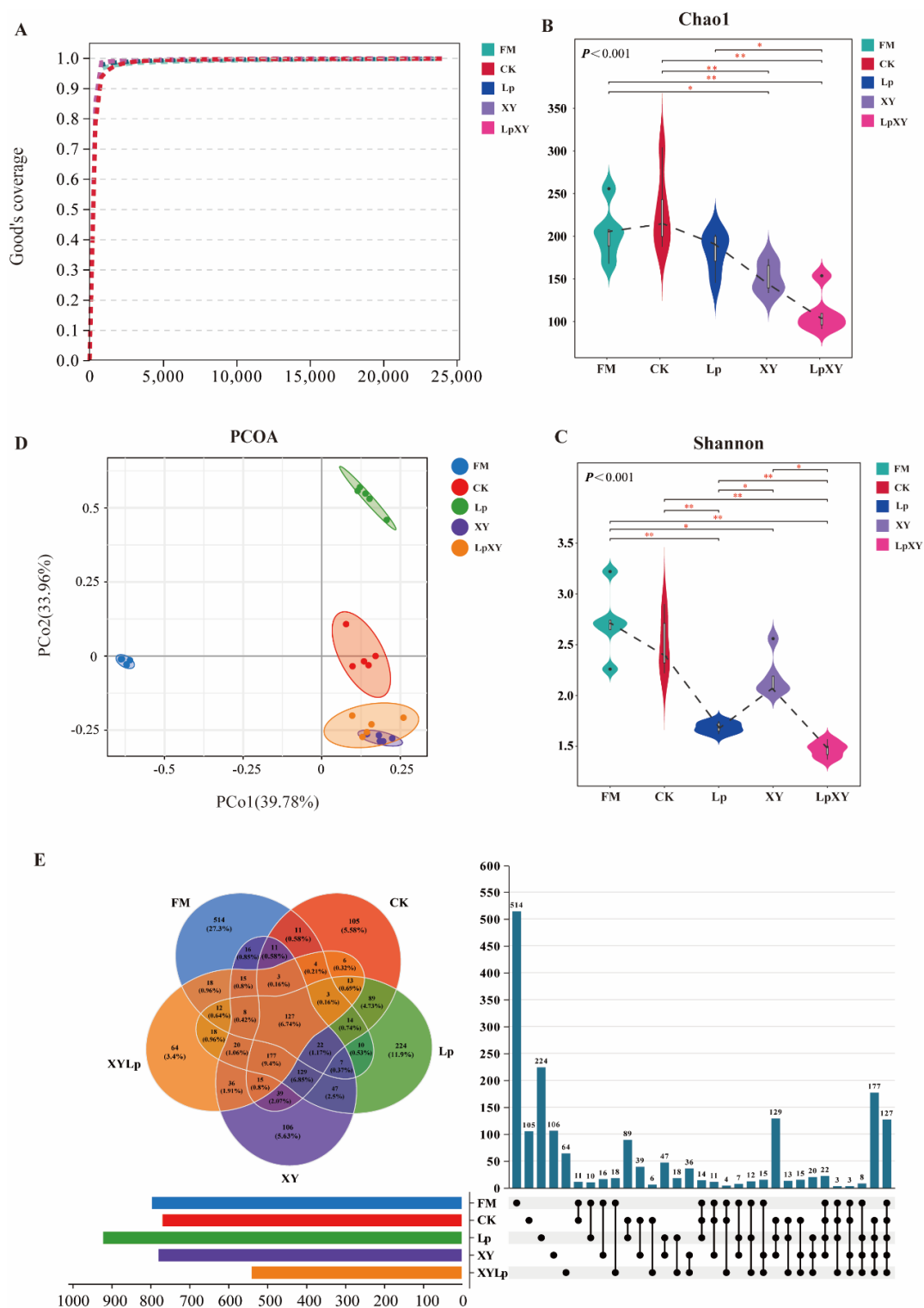


Figure 1. Illustrates the effects of different additives on the microbial community structure of rapeseed straw silage. Panel (A) displays the Good's coverage, highlighting the sequencing depth; Panels (B,C) show the differences in the Chao1 index and Shannon index among groups, respectively; Panel (D) presents the Principal Coordinates Analysis (PCoA) based on Bray–Curtis distances, revealing compositional differences between treatments; Panel (E) shows Venn and Upset plots, emphasizing the common and unique Operational Taxonomic Units (OTUs) between fresh material and silage. Asterisks denote statistical significance: * $p < 0.05$, ** $p < 0.01$.

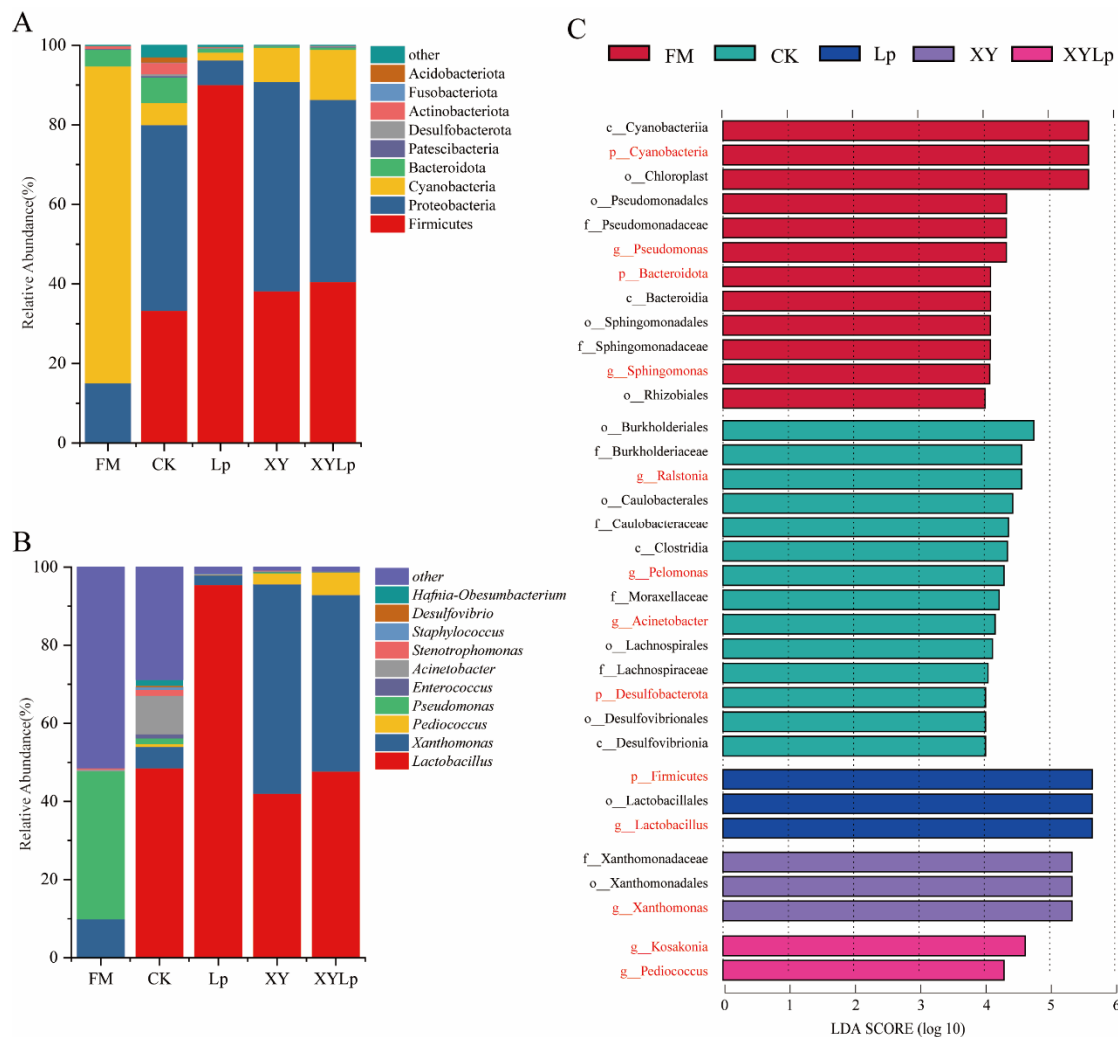


Figure 2. The relative abundance of microbial taxa is displayed at the bacterial phylum level (Panel A) and bacterial genus level (Panel B), indicating shifts in community structure. Panel C employs the LEfSe (LDA Effect Size) tool to identify statistically significant microbial biomarkers in the mixed silage. Red-marked taxa are the key phyla and genera with significant differences.

Bacterial community differences in rapeseed straw silage under different treatments were analyzed by LEfSe (Figure 2C). Compared with the silage groups, the FM group showed significant enrichment of Cyanobacteria, *Pseudomonas*, Bacteroidota, and *Sphingomonas*, which are generally considered detrimental or unfavorable microorganisms in silage fermentation [42–45]. In the CK group, significant enrichment was observed for *Ralstonia*, *Pelomonas*, *Acinetobacter*, and Desulfobacterota. While Zhao et al. reported a negative correlation between *Acinetobacter* and pH and a positive correlation with lactic acid (LA) content, the present study did not align with these findings [34]. A possible explanation lies in the delayed initiation of fermentation in the CK group of rapeseed straw silage, where residual oxygen and insufficient substrate competition promoted the proliferation of naturally attached aerobic/facultative anaerobic bacteria such as *Acinetobacter*. *Acinetobacter* competes with lactic acid bacteria for fermentable substrates, diverting water-soluble carbohydrates toward respiratory and other metabolic pathways rather than efficient conversion to lactic acid as in homofermentative lactic acid bacteria. This directly resulted in reduced lactic acid production [46].

A significant enrichment of Firmicutes and *Lactobacillus* was observed in the Lp group. Firmicutes, as the dominant phylum in anaerobic fermentation, collectively contribute to the establishment of an acidic environment, while *Lactobacillus* efficiently and rapidly

generates lactic acid, directly driving the decrease in pH [47,48]. The significant negative and positive correlations of *Lactobacillus* with pH and LA content, respectively, are in agreement with earlier studies [49,50]. In the XY group, *Xanthomonas* was significantly enriched. Correlation analysis revealed significant negative correlations between this genus and ADF, ADL, cellulose, hemicellulose, and holocellulose contents, providing clear evidence of its role in fiber degradation. The XYLp group showed significant enrichment of *Kosakonia* and *Pediococcus*. Significant negative correlations were observed between *Pediococcus* and ADF, ADL, cellulose, hemicellulose, and holocellulose, indicating its potential to degrade fibrous materials in rapeseed straw silage. Studies have shown that *Pediococcus acidilactici* possesses a superior growth rate and the ability to produce lactic acid in high-pH media compared to other strains. These traits enable it to inhibit spoilage microorganisms, ultimately improving fermentation quality and enhancing fiber degradability [51,52]. Previous research has demonstrated that using *Pediococcus* alone in silage induces a rapid initiation of fermentation in the early stage [35,53]. The combination of *Pediococcus* and cellulase may serve as an effective approach for enhancing silage fermentation quality and fiber degradability [54] (Figure 3).

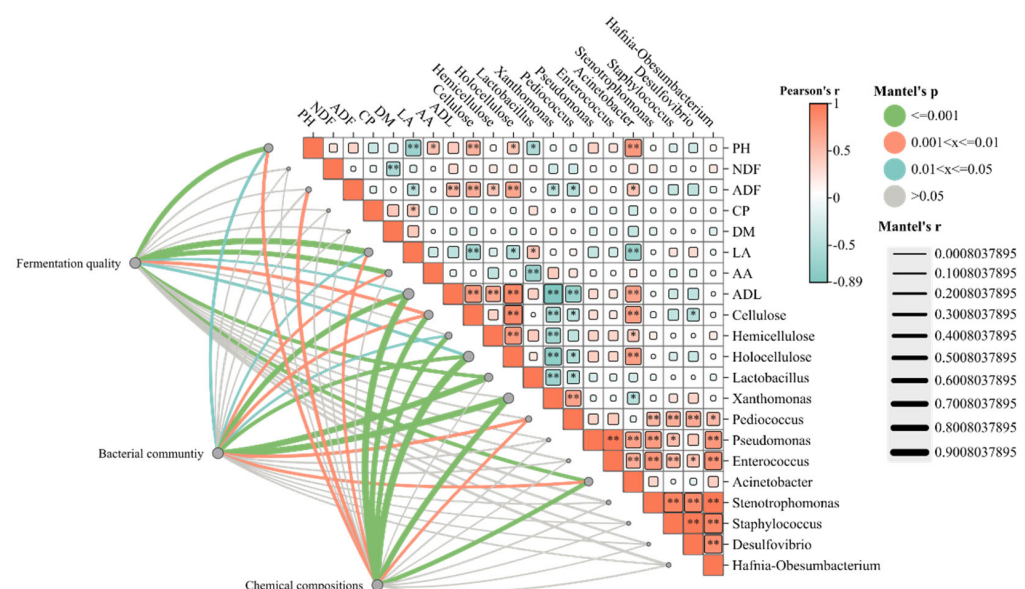


Figure 3. Mantel analysis of nutrient composition, fermentation parameters, and bacterial communities in rapeseed straw silage. Red indicates positive correlations; green indicates negative correlations. Asterisks denote statistical significance: * $p < 0.05$, ** $p < 0.01$. The square size represents the magnitude of correlation coefficients, while color intensity reflects the strength of the correlation.

3.3. Bacterial Community Function Prediction Analysis

Predicting functional changes in bacterial communities helps assess their impact on silage quality. Therefore, PICRUSt2 was employed to analyze the relative abundance of KEGG pathways in the bacterial community. At pathway level 1, metabolism was the dominant category, indicating that the ensiling process is primarily mediated by microbial metabolic pathways that convert fermentable substrates into various metabolites. At pathway level 2, the most abundant functional categories were identified as carbohydrate metabolism, amino acid metabolism, nucleotide metabolism, energy metabolism, and metabolism of cofactors and vitamins. According to Bai et al., these metabolic pathways are closely linked to the fermentation quality of silage [53]. At pathway level 3, this study found significant enrichment of glycolysis/gluconeogenesis in the XYLp group. A possible explanation is that xylanase supplementation degraded fibrous structures, providing *L. plantarum* with more abundant fermentable substrates and creating a more favorable

environment for its growth and metabolism. This strongly stimulated the glycolytic activity of *L. plantarum*, ultimately leading to the significant increase in the relative abundance of this pathway in the functional predictions of the microbial community [55,56]. The MAPK signaling pathway—yeast was enriched in the XY and XYLp groups. When yeast cells or other microorganisms perceive drastic environmental changes, they activate different MAPK pathways, triggering survival responses such as cell wall repair. This mechanism may contribute to nutrient preservation during ensiling, though it was not the focus of the present study and warrants future investigation. In addition, functional annotation of the FM group revealed significant activity in the nitrogen metabolism pathway, which is considered beneficial for silage. The production of harmful substances like ammonia and biogenic amines, resulting from nitrogen metabolism during ensiling, leads to true protein loss. This not only diminishes nutritional value and palatability but may also pose risks to animal health [57,58]. The bacterial secretion system pathway was significantly annotated in the XY and XYLp groups. Combined with LEfSe analysis, this enrichment was closely associated with *Xanthomonas*, *Kosakonia*, and *Pediococcus*. This suggests that these taxa may competitively secrete enzymes or other metabolites through an active secretion system, thereby gaining an ecological advantage in the silage environment (Figure 4).

3.4. Enzyme Function Prediction of Microbial Communities Using the KEGG Database

Starch and sucrose metabolism pathway was significantly annotated in XY and XYLp. Its core function involves the synthesis and degradation of two major storage polysaccharides, starch and glycogen, as well as their substrate sucrose, among which the pathways related to fiber degradation are particularly important. In this experiment, the expression of Cellulase (EC: 3.2.1.4) in the XY and XYLp groups were significantly higher than in the other groups ($p < 0.05$). Cellulase (EC: 3.2.1.4) is a key hydrolase that converts Cellulose into Cellodextrin and further into Cellobiose, playing an indispensable role in Starch and sucrose metabolism. Additionally, the expression of Beta-glucosidase (EC: 3.2.1.21) in the XYLp group was higher than in all other groups except the XY group. This enzyme is involved in two pathways within this metabolic process: one decomposes Cellodextrin into Cellobiose, and the other decomposes Cellobiose into D-Glucose. The latter is a critical step in the complete degradation of polysaccharides into monosaccharides, which can be utilized by microorganisms. In this study, the expression of Beta-glucosidase in the XYLp group was significantly higher than in the Lp group, indicating that the combined addition of xylanase and *L. plantarum* (XYLp) synergistically promotes more thorough cellulose degradation, with a superior effect compared to the sole addition of *L. plantarum* (Lp). This may more effectively drive the overall fermentation process and provide lactic acid bacteria with more abundant fermentation substrates (glucose).

Endo-1,4-B-xylanase (EC: 3.2.1.8) is involved in the reaction process where the internal (1→4)-β-D-xylosidic linkages in xylan undergo hydrolytic cleavage. The expression level in the XYLp group was significantly higher than that in the Lp group, indicating that the interaction between the bacterium and the enzyme is synergistic in the silage environment. This synergy enhances the hydrolysis of xylosidic linkages by increasing the expression of Endo-1,4-B-xylanase.

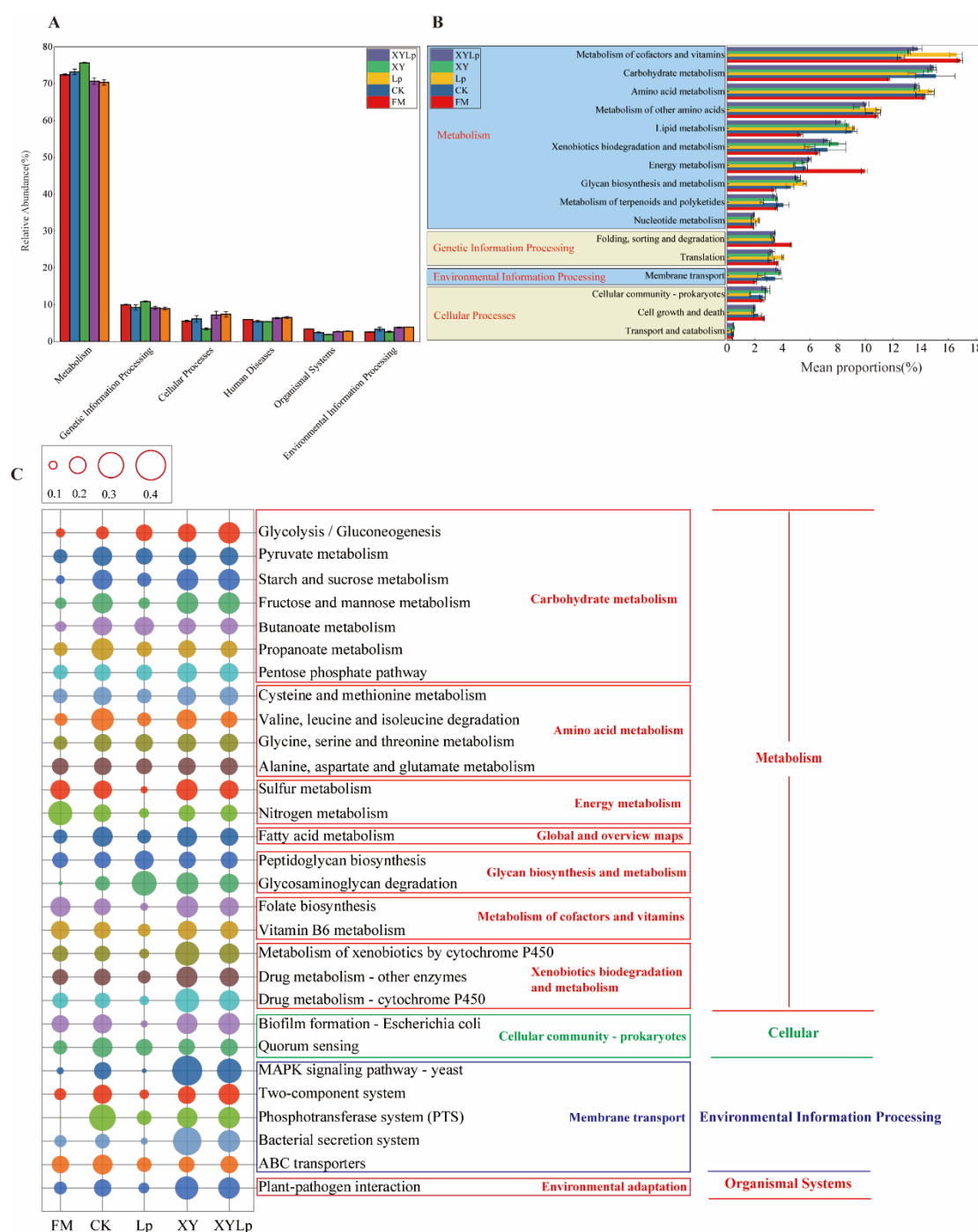


Figure 4. PICRUSt2 analysis of dynamic changes in bacterial functional profiles under different treatments (n = 3). (A) Level 1 metabolic pathways. (B) KEGG ortholog functional predictions at Level 2, showing the relative abundance of the top 16 significantly differential metabolic functions. (C) KEGG functional predictions at Level 3, displaying the relative abundance of the top 29 metabolic functions.

Feruloyl-esterase (EC: 3.1.1.73) participates in the reaction that hydrolyzes feruloyl-polysaccharide into ferulate and polysaccharide. In this experiment, the FM group exhibited the highest expression level of Feruloyl-esterase. A possible reason is that the naturally attached and diverse microbiota in the fresh forage underwent a brief but intense metabolic burst shortly after harvesting, producing high levels of Feruloyl-esterase. In contrast, the additive treatments rapidly established an acidic-dominated fermentation system, which suppressed the activity of these indigenous microorganisms. Consequently, the overall enzyme activity detected was lower than the initial levels.

Alpha-L-arabinofuranosidase (EC: 3.2.1.55) and Xylan 1,4-beta-xylosidase (EC:3.2.1.37) are two decomposing enzymes involved in the Amino sugar and nucleotide sugar metabolism pathway. The primary pathway of Alpha-L-arabinofuranosidase is the decomposition of Arabinan into L-Ara. L-Ara is often linked with arabinose and serves as side chains in the hemicellulose of the cell wall. The annotation of this enzyme indicates that the side chains connected to the xylan backbone in the plant cell walls within the silage can be effectively degraded. In this experiment, the expression level of this enzyme in the XY group was the highest and significantly higher than in all other groups ($p < 0.05$). There was no significant difference between the Lp group and the XYLp group, indicating that the bacterial–enzyme synergy had little impact on this pathway. The pathway involving Xylan 1,4-beta-xylosidase is the decomposition of 1,4-B-Xylan into Xyl. Xyl, as the backbone of hemicellulose in the cell wall, is a key indicator determining whether the cell wall is degraded. In this experiment, the expression level of Xylan 1,4-beta-xylosidase in the XYLp group was significantly higher than in all other groups except the XY group. This indicates that the bacterial–enzyme synergy can effectively break down the cell wall, thereby releasing the cell contents and providing favorable conditions for the silage (Figure 5).

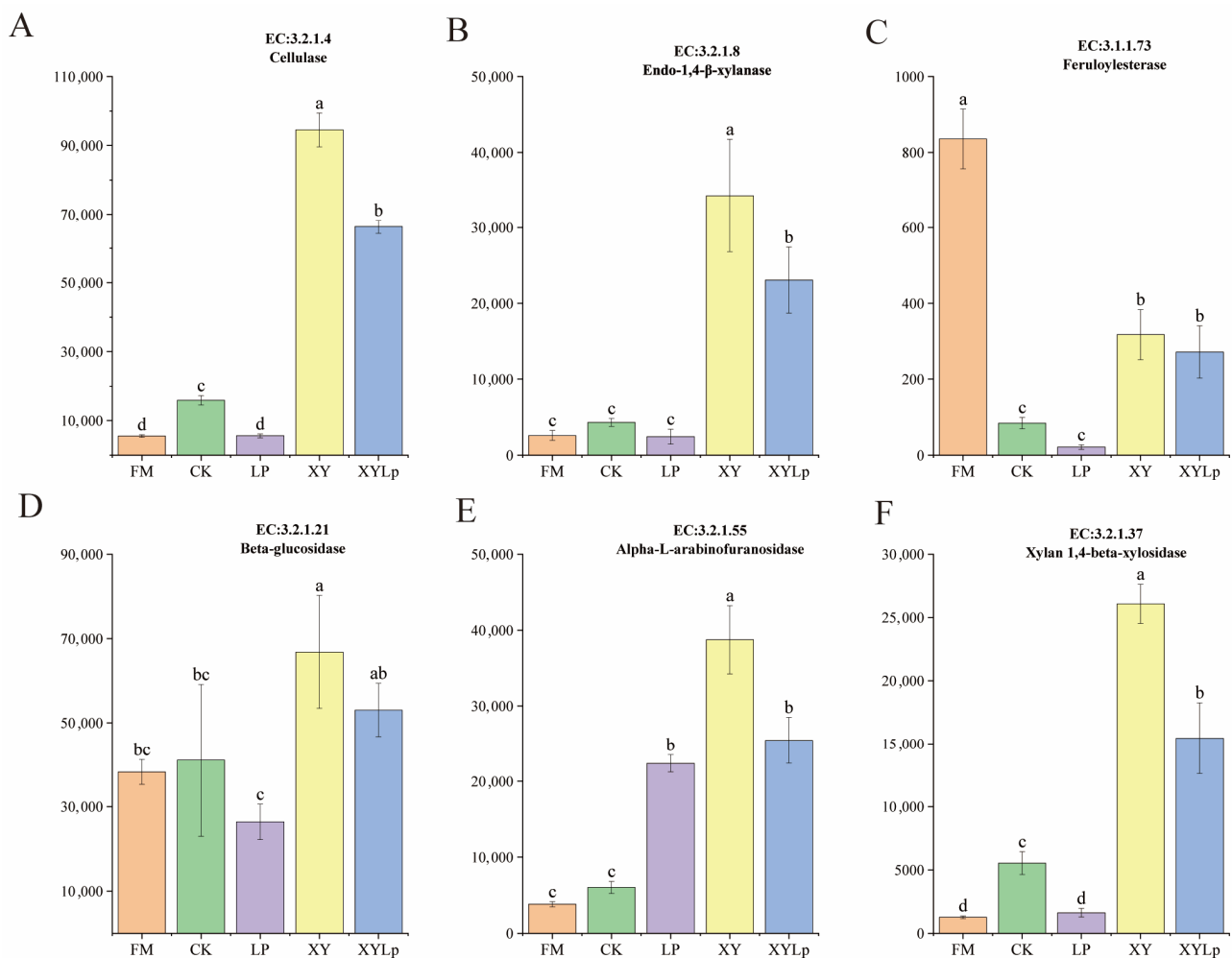


Figure 5. Changes in key enzymes of the cellulolytic bacterial community during silage fermentation of rapeseed straw with additives. (A): Cellulase; (B): Endo-1,4-β-xylanase; (C): Feruloyl esterase; (D): Beta-glucosidase; (E): Alpha-L-arabinofuranosidase; (F): Xylan 1,4-beta-xylosidase. The error bars in the bar chart represent standard deviation (SD), There are significant differences ($p < 0.05$) in the average values between different letters.

4. Conclusions

This study confirmed that the addition of *Lactiplantibacillus plantarum* or xylanase alone can improve the quality of rapeseed straw silage, but the combined bacterium–enzyme treatment (XYLp) showed the greatest potential in degrading the contents of ADF, NDF, cellulose, hemicellulose, and holocellulose. This bacterium–enzyme co-regulation promoted the proliferation of key fibrolytic genera (*Kosakonia*, *Pediococcus*) and upregulated the expression of critical fibrolytic enzymes, leading to the effective deconstruction of plant cell walls. Future research can combine methods such as metagenomics to further reveal the molecular mechanisms and regulatory networks of bacterial–enzyme synergistic fiber degradation.

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