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Novel mechanistic understanding that *Lactiplantibacillus plantarum* is more capable of improving the ensiling performance of wheat straw silage than xylanase by driving certain key metabolites

Haoran Yu[1,2], Richa Hu[3], Yushan Jia[1], Yanzi Xiao[3*] and Shuai Du[1*]

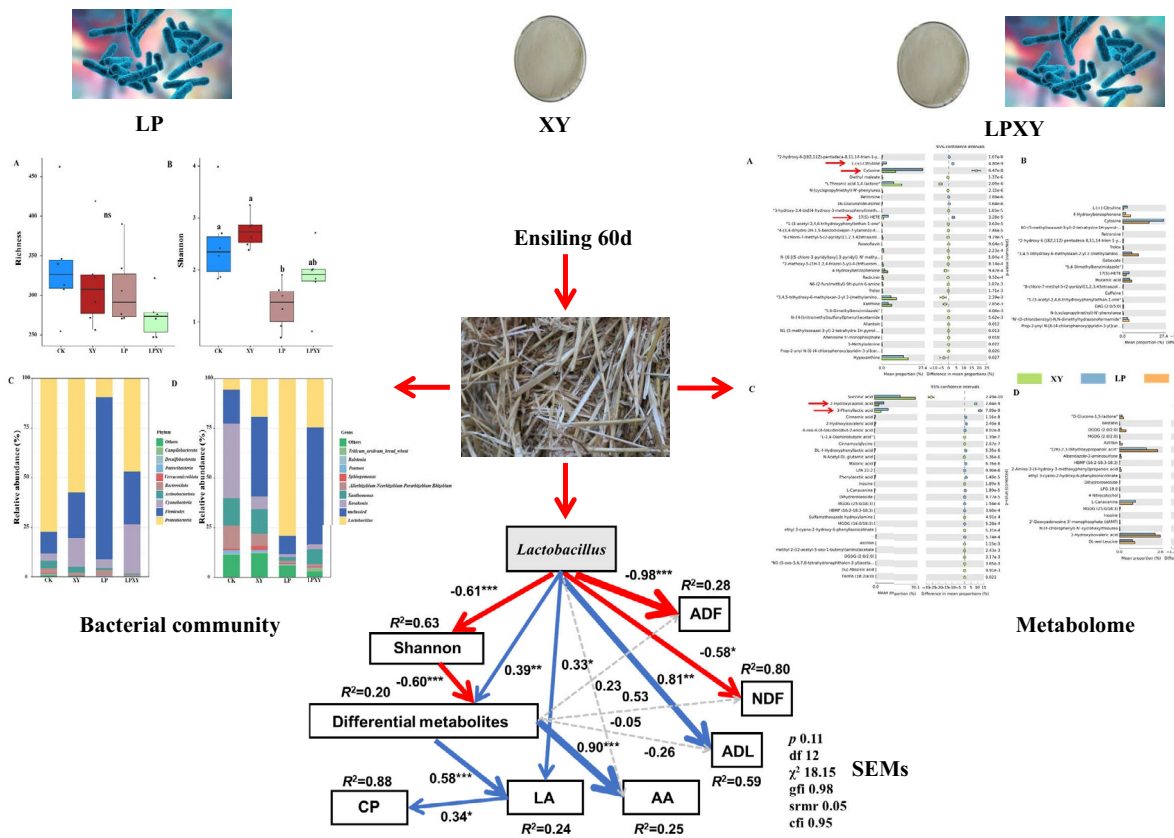
Abstract

Microbial and enzyme additives can improve silage performance, but there is limited comparative research on the effects of microbial and enzyme additives on improving silage fermentation quality, and the underlying microbial and metabolic pathways remain unclear. This study investigated the effects without inoculants (CK treatment) or with *Lactiplantibacillus plantarum* (LP treatment), xylanase (XY treatment) and their combination (LPXY treatment) on the fermentation quality, as well as on the microbial communities and metabolite profiles of the wheat straw silage. The results demonstrated that the LP treatment has a better effect on improving the fermentation quality of wheat straw silage compared to other treatments, as evidenced by markedly ($p < 0.05$) decreased the pH (4.06), acid and neutral fiber (ANF, NDF, 23.43 and 31.69%DM), and increased the lactic acid (LA, 965.89 mg/L) and acetic acid (AA, 656.10 mg/L) concentrations. After the fermentation process, the LP treatment significantly ($p < 0.05$) enhanced the abundance of *Lactobacillus*, reduced bacterial Shannon ($p < 0.05$) and increased some key metabolites content. The structural equation models (SEMs) and Pearson's correlation results proved that the LP treatment improved the wheat straw silage fermentation quality via increasing the abundance of *Lactobacillus*, decreasing the diversity of bacterial community and enriching the content of certain key metabolites. The present study provides mechanistic evidence that *Lactiplantibacillus plantarum* additive is superior to xylanase additive and their combination on improving fermentation quality of wheat straw silage, that is, by enriching certain key metabolites to increase AA and LA concentrations, providing a reference for the cross study of silage feed fermentation microbiome and metabolome.

Keywords Wheat straw, Ensiling, Microbiome, Metabolome, Fermentation quality

*Correspondence:
Yanzi Xiao
xiaoyz1113@126.com
Shuai Du
dushuai_nm@sina.com
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Wheat (*Triticum aestivum* L.) straw is a common agricultural residue utilized as ruminant feed owing to its high carbohydrate content [1]. Ensiling, as a method for large-scale preservation of wet materials, reduces dry matter loss in feed and has increasingly become a long-term utilization strategy for wheat straw [2]. Compared with other storage, ensiling not only improves biodegradability but also saves costs [1]. Nevertheless, wheat straw typically possesses low water-soluble carbohydrates (WSC) and lacks epiphytic lactic acid bacteria, making it challenging to produce high-quality silage through natural anaerobic fermentation [3]. Silage microbial additives are commonly employed in feed production to effectively enhance fermentation quality [4]. Lactic acid bacteria inoculants have the capability to rapidly accumulate lactic acid and lower pH during the early stages of silage, thereby enhancing fermentation quality [5]. Xylanase (XY) can improve the fermentation quality and rumen digestion rate of silage feed [6]. However, there is little research on whether the mixed additions of lactic acid bacteria and XY have a synergistic effect, as well as the comparative study of the two types of additives on the improvement of wheat straw silage quality.

Xylan is among the hemicelluloses that are not fully utilized in the rumen, leading to the inefficient utilization of feed energy [7]. XY can disrupt its internal structure, release soluble sugars, increase the concentration of fermentation substrates, and improve feed utilization efficiency [8]. Homofermentative bacteria (e.g., *Lactiplantibacillus plantarum*) are widely used as they are safe and easy to use [9]. Homofermentative bacteria can reduce the loss of silage fermentation quality by directly increasing lactate and acidification rates [9]. Therefore, both two types of additives may enhance the fermentation quality of wheat straw silage. Both additives can improve the quality of silage feed, but using one additive alone may have limitations. For example, lactic acid bacteria additives directly provide an increase in lactic acid, but ignore the energy required by microorganisms (like

WSC), therefore, mixed additives may have a synergistic effect on improving silage quality.

Silage is a fermentation process dominated and driven by microorganisms, so changes in microbial communities are usually related to fermentation quality [10]. Understanding the microbial community's contribution to silage feed not only provides insights into high-quality feed preparation techniques, but also helps maintain the quality of silage feed [11]. Lactic acid bacteria are considered beneficial bacteria in the production of organic acids such as lactic acid (LA) and are key to ensuring high-quality silage feed [12]. Throughout the silage fermentation process, the production of harmful bacteria like *Listeria* sp. and *Clostridia* can diminish feed quality [13]. The microbial community diversity of silage feed includes both beneficial and harmful bacteria [14]. Therefore, changes in lactate content in silage feed typically regulate microbial diversity. An increase in lactic acid bacteria content within the microbial community tends to reduce microbial diversity [15]. While some research has investigated the effects of microbial additives on microbial community changes during ensiling fermentation, there remains a lack of mechanistic understanding, particularly concerning microbial community changes in oat ensiling with lactic acid bacteria and XY additives [11, 16].

In addition to microbial community succession, the fermentation process of wheat straw silage also involves changes in metabolites and metabolic pathways [17]. The quality of feed fermentation is closely related to the relationship between silage microorganisms and metabolites, and fermentation quality is usually driven by microorganisms and metabolites [18]. Recently, metabolomics has been applied to the study of silage ecosystems [16, 19, 20], and these results indicate a strong interaction between the metabolites and microorganisms. Since the fermentation process of silage is dominated by microorganisms, microorganisms also determine the changes in metabolic products, ultimately affecting the fermentation quality [21]. However, there is a lack of comprehensive understanding regarding the microbial community's role in driving metabolic product changes in silage ecosystems, and the pathways through which microbes drive such changes and enhance fermentation quality remains elusive.

To date, there is limited literature on the fermentation quality and microbial community changes of wheat straw with the additions of XY and *Lactiplantibacillus plantarum* (LP), and the potential synergistic effect of combining these two additives remains unclear. And there has been no investigation into how the microbial community of silage influences metabolic products to enhance fermentation quality. The aim of the present study was to identify microbial additives that enhance the fermentation pathway and pathways of wheat straw based on metabolomic and microbiome analyses, and to assess their potential synergistic effect.

Materials and methods

Substrate and silage

Wheat straw was originated from the Hulunber Grassland Ecosystem National Observation and Research Station of the Chinese Academy of Agricultural Sciences in Hulunber, Inner Mongolia, China (E 119° 55′, N 49° 19′). The climate zone belongs to the temperate semi-humid zone, with an average annual precipitation of 380–400 mm, an average annual temperature of −2 °C to −1 °C and a humidity level of 0.49–0.50. The wheat straw was harvested at the late maturity stage, then chopped and immediately transferred to the laboratory for silage making. XY (total xylanase activity of 50,000 U/g) purchased from Hefei Bomei Biotechnology Co., Ltd, Hefei, China (BBMO831, size 500 g). *Lactiplantibacillus plantarum* purchased from Jiangsu Lvke Biotechnology Company, Gaoyou, China. The treatments were as follows: control (CK), XY, LP and LPXY (mixed LP and XY). The inoculants were diluted in distilled water and added according to the manufacturer's guidelines, the amount of XY was 100 U/g of FM, the LP was supplemented at 1.0×10^5 cfu/g of FM, the CK treatment was also treated with the same volume of distilled water. Both fresh materials wheat straw and wheat straw silage were stored at a small-scale fermentation system (260 cm × 380 cm; Hiryu KN type; Asahi kasei, Tokyo, Japan). A 200 g of the samples were packed into the polyethylene plastic bag, and removing air with a vacuum sealer (N-14886, Deli Group Co., Ltd., Zhejiang, China). A total of 24 bags (4 treatments × 6 replicates) of wheat straw were stored at room temperature (23 ± 2 °C). After 60 days of fermentation process, these bags were opened and the ensiling performance, bacterial community and metabolites profiles were analyzed [22].

Ensiling performance and nutritive values analyses

Clean containers were used to collect FM and wheat straw silage after being uniformly blended for ensiling performance and nutritive values analyses. The dry matter (DM) content of the FM and silage samples were measured after drying the sample for 72 h at 65 °C with an oven [12]. The dried samples were ground and through a 1-mm screen for the nutritive values analysis. The crude protein (CP) and acid detergent lignin (ADL) contents were analyzed according to the method of the Association of Official Analytical Chemists (AOAC, 2005). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by an ANKOM A200i Fiber Analyzer (ANKOM Technology,

Macedon, NY, USA) with the report [23]. The fraction of cell wall constituents, including cellulose, hemicellulose and holocellulose was calculated using methods briefed by [24]. The anthrone method was selected to evaluate the WSC content [25]. A 20 g of the wheat straw silage samples were mixed with 180 mL sterile water and stored for 24 h at 4 °C fridge, then the extracts were filtered through four layers of cheesecloth. A glass-electrode pH meter was used to measure the pH value of the filtrate (PHSJ-5; LEICI, Shanghai, China). The organic acids concentrations in the filtrate, mainly lactic acid, acetic acid, propionic acid and butyric acid, were measured by the high-performance liquid chromatography methods (Thermo, Ultimate 3000LC, Q Exactive) [26].

Microbial analysis

The genomic DNA of bacterial community was extracted from the FM and wheat straw silage samples by the CTAB method. The Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the concentrations and qualities of the extracted genomic DNA. The V3–V4 regions of 16S rDNA gene was targeted with the universal primer pair 341F and 806R. The Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) was used to sequence. The raw pair-end reads were analyzed by the Qiime2 platform (https://qiime2.org/). Amplicon sequence variants (ASVs) were obtained by eliminating low-quality data using DADA2 [27]. Subsequently, the ASVs were taxonomically annotated against the SILVA database (https://www.arb-silva.de/, Release 138) using mothur [28].

Metabolites profiles analyses

The metabolites in the wheat straw silage samples were extracted according to the previous methods, dissolved with 2-chlorobenzalanine methanol solution (150 μL), and filtered through a 0.22 μm membrane for LC–MC analysis [29]. The raw data files of the 24 wheat straw silage samples were generated by the liquid chromatography–mass spectrometry (LC–MS) platform (Thermo Fisher, Ultimate 3000LC, Q Exactive) using the compound Discover 3.1 (CD 3.1 Thermo Fisher) to perform peak picking, peak alignment and quantitation for each metabolite. After that, peak intensities were normalized to the total spectral intensity [30]. The normalized data were used to predict the molecular formula based on additive ions, molecular ion peaks and fragment ions [31]. Then peaks were matched with the mzCloud (https://www.mzcloud.org/) mz Vault and Mass List database to obtain the accurate qualitative and relative quantitative results. After mean centering and unit variance scaling, the principal component analysis (PCA) and (orthogonal) partial least squares discriminant analysis (O)PLS-DA were selected to show the differences of the metabolites among the treatments by R package (prcomp). The variable importance in the projection (VIP) ranks and VIP > 1.7 were considered as the relevant for treatment discrimination, and the results were displayed by the (O)PLS-DA plots. The plots package in R (version 4.3.2) was used for significant metabolites for expression pattern clustering using. Hierarchical clustering method was used for distance calculation algorithms. The metabolites set enrichment was analyzed with the Stats package in R and the SciPy package in Python using the MetaboAnalyst 6.0 (https://www.metaboanalyst.ca).

Statistical analysis

The chemical compositions and bacterial alpha diversity (Shannon, Richness) data of wheat straw and wheat silage were analyzed by a one-way ANOVA and Kruskal–Wallis test (HC and HoC do not follow a normal distribution). Tukey's test (follows a normal distribution) and Fisher's test (does not follow a normal distribution) were utilized to assess significant differences in comparisons at the 5% level. To better characterize the differences between genera, the linear discriminant analysis (LDA) effect size (LEfSe) with relative abundance data was utilized to assess the significance. To compare functional profiles among groups, metabolites from different treatments were analyzed using Duncan test; Duncan's test has a lower probability of error for metabolic product data. We correct for the false discovery rate by controlling FDR (false discovery rate) to not exceed 5%. Five differential metabolites were ultimately identified (enriched in LP), including positive and negative. To determine the relationship between differential metabolites and fermentation quality, Pearson's correlation analysis was conducted, using $\log_{10}$-transformed to differential metabolites. Structural equation modeling (SEMs) was employed to evaluate directly and indirectly effect of fermentation quality (CP, LA, AA, ADL, ADF, and NDF), including bacterial community and differential metabolites. Unlike regression or ANOVA, SEMs offers the ability to separate multiple pathways of influence and view them as parts of a system, and thus is useful for investigating the relationship complex networks found in silage fermentation ecosystems [32]. We calculated the standardized of all index in SEMs. All the data were analyzed using open-source tools for R software, packages including vegan, *piecewiseSEM*, ggplot2 and Microeco (version 4.3.2).

Result

Chemical characteristics and microbial population of raw materials

The DM of the fresh wheat straw was 50.30%, ADF, NDF, CP contents were 29.50, 37.10 and 12.90%DM, respectively (Table 1). The counts of lactic acid bacteria, yeasts, aerobic bacteria, and coliform bacteria were 4.27, 8.47, 8.35, 8.40 and 8.32 log10 cfu/g of FM, respectively.

Table 1 Chemical and microbial characteristics of substrates before ensiling

Items	Wheat straw
Dry matter (%)	50.30
Water-soluble carbohydrates (% DM)	3.42
Crude protein (% DM)	12.90
Acid detergent fiber (% DM)	29.50
Neutral detergent fiber (% DM)	37.10
Lactic acid bacteria (log_{10} cfu/g FM)	4.27
Yeast (log_{10} cfu/g FM)	8.47
Aerobic bacteria (log_{10} cfu/g FM)	8.35
Coliform bacteria (log_{10} cfu/g FM)	8.40
Mold (log_{10} cfu/g FM)	8.32

DM dry matter, *cfu* colony-forming units, *FM* fresh matter

Fermentation quality of different treatments

The fermentation characteristics of the wheat straw silage with different treatments are shown in Fig. 1. All the additions significantly ($p < 0.05$) decreased the pH value. Compared to the CK and XY treatments, the LP and LPXY treatments enhanced LA and AA ($p < 0.05$). XY-treated and LAB-treated groups significantly reduced ADF, NDF and ADL ($p < 0.05$). While there was no distinction between additives and CK on the CP content in wheat straw silage.

Bacterial community of wheat straw silage

The bacterial community in the different treated silages were clearly distinguished at 60 days of wheat straw (Fig. S1). Alpha diversity of wheat straw silage bacteria is shown in Fig. 2A, B. LP-treated silage significantly reduced bacterial Shannon diversity ($p < 0.05$), while XY-treated silage and LPXY-treated silage did not exhibit a

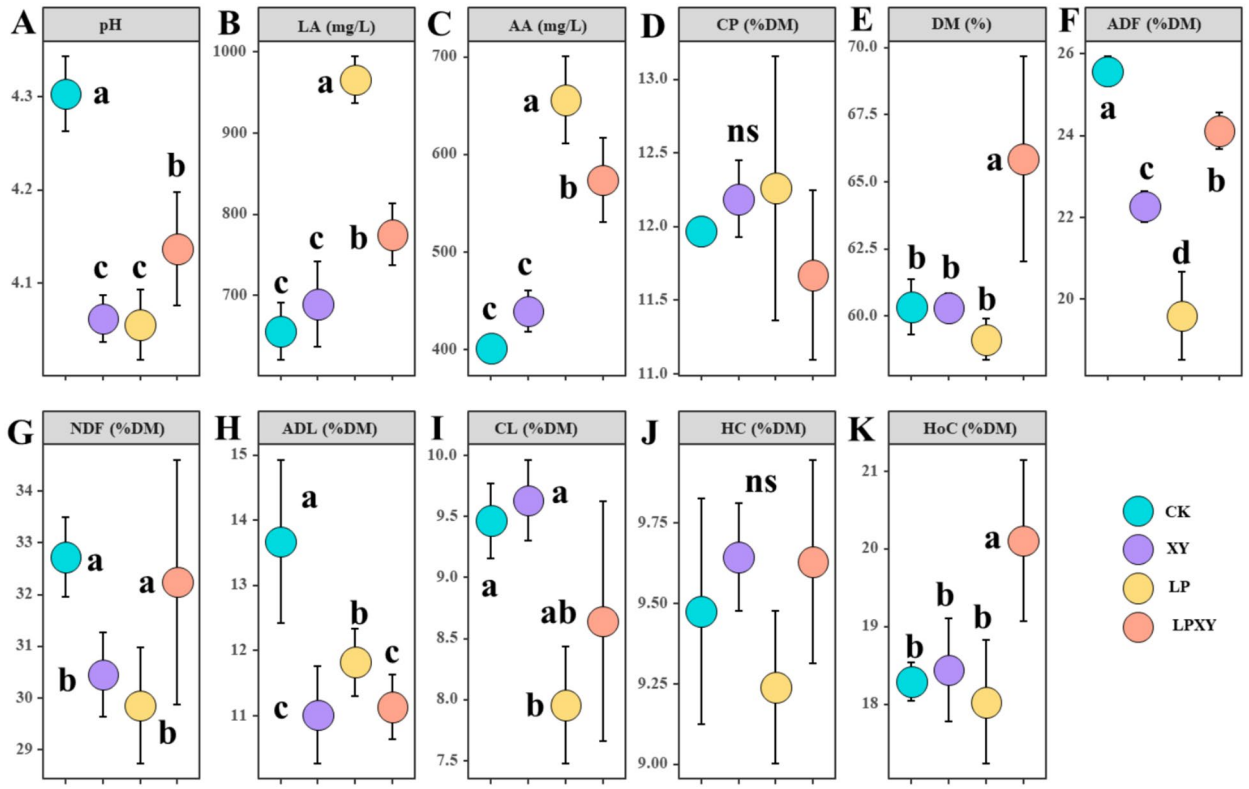


Fig. 1 Fermentation quality and chemical composition in wheat straw silage (*LA* lactic acid, *AA* acetic acid, *DM* dry matter, *CP* crude protein, *ADF* acid detergent fiber, *NDF* neutral detergent fiber, *ADL* acid detergent lignin, *CL* cellulose, *HC* hemicellulose, *HoC* holocellulose. Propionic acid and butyric acid were not detected in all wheat straw silages. *CK* control treatment, *XY* wheat straw inoculated with xylanase treatment, *LP* wheat straw inoculated with *Lactiplantibacillus plantarum* treatment, *LPXY* wheat straw inoculated with *Lactiplantibacillus plantarum* and xylanase treatment)

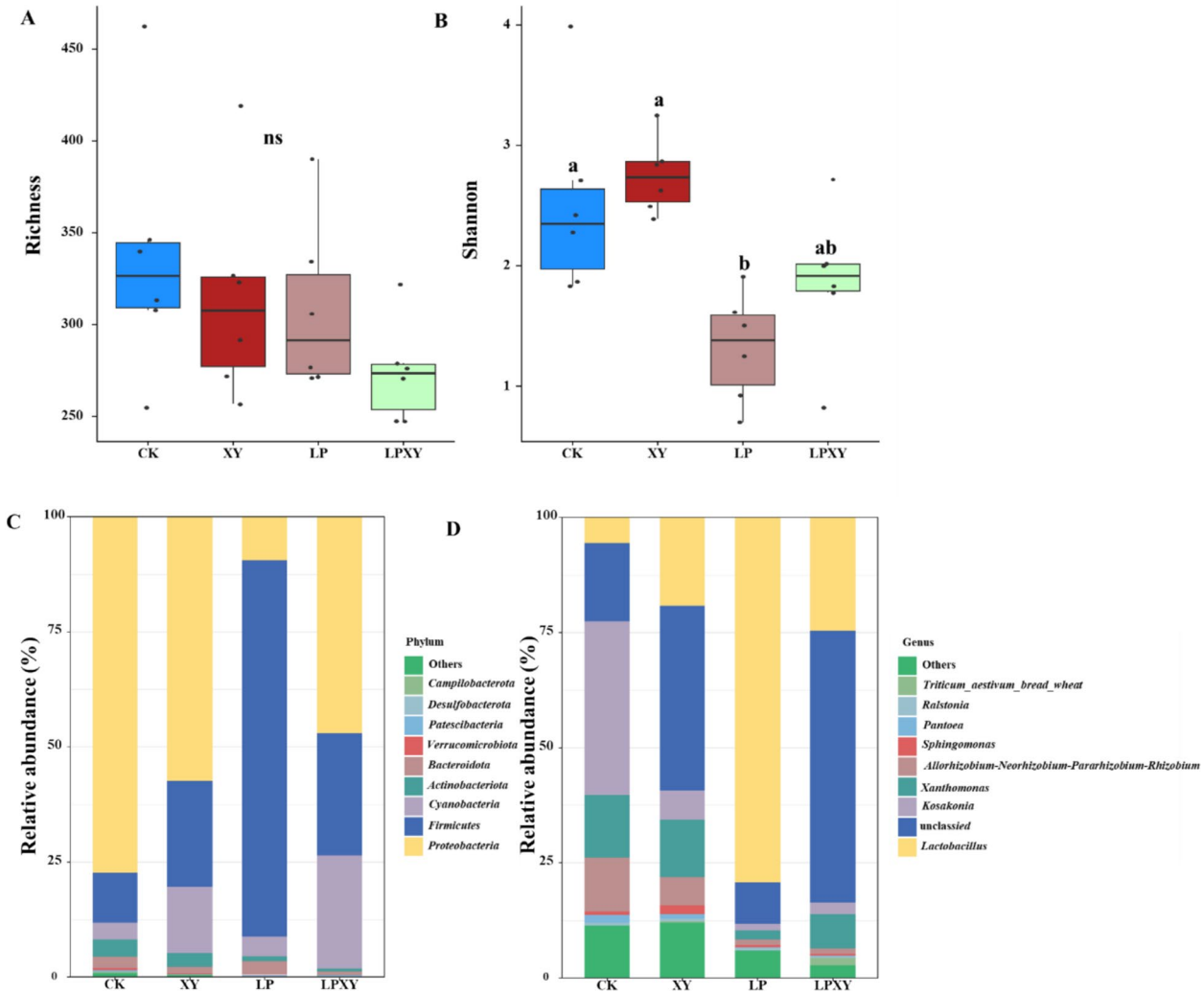


Fig. 2 Alpha diversity (**A**, **B**) and the relative abundance of bacterial phyla (**C**) and genus (**D**) of the wheat straw silage indices of wheat straw with different treatments. *CK* control treatment, *XY* wheat straw inoculated with xylanase treatment, *LP* wheat straw inoculated with *Lactiplantibacillus plantarum* treatment, *LPXY* wheat straw inoculated with *Lactiplantibacillus plantarum* and xylanase treatment

decrease in bacterial Shannon diversity compared to CK. Furthermore, all three additives had no impact on bacterial Richness.

Metabolomic profiles

Metabolomes in the different treated silages were clearly separated at 60 days of wheat straw (Fig. S2). Overall, 2557 metabolites were identified in the wheat straw silage samples. Based on Duncan tests and variable importance in projection (VIP) filtering of the relative contents of wheat straw silage samples, 57 metabolites exhibited significant differences between the two groups ($p < 0.05$ and VIP > 1.7). Among these, 30 were positively ionized metabolites (Fig. 3A, B), and 27 were negatively ionized metabolites (Fig. 3C, D), including carboxylic acids and derivatives, amino acids, peptides, analogues, and other metabolites. Specifically, three metabolites were enriched in positive ionization mode, and two metabolites were enriched in negative ionization mode in LP-treated silage. Additionally, combined with LDA analysis, the results confirmed the enrichment of five metabolites in LP-treated silage (Figs. S3 and S4).

Lactobacillus-driven fermentation quality of wheat straw

Results from the SEMs showed that 88% (CP), 24% (LA), 25% (AA), 59% (ADL), 28% (ADF) and 80% (NDF) of the variance in fermentation quality could be explained by *Lactobacillus* of wheat straw silage, respectively (Fig. 4). *Lactobacillus* had a negative and large effect on bacterial Shannon (63%), indicating a

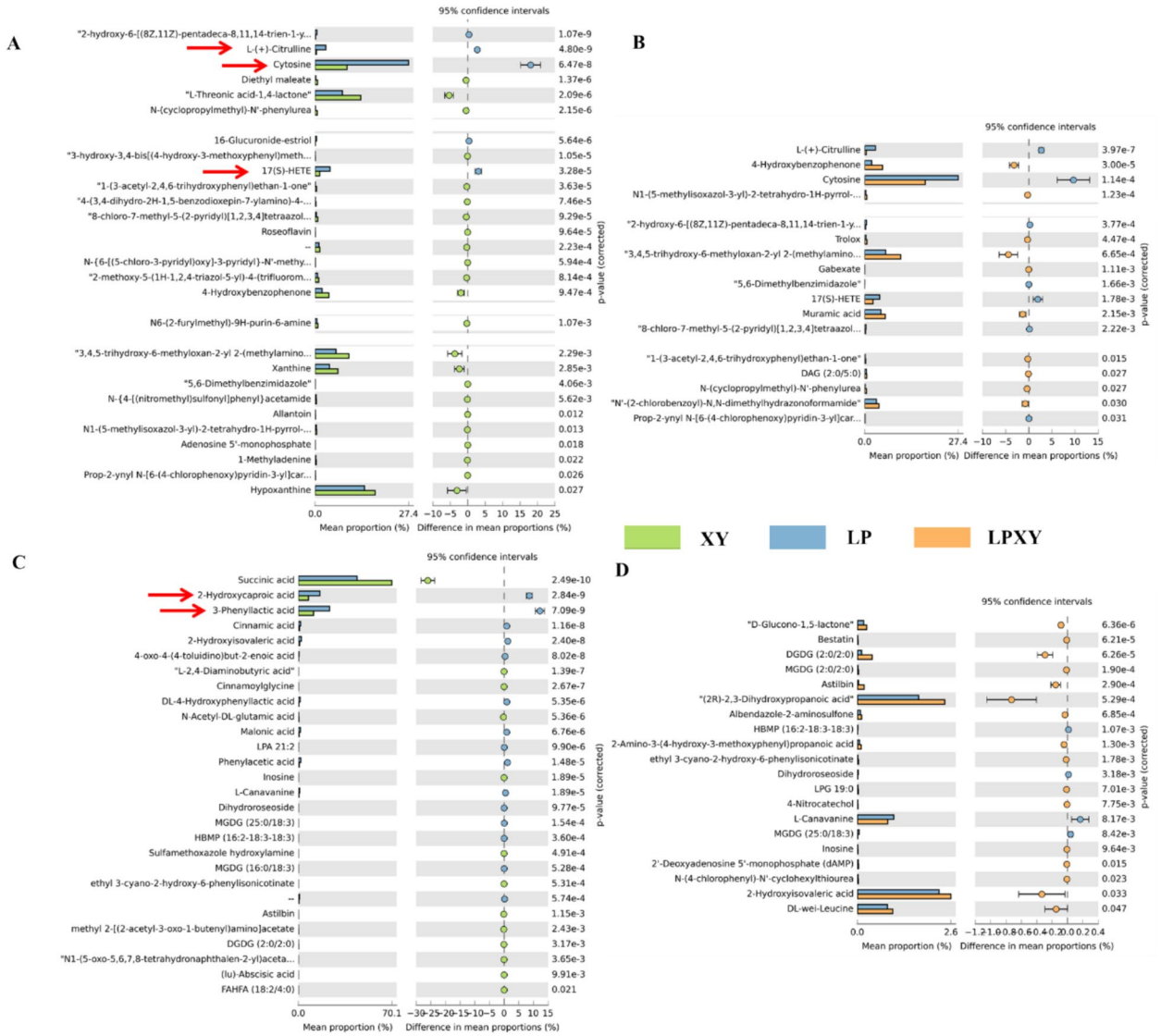


Fig. 3 Bar plot with significantly differential metabolites among the wheat straw silage. **A**, **B** Positive mode ionization; **C**, **D** negative mode ionization. **A**, **C** LP vs XY; **B**, **D** LP vs LPXY ($p < 0.05$). Marked red indicate enriched in LP treatment. *XY* wheat straw inoculated with xylanase treatment, *LP* wheat straw inoculated with *Lactiplantibacillus plantarum* treatment, *LPXY* wheat straw inoculated with *Lactiplantibacillus plantarum* and xylanase treatment

reduction in bacterial diversity in silage *Lactobacillus* had a direct positive effect on differential metabolites, while bacterial diversity had a negative effect on differential metabolites. In summary, *Lactobacillus* promotes the production of differential metabolites. In addition, our SEMs demonstrate that LA and AA are influenced by differential metabolites. LP-treated silage decreased bacterial diversity and enriched some metabolites, thereby affecting LA and AA production.

Discussion

The WSC and LA of wheat straw before ensiling was much higher than the detected by other research [33], may due to the factors like climate and season of harvest, which have influence on the feeding value of the forage. LP treatment has shown a stronger promote effect than other treatments in LA and AA. After anaerobic fermentation process, the organic acid (especially the LA and AA concentrations) is the

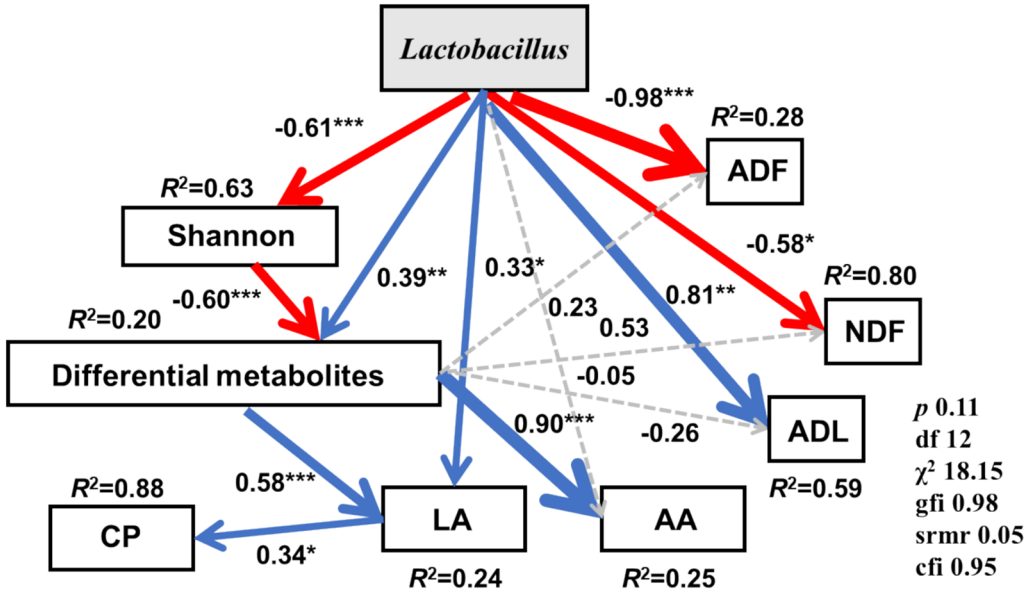


Fig. 4 Structural equation models (SEMs) show the direct and indirect effects of *Lactobacillus* on wheat straw silage fermentation quality. Solid and dashed arrows, respectively, represent significant ($p \le 0.05$) and non-significant ($p > 0.05$) paths. Blue and red arrows, respectively, represent positive and negative effects. Numbers adjacent to arrows represent the standardized path coefficients. R^2 indicates the proportion of variance explained. There was non-significant deviation of the data from the models ($p = 0.11$; $df = 12$; $\chi^2 = 18.15$; gfi = 0.98; srmr = 0.05; cfi = 0.95). *CP* crude protein, *ADL* acid detergent lignin, *ADF and NDF* acid and neutral detergent, *AA and LA* acetic and lactic acid

largest contributor for pH value, previous studies have also provided the same result [34]. These results found that the LA and AA contents in LP-treated and LPXY-treated silage were higher than in other treatments, confirming the significant influence of LA and AA on the pH value of silage. This may be due to the increase of content of organic acids accelerating the decrease in pH [11, 35]. Besides, the higher AA concentration in LP-treated silage may reflect a higher count of LA-produced bacteria in the process. Interestingly, the pH value of LPXY-treated silage was higher than that of XY-treated and LP-treated silage, possibly due to higher levels of bioactive components in XY-treated and LP-treated silage, such as phenolic acids [36]. However, the NH_3–N during the fermentation process may also neutralize acids and prevent pH reduction [37]. Therefore, LPXY-treated silage may also exhibit higher NH_3–N content to neutralize LA and AA [38]. LP treatment has the highest content of LA and AA, for wheat straw silage, directly increasing lactic acid bacteria has a stronger promoting effect on the enhancement of volatile fatty acids. Furthermore, additives did not significantly increase the crude protein content of silage feed that may be due to degradation of proteins by some undesirable microorganisms [39]. Proteobacteria species were detected in all treatments, and even accounted for a significant proportion in the XY and LPXY treatments. Therefore, this may be due to the high proportion of Proteobacteria in the straw microbial community we selected for our experiment.

LP and XY treatment have more impact on silage fiber. Ruminant animals prefer high protein and low-fiber feed because these feeds can have higher nutrition and energy [40]. According to our results, LP additives exhibited the most significant degradation effect on fibers in wheat straw feed, which may be more favored by ruminants, despite not increasing protein content. The reason is that lactic acid bacteria inoculum contains cellulase, reducing fiber content [38]. The increase of lactic acid bacteria directly enhances the utilization of compounds to improve the efficiency of lactic acid production in feed, thereby enhancing fermentation quality [12]. Both these additives can lower silage ADF and NDF. NDF is negatively correlated with animal feed intake, and ADF is negatively correlated with feed digestibility, indicating that additives have a potential promoting effect on animal feeding and digestion [41, 42]. In addition, based on the comprehensive analysis of ADL, CL, HC, and HoC, the LP additive exhibits the strongest effect on fiber decomposition in wheat silage feed, surpassing that of LPXY-treated silage. The promotion effect of XY additive on silage quality is weaker than LP additive. Wheat straw contains a large amount of lignin. Lignin is a complex polymer composed of phenolic monomers, which can prevent glycoside hydrolases from coming into contact with their substrates [43, 44]. Lignin is a

difficult substance to decompose, and fungi with strong decomposition ability usually have good effects on lignin decomposition, such as saprophytic fungi (e.g., white-rot fungi) [45, 46]. The XY additive primarily decomposes hemicellulose and effectively utilizes free xylan, thus its impact on lignin decomposition may be minimal [47]. Contrary to expectations, there was no synergistic effect between XY additive and LP additive. Wheat straw contains a significant amount of lignin [43].

Our analysis of dominant bacterial relative abundance revealed that Lactobacillus is the predominant genus in LP-treated silage. The abundance of *Lactobacillus* exceeds half of all bacterial communities' genus. According to the principle of 'competitive exclusion', the dominant microbial community is abundant, while the non-dominant microbial community is reduced, resulting in a corresponding decrease in microbial diversity [48, 49]. Thus, inoculation with LP increased the number of dominant bacterial genera (*Lactobacillus*) and reduced bacterial diversity. Similarly, previous results also revealed the same results [50]. Although the XY and LPXY treatments increased the abundance of *Lactobacillus* compared to CK, the differences were not significant. Firmicutes was the dominant phylum in LP-treated silage, whereas Proteobacteria were dominant in the other treatments [51]. Many spoilage and harmful microorganisms belong to Proteobacteria (e.g., *Escherichia coli*). The high pH value of silage feed is conducive to the growth of other spoilage or pathogenic microorganisms [52]. LPXY-treated silage and CK exhibited higher pH levels than LP-treated silage, providing conditions conducive to the growth of harmful microorganisms, which may explain why Proteobacteria are the predominant phylum. Meanwhile, fermentation of silage is mainly carried out by lactic acid bacteria [53].

Our experiment also provides evidence from the perspective of silage microorganisms, that LP additives can directly increase the number of Lactobacillus and improve fermentation quality of wheat straw silage. The abundance of *Lactobacillus* in the silage with mixed LP and XY addition is not sufficient, thus the synergistic effect of bacteria and enzymes is not significant. Additionally, *Lactobacillus* was found to be relatively abundant in LP-treated silages during ensiling, as indicated by LEfSe analysis (Fig. 5A), further highlighting the difference of LAB treatment compared to other treatments due to the enrichment of *Lactobacillus* ($p < 0.05$).

The enriched metabolites in LP-treated silage include furoic acid and derivatives and pyridines and derivatives. Varied microbial communities and metabolites influence fermentation quality [54]. The microbial community of inoculated microbes in silage feed, such as LP, produces more complex metabolites [16]. Our results indicate that LP treatment resulted in better fermentation quality compared to other treatments. Therefore, the bacterial community in LP-treated silage may generate distinct metabolites, previous studies have confirmed that bacteria can synthesize arginine and citrulline [55, 56]. Five metabolites enriched in LP-treated silage exhibited significant differences compared to other treatments. Metabolomic data suggest differences in microbial activity among silage feed treated with different additives [57]. A study identified that culturable anaerobic bacteria culture supernatant revealed major compounds, including hydroxycaproic acid and phenyllactic acid [58]. This is

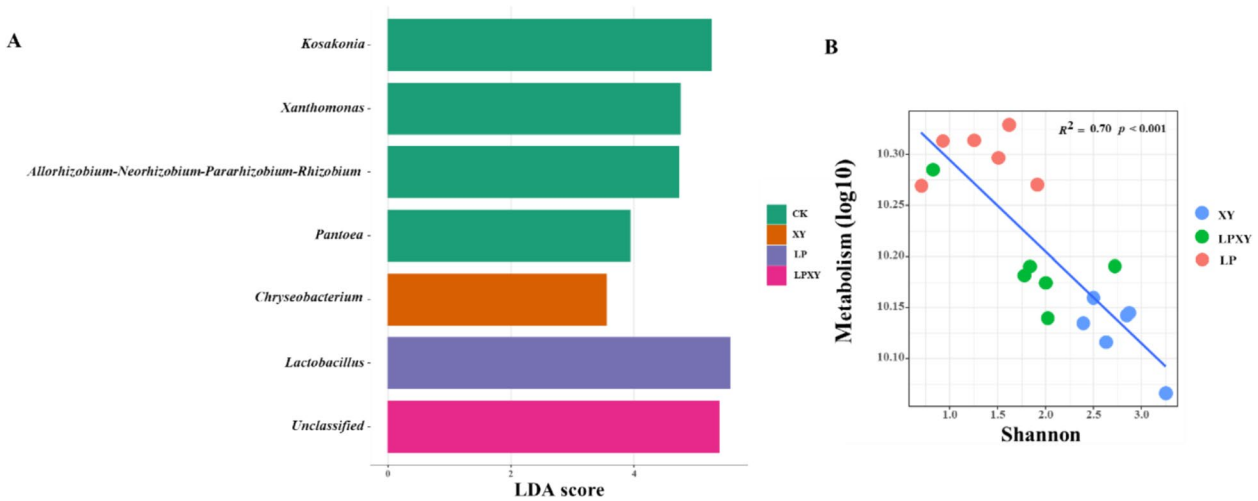


Fig. 5 Comparison of bacterial variations using the LDA analysis for wheat straw silages (**A**). **B** Pearson's analysis shows the relationship between bacterial Shannon and differential metabolism (log10). *XY* wheat straw inoculated with xylanase treatment, *LP* wheat straw inoculated with *Lactiplantibacillus plantarum* treatment, *LPXY* wheat straw inoculated with *Lactiplantibacillus plantarum* and xylanase treatment

similar to our research findings, confirming that bacteria drive differential metabolites. Our study from the perspective of specific metabolites also helps explain why LP additives are superior to XY. In other agricultural waste management, *Lactobacillus* can be considered as a means to improve waste utilization.

To our knowledge, this is the first application of SEMs in silage research to elucidate the pathways and mechanisms underlying changes in fermentation quality of silage feed by integrating metabolomics and microbiome data. More importantly, we have provided compelling evidence supporting the use of microbial additives to enhance the quality of silage feed, as Lactobacillus altered bacterial diversity and enriched certain metabolites. Abundant organic acids can usually improve the palatability of feed, high LA indicates good fermentation quality, and high AA contributes to the aerobic stability of feed [59, 60]. Previous studies combined with metabolomics, have shown that LP-treated produces more organic acids, but specific mechanisms and pathways have not been proposed [20]. Our Pearson's correlation analysis also revealed a positive linear relationship between LA, AA, and differential metabolites, respectively (Fig. 6A, B). With comprehensive Pearson's analysis and SEMs, our results are more reliable, providing a compelling explanation of the role of differential metabolites in LA and AA production.

However, the results of the present study also confirm that carbohydrates in feed are not influenced by differential metabolites (Figs. 4 and 6). This may be attributed to the production of certain enzymes by *Lactobacillus* during the silage fermentation process, such as cellulase and feruloyl esterase, which degrade structural carbohydrates [61]. Therefore, *Lactobacillus* directly explained ADF, NDF and ADL (Fig. 4). It is important to acknowledge objectively that differential metabolites cannot fully account for the changes in AA and LA. Out of the 2557 metabolites detected, only 5 differential products accounted for a very small proportion, less than 0.2%, yet they explained over 20% of LA and AA. There may be other pathways driving changes in lactate, which are currently unclear, but this does not conflict with the significance of these 5 differential metabolites of our study. Ultimately, silage can be viewed as a complex ecosystem, where multiple biotic and abiotic factors interact to shape its composition [62, 63].

Conclusion

Our study findings suggest that *Lactiplantibacillus plantarum* additives enhanced the abundance of Lactobacillus, reduced bacterial diversity, and led to an increase in specific metabolites. These specific metabolites effectively enhanced LA and AA, thereby improving the fermentation quality of wheat straw silage. Overall, our study not only confirms the positive effect of *Lactiplantibacillus plantarum* additives on wheat straw silage, but also provides a mechanistic explanation for the improved quality of silage feed due to these additives.

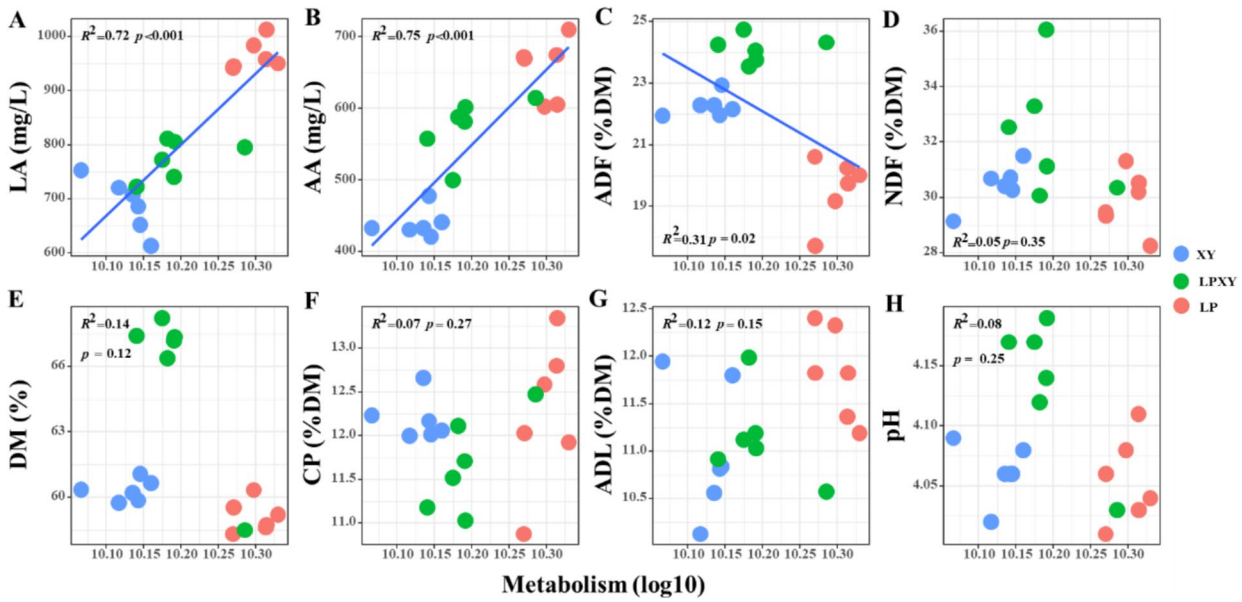


Fig. 6 Pearson's analysis shows the relationship between differential metabolism (log10) and fermentation quality. *XY* wheat straw inoculated with xylanase treatment, *LP* wheat straw inoculated with *Lactiplantibacillus plantarum* treatment, *LPXY* wheat straw inoculated with *Lactiplantibacillus plantarum* and xylanase treatment

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00677-8.

Supplementary Material 1.

Author contributions

D.S contributed to securing financial support, designing the study, and preparing the first manuscript draft; Y.H, H.R, J.Y, and X.Y contributed to do this study and revised the manuscript draft; Y.H and D.S performed data collection and statistical analysis. All authors have read and approved the final manuscript.

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Availability of data and materials

No datasets were generated or analyzed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Author details

[1]Key Laboratory of Forage Cultivation, Processing and High Efficient Utilization, Ministry of Agriculture, College of Grassland Science, Inner Mongolia Agricultural University, Hohhot 010019, Inner Mongolia, China. [2]School of Life Science, Northeast Normal University, Changchun 130021, China. [3]Grass Industry Collaborative Innovation Research Center, Hulunbuir University, Hulunber 021000, China.

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