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Enhancing alfalfa and sorghum silage quality using agricultural wastes: fermentation dynamics, microbial communities, and functional insights

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Abstract

At present, there are many researches on the nutritional quality and fermentation quality of forage silage by adding distillers' grain and fruit residue, but few researches on the succession and function prediction of microbiotic community. In this study, the potential of Moutai distillers' grain (MDG), *Rosa roxburghii* pomace (RP) and *Lactobacillus acidophilus* (LAB) to improve silage quality during anaerobic storage of alfalfa and sorghum was investigated. Harvested alfalfa and sorghum were ensiled without (CK) or with MDG, RP, LAB, LAB + MDG or LAB + RP for 45 days at 21–25 °C. Compared with the uninoculated control, alfalfa silage inoculated with LAB + MDG presented better nutrient retention, where the lactic acid (LA) content was increased by 84.62% and the ammonia nitrogen (AN) content was reduced by 38.52%. Similarly, in sorghum silage, both inoculation with LAB + MDG and inoculation with LAB + RP effectively increased nutrient retention, increased the LA content and reduced the AN content. The proportion of *Lactobacillus* increased in sorghum and alfalfa silage after 45 days of fermentation. Inoculation of alfalfa and sorghum with RP or LAB + MDG significantly increased the relative abundance of lactic acid bacteria in silage, especially *Lactobacillus plantarum*, which was the main dominant strain. The addition of MDG to the feeds not only effectively retained the crude protein (CP) content of the feeds for better retention of their nutritional value but also significantly reduced the contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF), which improved the digestibility and utilization of the feeds. In addition, the addition of MDG further promoted the proliferation of *Lactobacillus* and increased their abundance in the silage, thus contributing to the improvement of fermentation quality and preservation of silage. In summary, MDG, LAB + MDG, and RP + LAB resulted in higher-quality silage, but the addition of MDG was more cost effective and therefore is recommended for application in production.

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Introduction

China, the world's second most populous country, generates substantial agricultural waste annually, posing environmental risks if improperly managed [1]. Meanwhile, rapid growth in animal husbandry since the 21st century has intensified feed grain shortages, a key constraint on agricultural development [2]. To address these challenges, we propose enhancing forage quality through silage combined with agricultural byproducts.

Rosa roxburghii (Roxburgh rose), a thorny, nutrient-rich plant endemic to China, has seen significant cultivation expansion in Guizhou Province, reaching 140,000 hectares and producing over 300,000 tons of fruit annually by 2022 [3–4]. Its fruit, valued for active compounds, is primarily processed into juice, generating large amounts of *Rosa roxburghii* pomace (RP) with 50% water content [5]. This pomace is often disposed of by simple piling, landfilling or incineration, which not only results in a large amount of unused natural resources but also raises serious ecological pollution problems. While studies show RP's potential as dietary fiber [3] and livestock feed (improving protein efficiency in Hu sheep at 30% inclusion [6]), its application in silage remains underexplored. The present study shows that the quality of alfalfa silage can be improved by adding astragalus and hawthorn residues [7] or grape pomace [8]. Distillers' grain (DG), a byproduct of alcohol production, contains residual grains, yeast, sugars, crude protein (CP), fiber, and micronutrients [9]. China generates about 35 million tons of DG annually from liquor production [10], with Guizhou being a major contributor. Due to its high moisture content, DG spoils easily, posing environmental risks if not disposed of promptly [11]. While most research focuses on using dried DG as animal feed, excessive heat during drying can degrade proteins and amino acids, limiting the utilization of its bioactive components and economic value [12]. Although RP and MDG have silage application potential, most of the existing studies are limited to nutritional analysis, and the specific mechanisms of their regulation of fermentation microbial communities and inhibition of spoilage are not clear, and need to be further analyzed through pairing experiments and microbiomes. Therefore, we propose that RP and DG be added to forage silage to improve its quality, thus increasing waste utilization and reducing environmental pollution.

Alfalfa (*Medicago sativa* L.) is a nutrient-rich perennial legume forage, high in protein, minerals, and vitamins, making it ideal for ruminants like cattle and sheep. In southern China, it is often preserved as silage due to hot, humid summers [13]. However, alfalfa silage typically has poor quality due to high buffering capacity, low water-soluble carbohydrates (WSC), and harmful microbes [14, 15]. Studies show that adding byproducts like sea buckthorn [16], lemon pomace [17], grape residue [8] improves silage quality by reducing harmful microorganisms. Therefore, incorporating other bioactive byproducts, such as RP and DG, may similarly enhance alfalfa silage quality, extending shelf-life and boosting nutritional value. Sorghum (*Sorghum bicolor* L.), an annual grass native to Africa, is cultivated globally for its nutritional and high-sugar stalks, making it valuable for feed [8]. Sorghum grows vigorously in summer, with excess amounts of fresh grass produced, and southern China is humid and rainy, so fresh grass is not easy to preserve. Ensiling whole sorghum helps retain nutrients and extend storage, addressing seasonal forage shortages. However, direct sorghum silage has poor fermentation quality, though lactic acid bacteria can improve it [18]. The impact of RP and DG on alfalfa and sorghum silage remains unstudied, and their mechanisms on different forages are unclear.

Silage preserves fresh feed via lactic acid bacteria fermentation, where organic acids (mainly lactic acid (LA)) lower pH, ensuring long-term stability. However, alfalfa and sorghum are unsuitable for solo ensiling, while RP and Moutai distillers' grain (MDG) spoil easily due to high moisture. This study investigates how RP, MDG, *Lactobacillus acidophilus* (LAB), LAB + RP, and LAB + MDG influence silage quality, microbial diversity, and functional profiles in Leguminosae (alfalfa) and Poaceae (sorghum).

By analyzing these interactions, we aim to demonstrate how agricultural byproducts can optimize fermentation by suppressing harmful microbes, improving forage quality. This research supports sustainable waste utilization and advances silage technology innovation.

Materials and methods

Preparation of alfalfa and sorghum before silage

Whole plants of alfalfa and sorghum were artificially harvested from Anshun city, Guizhou Province, on August 15, 2022. The samples were cut into 10–20 mm lengths before silage. *Rosa roxburghii* pomace was obtained from Guizhou Hengliyuan Natural Biological Technology Co., Ltd., in Guiyang city, Guizhou Province, China. The MDG was acquired from the Kweichow Moutai Group in Moutai town, Renhuai city, Guizhou Province, China. The chemical compositions of alfalfa, sorghum, MDG and RP are shown in Table 1. Each bag contains

Table 1 Chemical compositions of fresh Alfalfa, Sorghum, Moutai distillers' grain and *Rosa roxburghii* pomace

Item	Fresh alfalfa	Sorghum	Moutai distillers' grain	Rosa roxburghii pomace
DM (%FM)	27.88 ± 1.32	38.45 ± 0.36	44.92 ± 0.03	29.55 ± 1.67
CP (%DM)	26.64 ± 0.57	5.20 ± 0.04	28.47 ± 0.11	6.21 ± 0.43
NDF (%DM)	43.51 ± 0.00	57.33 ± 0.03	43.72 ± 0.68	46.65 ± 0.03
ADF (%DM)	29.21 ± 0.08	32.23 ± 0.00	30.63 ± 0.71	25.14 ± 0.06
WSC (%DM)	4.62 ± 0.09	12.28 ± 0.03	0.92 ± 0.24	5.03 ± 0.04

The data are expressed as the means ± standard errors. DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water soluble carbohydrate; FM, fresh matter

330 g of chopped samples, which were then vacuumed and sealed with a vacuum packaging machine (Mag, DZ-400/2S, Qingdao). This process was repeated for alfalfa. (1) CK (without additives), applied as 5% fresh weight (FW) distilled water; (2) LAB, applied as *Lactobacillus acidophilus* (Sourced from Guangzhou Hua Microbial Technology Co., Ltd., Guangzhou, China, the effective microbial count is 5×10^9CFU/g) at 10^5 cfu/g FW; (3) RP, applied as 25% FW RP (Guizhou Hengliyuan Natural Biological Technology Co., Ltd., Guizhou, China); (4) MDG, applied as 25% FW MDG; (5) LAB + RP, combined with the application of 25% FW RP with LAB at 10^5 cfu/g FW (Guangzhou Hua Microbial Technology Co., Ltd., Guangzhou, China); and (6) LAB + MDG, combined with the application of 25% FW MDG with LAB at 10^5 cfu/g FW. Three replicates are set for per treatment. In this work, S is used to represent sorghum, and A is used for alfalfa. Based on the findings of Dai et al. (2022), we selected 25% FW MDG and RP [19]. All bags containing silage were stored at 21–25 °C for 45 days, after which the fermentation indicators, nutritional indicators, microbial communities, and functional predictions of the silage were determined.

Chemical composition analysis of sorghum and alfalfa silage samples

A total of 10 g of each sample was added to 90 mL of water, mixed well, allowed to stand for 24 h, and filtered with cheesecloth. Organic acids, ammonia nitrogen (AN), and pH were measured in the supernatant. The pH was measured via a glass electrode pH meter. The content of AN was determined by the phenol-sodium hypochlorite method of Broderick & Kang (1980) [20]. LA, acetic acid (AA), propionic acid (PA) and butyric acid (BA) levels were determined via high-performance liquid chromatography [21]. Each sample, weighing approximately 200 g, was dried at 65 °C until a constant weight was achieved to determine the dry matter (DM) content. Afterward, the samples were ground through a 1 mm mesh sieve for chemical component analysis. The CP content was examined with a Kjeldahl analyzer in accordance with the Association of Official Analytical Chemists (AOAC) procedures [22]. Using an Ankom 2000 fiber analyzer, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined following the Van Soest method, as detailed in Van et al. [23]. WSC content was determined by anthrone - sulfuric acid colorimetric method [24].

Microbial population analysis

Total genomic DNA from each sample was extracted via the CTAB method [25]. The full-length 16S ribosomal RNA (rRNA) gene was amplified via PCR for SMRT sequencing via the forward primer 27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and the reverse primer 1541R (5'-AAGGAGGTGATCCAGCCGCA-3'), which contained a set of 16-nucleotide barcodes [26]. DNA libraries were generated with the SMRTbell Template Prep Kit (PacBio, Menlo Park, 135 CA, USA) and sequenced via the PacBio Sequel system. The processing of the raw sequences was performed by Novogene Bio Technology 136 Co., Ltd. (Beijing, China). The data were analyzed via the Novogene Magic Cloud Platform (https://magic.novogene.com). Kyoto Encyclopedia of Genes and Genomes (KEGG) functional prediction analysis was used to predict bacterial community function in sorghum and alfalfa silage via the Tax4fun tool.

Statistical analysis

Two-factor ANOVA was performed via SPSS 19 software to analyze chemical composition and fermentation quality data, and multiple comparisons were subsequently performed via Duncan's test to determine the significance of differences between treatments. Data on microbial communities were analyzed and plotted via GraphPad Prism. 8.

Results

Chemical composition of fresh samples

The chemical compositions of the fresh samples were shown in Table 1. The DM levels of alfalfa, sorghum, MDG and RP were 27.83%, 38.45%, 44.92% and 29.57%, respectively, before ensiling. The CP contents of the fresh alfalfa and sorghum samples were 26.64% and 5.20%, respectively. The NDF and ADF contents of alfalfa were 43.51% and 29.21%, respectively, whereas the NDF and ADF contents of sorghum were 57.33% and 32.23%, respectively.

Chemical composition of alfalfa and sorghum silage

For sorghum silage, the DM content in the CK treatment was significantly lower than that in the LAB and MDG treatments; for alfalfa silage, the DM content was significantly greater in the LAB + RP and LAB + MDG treatments than in the CK treatment (Table 2). Higher

Table 2 Chemical composition of silage

Forage species (F)	Treatment (T)	Items				
		DM%FM	CP%DM	NDF%DM	ADF%DM	WSC%DM
Sorghum silage	S-CK	36.48^{cd}	5.82^{e}	61.33^{a}	32.69^{abc}	2.05^{ef}
	S-LAB	41.19^{ab}	6.69^{e}	46.07^{cde}	32.24^{abc}	4.03^{a}
	S-RP	40.13^{bc}	7.12^{e}	49.00^{bcd}	29.00^{abc}	1.80^{f}
	S-MDG	44.02^{a}	8.85^{d}	45.01^{de}	26.50^{c}	2.07^{ef}
	S-LAB + RP	38.37^{bc}	6.14^{e}	54.00^{b}	32.67^{abc}	2.07^{ef}
	S-LAB + MDG	39.46^{bc}	9.32^{d}	52.51^{b}	29.33^{abc}	3.06^{bc}
Alfalfa silage	A-CK	28.45^{f}	24.89^{a}	43.33^{de}	30.67^{abc}	2.09^{ef}
	A-LAB	28.75^{f}	25.64^{a}	42.00^{e}	29.33^{abc}	3.20^{b}
	A-RP	28.68^{f}	21.37^{c}	51.66^{bc}	35.74^{a}	2.90^{bcd}
	A-MDG	32.10^{ef}	25.71^{a}	45.63^{de}	33.56^{ab}	2.41^{de}
	A-LAB + RP	33.25^{de}	22.76^{b}	42.00^{e}	29.33^{abc}	2.67^{bcd}
	A-LAB + MDG	32.84^{de}	25.18^{a}	40.67^{e}	28.00^{bc}	2.63^{cd}
P value	T	0.006	< 0.001	0.003	0.710	< 0.001
	F	< 0.001	< 0.001	< 0.001	0.558	0.168
	T×F	0.022	< 0.001	< 0.001	0.030	< 0.001

Different lowercase letters indicate significant differences between different treatments ($P<0.05$). CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, moutai distillers' grain combined with *Lactobacillus acidophilus*; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate; FM, fresh matter; T, treatment; F, forage species; T × F, interaction of treatments and forage species

Table 3 Fermentation characteristics of silage

Forage species (F)	Treatment (T)	Items					
		pH	LA%DM	AA%DM	PA%DM	BA%DM	AN%DM
Sorghum silage	S-CK	4.82^{a}	0.71^{e}	0.47	2.15^{a}	0.16^{c}	1.08^{a}
	S-LAB	3.87^{c}	1.26^{b}	0.38	1.35^{b}	0.09^{d}	0.41^{d}
	S-RP	4.89^{a}	0.39^{f}	0.44	1.64^{b}	0.00^{e}	0.78^{b}
	S-MDG	4.78^{a}	0.83^{d}	0.35	1.18^{b}	0.28^{a}	0.55^{c}
	S-LAB + RP	4.46^{b}	1.07^{c}	0.46	1.49^{b}	0.03^{e}	0.79^{b}
	S-LAB + MDG	4.51^{b}	1.73^{a}	0.47	2.30^{a}	0.23^{b}	0.48^{cd}
Alfalfa silage	A-CK	5.56^{a}	0.39^{c}	0.83^{d}	0.65^{b}	0.00^{c}	3.08^{a}
	A-LAB	4.83^{c}	1.51^{a}	0.96^{bcd}	0.73^{a}	0.08^{b}	2.55^{b}
	A-RP	5.49^{a}	0.17^{d}	1.04^{b}	0.50^{c}	0.16^{a}	2.41^{bc}
	A-MDG	4.96^{b}	0.14^{d}	0.89c^{d}	0.03^{d}	0.21^{a}	2.12^{cd}
	A-LAB + RP	5.09^{b}	0.63^{b}	1.19^{a}	0.69^{ab}	0.07^{b}	2.63^{b}
	A-LAB + MDG	4.70^{c}	0.72^{b}	1.03^{bc}	0.05^{d}	0.00^{c}	1.77^{d}
P value	T	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001
	F	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	T×F	< 0.001	< 0.001	0.016	< 0.001	< 0.001	0.001

Different lowercase letters indicate significant differences between different treatments ($P<0.05$). CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*; LA, Lactic acid; AA, Acetic acid; PA, Propionic acid; BA, Butyric acid; AN, Ammonia nitrogen; DM, dry matter; T, treatment; F, forage species; T × F, interaction of treatments and forage species

CP contents ($P<0.001$) were clearly observed for sorghum silage with the addition of MDG and LAB + MDG than for S-CK. Compared with the LAB and LAB + MDG treatments, the CK treatment resulted in significantly lower levels of WSC in sorghum silage. In contrast, in alfalfa silage, we found a similar trend. Specifically, all the treatment groups with different substances added, except the MDG treatment group, presented significantly greater WSC contents than did the control group without any substances added.

Fermentation quality of alfalfa and sorghum silage

The silage fermentation quality results are shown in Table 3. The pH values measured for sorghum silage were generally lower than those for alfalfa silage in the treatment groups where the same types and amounts of additives were added. For both sorghum silage and alfalfa silage, the pH values were significantly lower in the LAB treatment than in the other treatments. In both the alfalfa and sorghum silages, the LA contents in the LAB, LAB + RP and LAB + MDG treatments were significantly greater than those in the other treatments. All

Table 4 The bacterial alpha diversity indices of sorghum and alfalfa

Forage species (F)	Treatment (T)	Items		
		observed species number	ACE index	shannon index
Sorghum silage	S-Fresh	73.00^{b}	113.14^{a}	3.29^{a}
	S-CK	92.00^{a}	105.49^{a}	2.65^{ab}
	S-LAB	33.67^{e}	53.47^{d}	0.75^{d}
	S-RP	64.00^{bc}	85.45^{b}	2.79^{a}
	S-MDG	51.33^{d}	68.81^{cd}	1.80^{c}
	S-LAB + RP	49.00^{d}	65.48^{d}	1.72^{c}
	S-LAB + MDG	58.00^{cd}	84.00^{bc}	1.96^{bc}
Alfalfa silage	A-Fresh	81.33^{b}	131.99^{a}	1.31^{c}
	A-CK	84.00^{b}	129.53^{a}	2.69^{a}
	A-LAB	68.00^{c}	89.14^{b}	2.04^{ab}
	A-RP	95.33^{a}	126.58^{a}	2.72^{a}
	A-MDG	38.00^{d}	55.58^{c}	1.76^{bc}
	A-LAB + RP	65.00^{c}	84.47^{b}	2.45^{ab}
	A-LAB + MDG	44.67d	63.43^{c}	1.85^{bc}
P value	T	< 0.001	< 0.001	< 0.001
	F	< 0.001	< 0.001	0.842
	T×F	< 0.001	< 0.001	< 0.001

Different lowercase letters indicate significant differences between different treatments ($P<0.05$). CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*; T, treatment; F, forage species; T × F, interaction of treatments and forage species

the alfalfa treatments presented significantly greater AA contents than did the sorghum treatments. In contrast, for sorghum, the AA content did not significantly differ after the different treatments. The LAB + RP treatment stood out among the other treatments in terms of alfalfa silage, with a noticeably higher AA content. the PA levels were significantly greater in all the sorghum treatment groups than in the alfalfa treatment groups, Interestingly, we found that PA was not detected in alfalfa silage in the MDG and LAB + MDG treatments. In our exploration, alfalfa silage was found to produce significantly higher levels of AN than sorghum silage. For both the alfalfa and sorghum silages, all the additive treatments led to a lower AN content than did the CK.

Changes in the microbial communities of sorghum and alfalfa during silage

The bacterial alpha diversity of the alfalfa samples during ensiling is illustrated in Table 4. The lowest number of strains was observed under the A-LAB treatment, which resulted in a decrease in bacterial diversity indices such as the ACE and Shannon indices. Compared with the S-CK treatment, the S-MDG treatment resulted in the greatest decrease in the ACE index. Compared with sorghum, the addition of LAB and LAB + RP to alfalfa resulted in a greater Shannon index, which indicates a significant difference in alpha diversity between sorghum and alfalfa. The bacterial communities at the phylum level in the alfalfa and sorghum raw materials after 45 days of ensiling are shown in Fig. 1A. Fresh alfalfa and fresh sorghum were found to be teeming with a diverse array of bacterial communities, with Cyanobacteria and Proteobacteria being predominant at the phylum level, and sorghum contained small amounts of Bacteroidetes. Prior to silage, the dominant phylum in sorghum was Proteobacteria (66.97%). The relative abundance of Firmicutes increased to 57.29% after 45 d of silage in the S-CK treatment. After 45 days of alfalfa silage, Firmicutes replaced Cyanobacteria as the dominant phylum, and its relative abundance reached 78.81%. After fermentation of both materials, the dominant phylum was Firmicutes. As shown in Fig. 1B, *Sphingomona*s, unidentified *Cyanobacteria* and

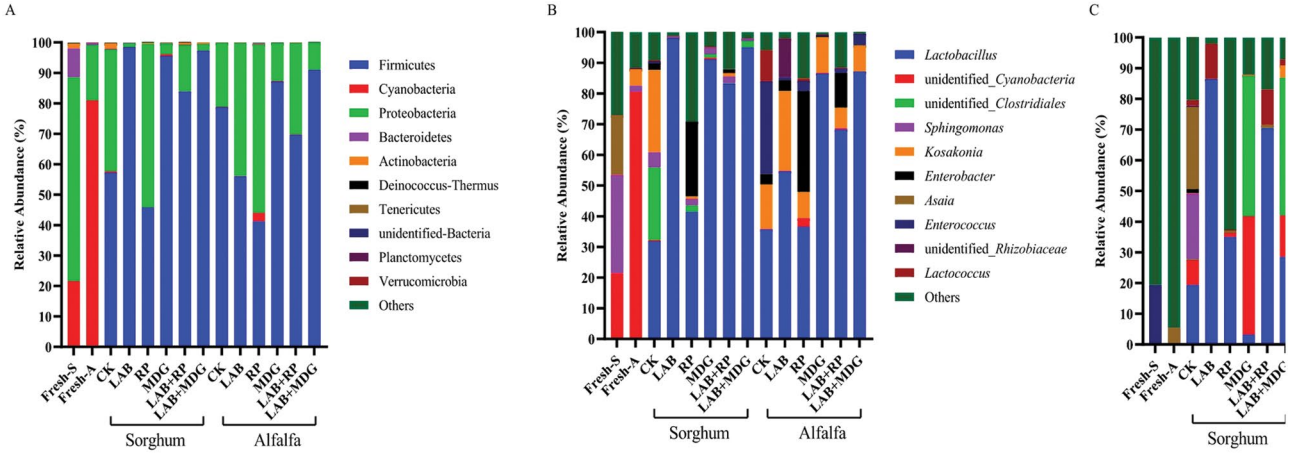

Fig. 1 Relative abundances of bacteria at the phylum level (**A**), genus level (**B**) and species level (**C**) in alfalfa and sorghum after 45 days of anaerobic storage. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

Asaia were dominant in fresh sorghum, and unidentified *Cyanobacteria* (80.51%), *Kosakonia* (5.46%) and others (11.51%) were dominant in fresh alfalfa. The dominant genus in sorghum and alfalfa after ensiling was *Lactobacillus*, *Kosakonia* (26.75%) and unidentified *Clostridiales* (23.63%) were more abundant in sorghum silage without additives, and *Kosakonia* (14.51%) and *Enterococcus* (29.9%) were more abundant in alfalfa silage without additives. The bacterial species present in the alfalfa and sorghum samples during anaerobic storage are shown in Fig. 1C. The main bacterial species treated with S-CK were *Lactobacillus plantarum*, *Clostridium acetobutylicum*, and *Kosakonia cowanii*. The A-CK treatment resulted in relatively greater abundances of *Lactobacillus acidipiscis*, *Kosakonia cowanii*, and *Enterococcus mundtii*. All the treatments (A-MDG, A-LAB + MDG, S-MDG and S-LAB + MDG) with the addition of MDG contained high levels of *Lactobacillus homohiochii*. After 45 days of sorghum ensiling, the abundances of *Kosakonia cowanii* and *Clostridium acetobutylicum* were greater in the S-CK control. With the addition of LAB, RP, and MDG, the harmful microorganisms *Kosakonia cowanii* and *Clostridium acetobutylicum* were replaced by beneficial *Lactobacillus* species. In S-RP, nine groups of bacterial species were enriched, and *Lactobacillus agilis* had the highest LDA score. However, there were no significantly enriched bacteria in either the S-LAB + MDG or the S-LAB + RP treatment. According to the data presented in Fig. 2B, it is evident that following a 45-day period of alfalfa ensiling, the silage produced under the A-RP treatment presented an increase in *Tatumella citrea* and *Escherichia coli*. *Lactobacillus plantarum* and *Weissella paramesenteroides* were more abundant in the A-LAB + RP-treated silage, whereas *Lactobacillus panis* was more abundant in the A-LAB + RP-treated silage. The addition of RP and MDG to both sorghum and alfalfa significantly increased the content of lactic acid bacteria.

Changes in the correlations of microbial communities with the ensiling characteristics of silage

According to the PCoA results (Fig. 3A), at 45 days of sorghum ensiling, the contributions of PC1 and PC2 were 44.9% and 18.04%, respectively. According to the PCoA results (Fig. 3B), at 45 days of alfalfa ensiling, the contributions of PC1 and PC2 were 29.69% and 25.17%, respectively. For sorghum, the ADF, AA and LA levels were positively correlated with the S-LAB and S-LAB + RP treatment levels; the pH and CP levels were positively correlated with the S-MDG treatment level; the WSC, NDF and AN levels were positively correlated with the S-CK treatment level; and the S-LAB and S-LAB + RP treatment levels were negatively correlated with both the pH and CP levels. The contents of ADF, NDF, and AN in alfalfa, as well as pH, were positively correlated with those in treatments A-CK. Moreover, the contents of WSC and AA were positively correlated with those in the A-LAB and A-LAB + RP treatments. On the other hand, the contents of CP and LA were positively correlated with treatment A-MDG. Notably, the A-MDG and A-LAB + MDG treatments, on the other hand, presented negative correlations with pH and AN levels. Through Spearman correlation analysis, we found that the pH of the silage samples was positively correlated with the abundance of *Kosakonia*, *Enterobacter*, *Enterococcus*, and unidentified *Enterobacteraceae* bacteria, whereas a significant negative correlation was observed with the abundance of *Lactobacillus* ($P < 0.05$; Fig. 4A). The concentration of AN was positively correlated with the abundances of *Kosakonia*, *Enterococcus*, unidentified *Rhizobiaceae*, and *Enterobacter* but negatively correlated with the abundance of *Lactobacillus* ($P < 0.05$). The abundance of *Lactobacillus plantarum* was negatively correlated with pH and the AN concentration ($P < 0.05$; Fig. 4B).

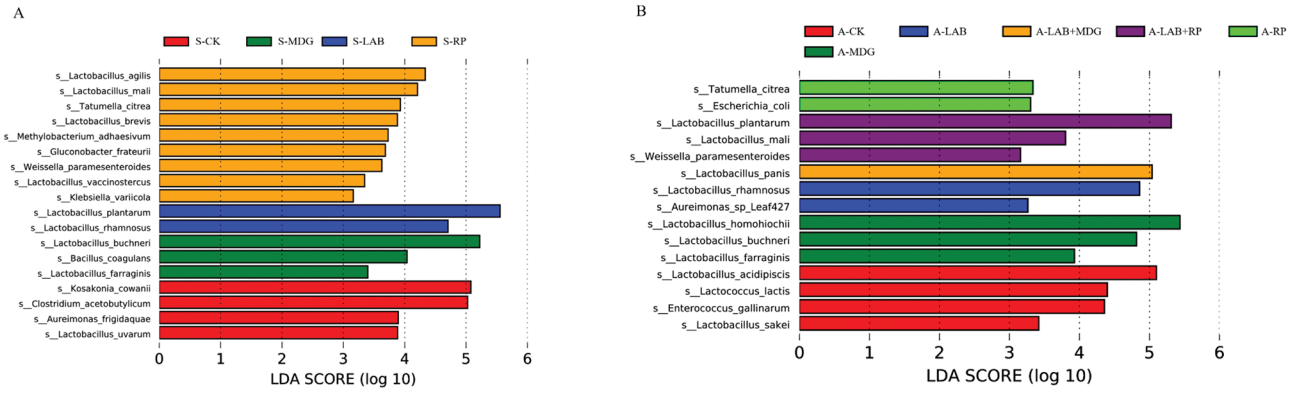

Fig. 2 The identified differentially abundant bacteria ($P < 0.05$ and linear discriminant analysis [LDA] score > 3) among the silage groups. (**A**) Differentially abundant bacteria identified in sorghum after 45 days of anaerobic storage. (**B**) Differentially abundant bacteria identified in alfalfa after 45 days of anaerobic storage. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

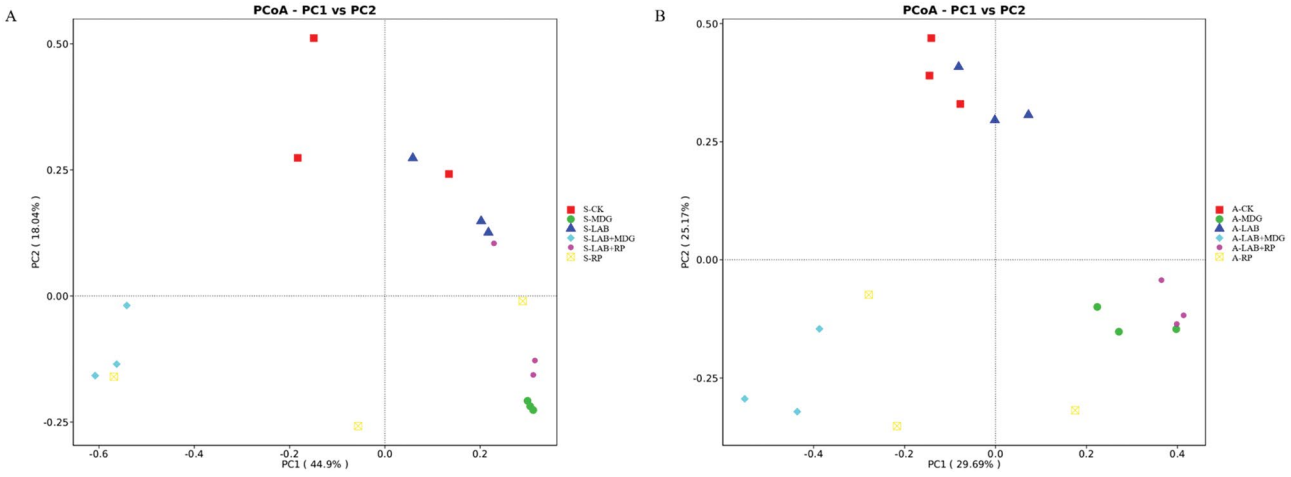

Fig. 3 Principal coordinate analysis (PCoA) plot at the bacterial operational taxonomic unit (OTU) level in sorghum (**A**) and alfalfa (**B**) after 45 days of anaerobic storage. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*;

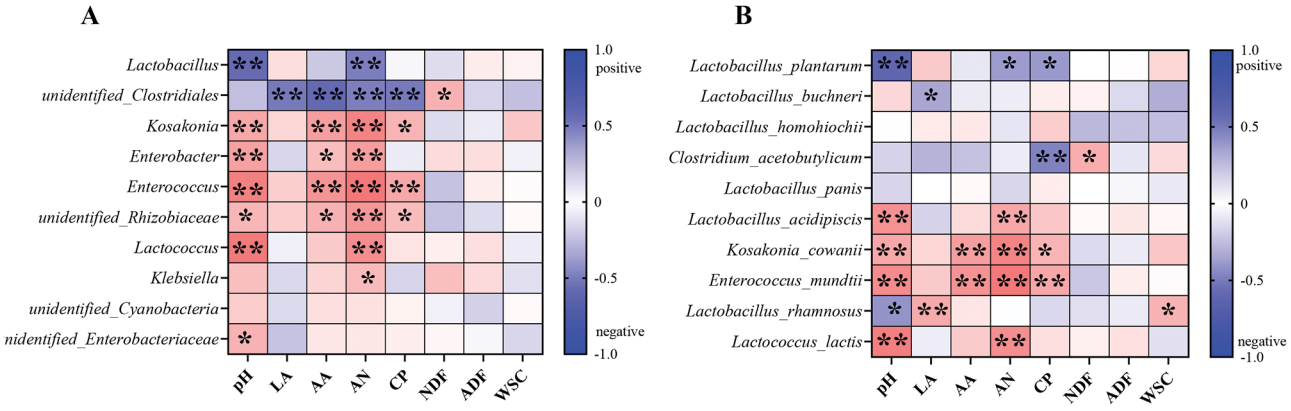

Fig. 4 Spearman correlation heatmap between silage parameters and bacterial genera (**A**) and species (**B**) in sorghum and alfalfa after 45 days of anaerobic storage. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate; LA, lactic acid; AA, acetic acid; AN, ammonia nitrogen; *, $P < 0.05$; **, $P < 0.01$. Red squares represent positive correlations, whereas blue squares represent negative correlations

Function prediction and genomic metabolic pathways of the bacterial community in the silages

Figure 5 shows the functional prediction of bacterial communities in silage. The functions of the bacterial community in fresh sorghum were "chemoheterotrophy", "aerobic_chemoheterotrophy", "phototrophy", "oxygenic_photoautotrophy", "cyanobacteria", and "photoautotrophy". The functions of the bacterial community in fresh alfalfa were "phototrophy", "oxygenic_photoautotrophy", "cyanobacteria", and "photoautotrophy". The main function of the bacterial community in silage was "chemoheterotrophy", followed by "fermentation". The functional prediction of bacterial communities enabled us to evaluate how these communities influence the alterations in the metabolic pathways involved in silage formation.

As shown in Figs. 6a and 7a, both the sorghum and alfalfa metabolic pathways presented significantly greater relative abundances of "metabolism" than did the other pathways. As shown in Figs. 6b and 7b, in the metabolic network of sorghum and alfalfa, the relative abundance of the two pathways "amino acid metabolism" and "carbohydrate metabolism" clearly occupied absolute dominance, which far exceeded all other metabolic pathways. Figure 8 shows "amino acid metabolism" (a) and "carbohydrate metabolism" (b) at the tertiary pathway level before and after sorghum silage. The levels of "histidine metabolism," "tyrosine metabolism," "phenylalanine metabolism," "tryptophan metabolism," "arginine metabolism," "proline metabolism," "lysine degradation," "valine metabolism," "glycine metabolism," and "alanine metabolism" were lower in the additive-treated group than in the CK and fresh samples; however, "cysteine metabolism" and "methionine metabolism" were increased. Moreover, the level of "amino acid-related enzymes" increased in all

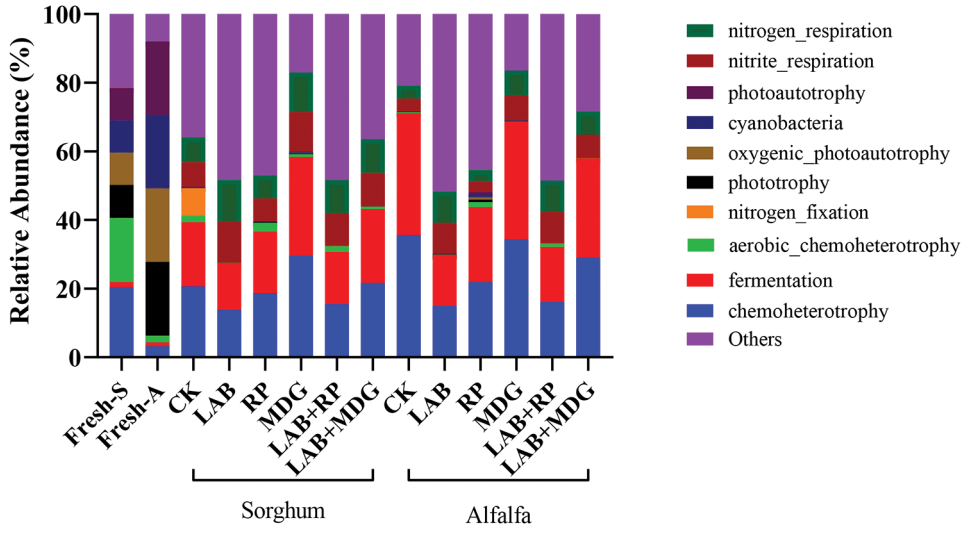

Fig. 5 Functional prediction for the bacterial community in fresh materials and silages. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

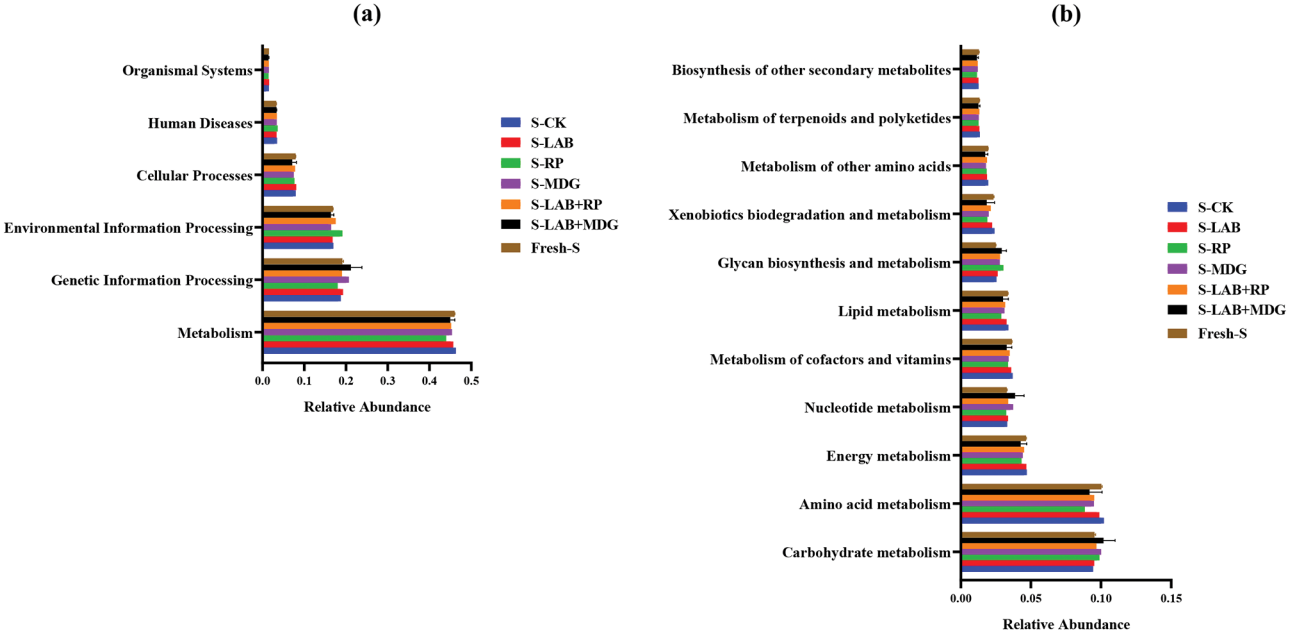

Fig. 6 Bar graphs showing 16 S rRNA gene-predicted functional profiles of the level 1 metabolic pathways (**a**) and level 2 metabolic pathways (**b**) obtained with Tax4Fun for sorghum silage after 45 days of ensiling. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

the treatment groups except S-RP. Ensiling and additives had no effect on the metabolism of "inositol phosphate", "ascorbate and aldarate" or "C5 branched dibasic acid". The addition of RP significantly promoted only the "interconversion of pentose and glucuronate". The addition of RP and MDG promoted the metabolism of "fructose", "mannose", "galactose", "starch", "sucrose", "amino sugars and nucleotide sugars" and the "pentose phosphate pathway".Ensiling inhibited "lysine degradation", "alanine metabolism", "glycine metabolism", "alanine metabolism", "histidine metabolism", "tyrosine metabolism", "phenylalanine metabolism", "tryptophan metabolism", and "arginine and proline metabolism", as shown in Fig. 9(a). As demonstrated in Fig. 9(b), silage significantly increased the activity of *Lactobacillus* in "carbohydrate metabolism", which includes "fructose and mannose metabolism", "starch and sucrose metabolism", "galactose metabolism" and the "pentose phosphate pathway" (PPP), strongly confirming the ability of *Lactobacillus* to utilize multiple different carbon sources for its own proliferation.

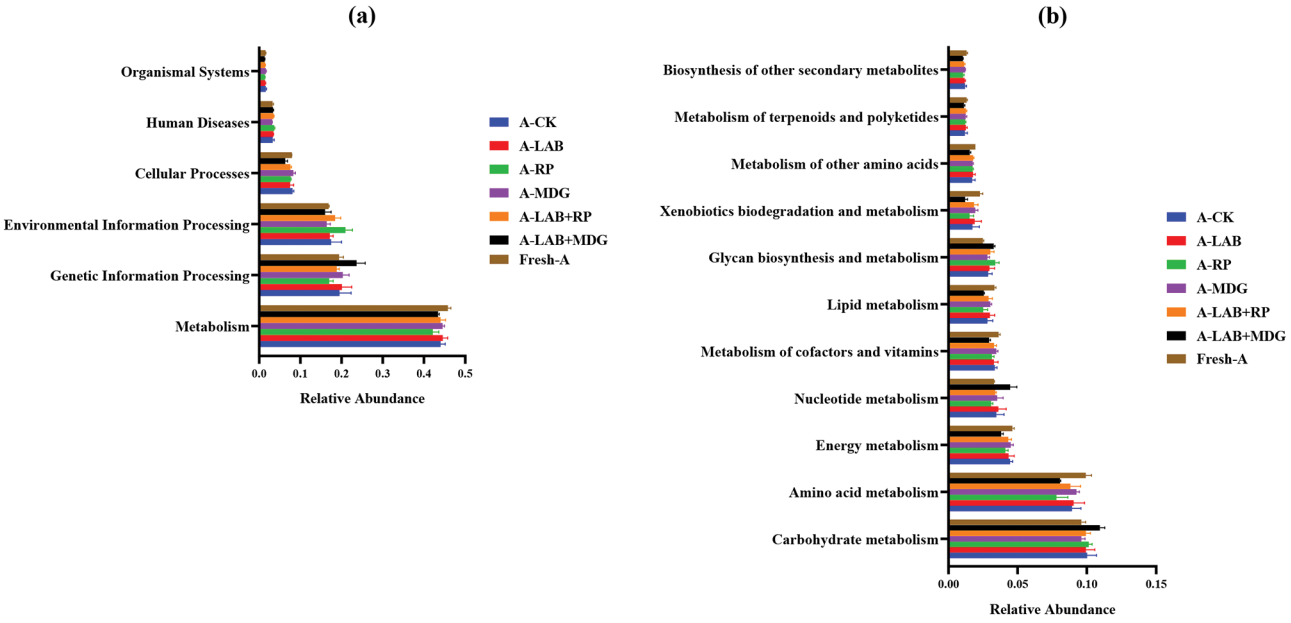

Fig. 7 Bar graphs showing 16 S rRNA gene-predicted functional profiles of the level 1 metabolic pathways (**a**) and level 2 metabolic pathways (**b**) obtained with Tax4Fun for alfalfa silages after 45 days of ensiling. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

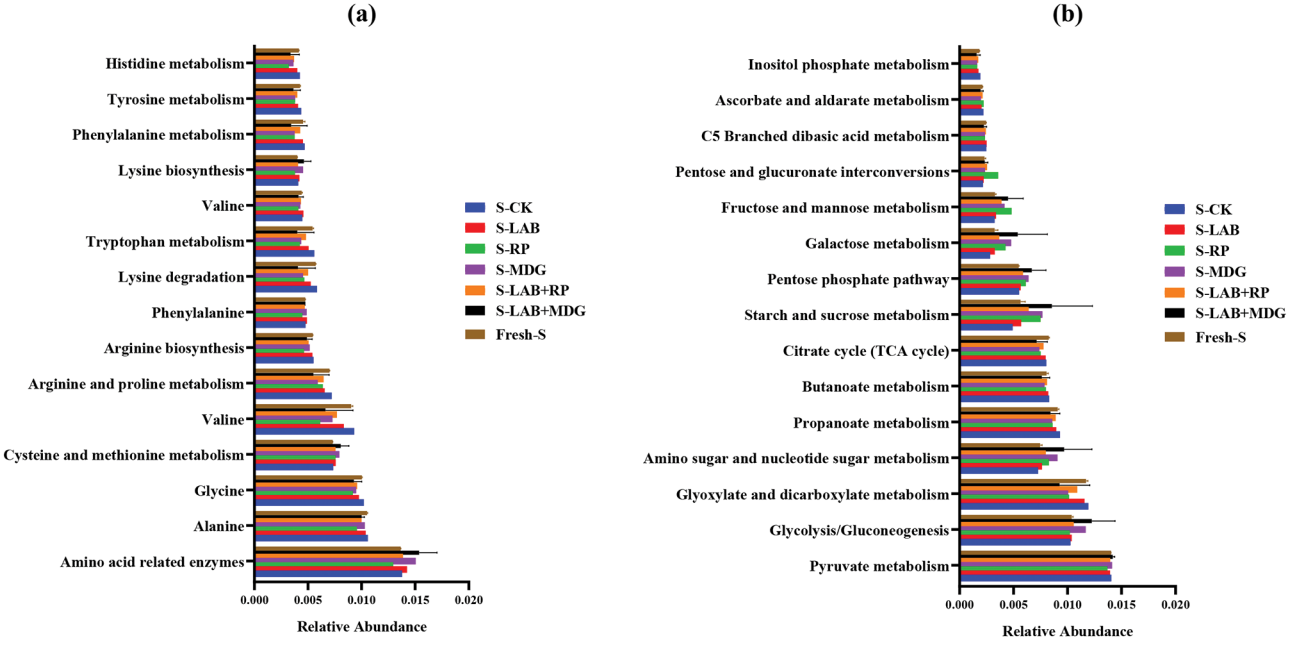

Fig. 8 Bar graphs showing 16 S rRNA gene-predicted functional profiles for amino acids metabolism (**a**) and carbohydrate metabolism (**b**) in the level 3 metabolic pathways obtained with Tax4Fun for sorghum silages after 45 days of ensiling. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

Discussion

Chemical composition of alfalfa and sorghum

The addition of MDG (28.47%) or RP (6.21%) increased the CP content of sorghum. The MDG and RP treatments resulted in lower NDF and ADF contents, and these two ingredients could reduce the fiber content of sorghum. One study has shown that when the WSC content is > 5% DM, high-quality silage can be obtained [27]. The WSC content of sorghum (12.28%) was sufficient for *Lactobacillus* fermentation, whereas alfalfa had a low WSC content (4.62%) and required silage additives to improve its fermentation quality, which is in accordance

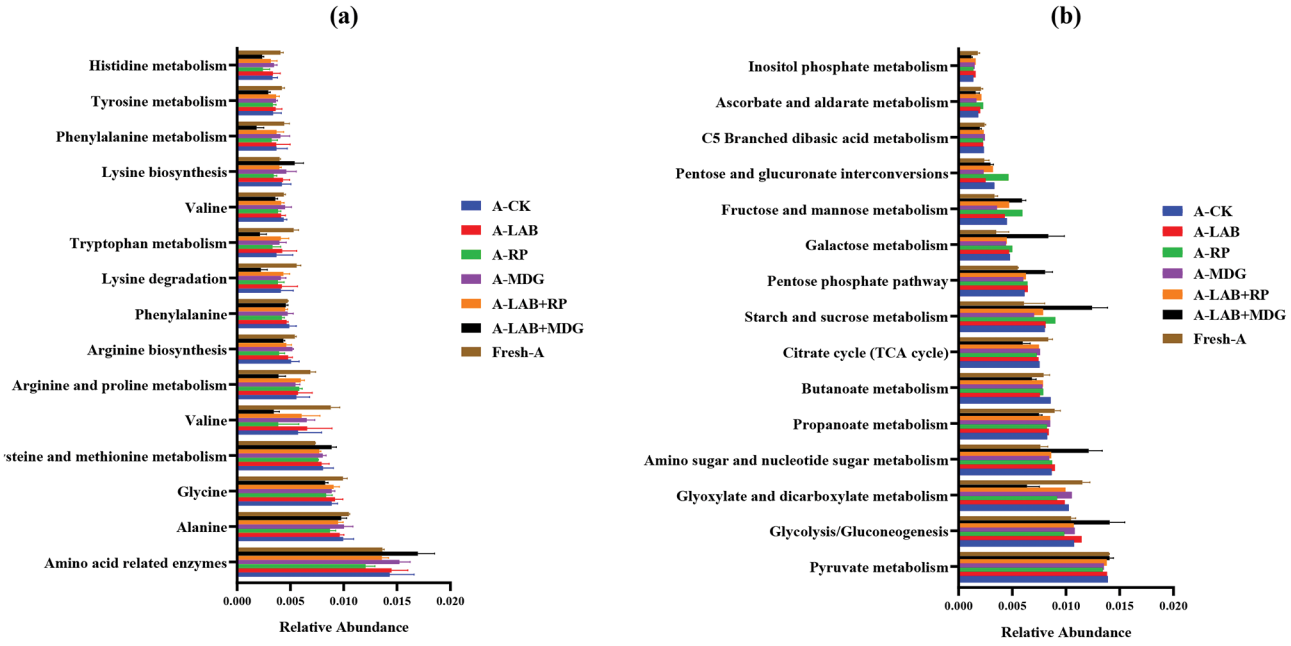

Fig. 9 Bar graphs showing 16 S rRNA gene-predicted functional profiles for amino acids metabolism (**a**) and carbohydrate metabolism (**b**) in the level 3 metabolic pathways obtained with Tax4Fun for alfalfa silages after 45 days of ensiling. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*

with the research of Jiang et al. [28]. However, adding RP can enhance the fermentation effect of alfalfa because the WSC content of RP is > 5%. In conclusion, the addition of RP and MDG may improve the nutritional quality and fermentation quality of alfalfa and sorghum to some extent.

(T×F) indicates that the effects of treatments and forage species on each index were not independent, but act together. The addition of lactic acid bacteria significantly increased the concentration of LA in silage, which in turn effectively lowered its pH and significantly reduced DM loss [29–30]. The low-pH (3.76) environment in the MDG inhibited protease activity, directly reducing protein hydrolysis [31]. LAB + MDG did not improve CP in alfalfa because the CP content of alfalfa itself was high, and the CP content of MDG was not different. After comparing alfalfa silage under different treatments, we found that alfalfa silage under two treatments, RP and LAB + RP, presented significantly lower CP contents than those in all the other treatments. The potential reason for this phenomenon may be the relatively high abundance of *Enterobacter* contained in RP, which leads to increased hydrolysis of CP. As the NDF and ADF contents continue to increase, the texture or acceptability of the feeds tends to decrease and become less palatable, which tends to result in a corresponding decrease in the amount of food consumed by the animals, while the digestion and absorption process of the feeds becomes slower as a result. When we examined alfalfa silage under the RP treatment alone, we found that its NDF content was significantly greater than that under all the other treatments, possibly because RP itself contains a relatively high proportion of NDF, with a specific value of 46.65% (Table 1). This significant difference was attributed to the fact that LAB effectively inhibited the multiplication of harmful microorganisms, leading to a reduction in the consumption of WSC by these harmful bacteria. These results suggest that LAB or other types of additives work effectively in alfalfa silage to inhibit the activity of harmful microorganisms that may consume WSC. Therefore, we can reasonably infer that appropriate additive use strategies are of great practical importance in protecting the WSC content in alfalfa silage from being overconsumed by harmful microorganisms, which is in accordance with the research of Tao et al. [32]. The inclusion of MDG and RP enhanced the nutritional quality of alfalfa and sorghum to some degree, although their effects were driven by different mechanisms.

Fermentation quality of alfalfa and sorghum silage

Chen et al. (2020) added sea buckthorn pomace to alfalfa to improve its silage quality, and this experiment also showed similar results when RP and MDG were added [16]. pH is one of the key indicators of silage quality, which is influenced by two main factors: the organic acids naturally produced during fermentation, which inhibit the activity of undesirable microorganisms by lowering the pH of the environment, and the buffering capacity of

the silage material itself, which resists, to a certain extent, the lowering of the pH [33–34]. The pH values measured for sorghum silage were generally lower than those for alfalfa silage. The reason for the lower pH of sorghum is its high WSC content, as well as having higher nutrients to control the bacterial population that produces LA and LA production [35]. Specifically, alfalfa has a relatively low WSC content, which means that during fermentation, it has limited substrate availability for microorganisms such as lactic acid bacteria to utilize and produces relatively little organic acid, which has a less significant effect on pH reduction than sorghum does. On the other hand, alfalfa has a strong buffering capacity, which is due mainly to its rich chemical composition, such as cellulose, protein and other substances with acid–base neutralizing effects, which are able to resist, to a certain extent, the pH-lowering effect of organic acids produced, resulting in a relatively high pH value in alfalfa silage [36]. This makes fermentation more difficult, resulting in low LA production. This phenomenon strongly suggests that the addition of *Lactobacillus* to silage is extremely effective at increasing the number of lactic acid bacteria during fermentation, which further promotes the proliferation of lactic acid bacteria and the production of abundant LA. This biochemical process not only accelerated the silage fermentation process but also significantly improved the overall quality of fermentation, resulting in optimized silage quality, which is in accordance with the results of Xu et al. [37]. The content of LA treated with LAB is higher, which effectively lowered the pH of the silage by promoting the high production of LA, which in turn enhanced the fermentation quality and preservation stability of the silage. These findings indicate that LAB increased LA production in alfalfa and sorghum, which is in accordance with the findings of Feng et al. [38]. Initially, silage is dominated by *Lactobacillus* homofermentation, but at later stages, fermentation is dominated by *Lactobacillus* heterofermentation, which leads to an increase in the AA content. The AA content of alfalfa was higher, which is most likely due to the relatively low DM content of alfalfa and its high buffering capacity, which together result in the further conversion of LA and PA already produced during silage to AA [39]. The addition of LAB + RP to alfalfa silage significantly increased its aerobic stability because of the remarkable ability of AA. Notably, the PA content of sorghum was higher, indicating the stronger antifungal resistance and superior nutrient retention potential of sorghum [40], which was attributed to the presence of *Clostridium* in sorghum postsilage fermentation. We speculate that the addition of MDG to alfalfa may have resulted in the production of new chemicals, thereby blocking PA production. The presence of BA in silage can exacerbate DM loss, and unfortunately, trace amounts of BA were detected in some silage samples. During the early stages of silage fermentation, the pH in the system decreases more slowly, providing a longer period for the sugars to undergo incomplete fermentation, which in turn encourages the conversion of some of the sugars to BA or the conversion of LA under unfavorable conditions, which results in the production of more BA. An increase in the BA content is usually detrimental to maintaining the quality of silage. However, BA production can be significantly inhibited by the addition of RP to sorghum silage; this may have occurred because some active substances in the RP inhibited the growth of *C. perfringens*.

The AN content is used to indicate the degree of breakdown of crude protein in silage, with lower levels representing less protein breakdown and better fermentation quality. During ensiling, the gradual accumulation of AN occurs as a result of microbial deamidation of amino acids, while the decrease in AN content is due to the formation of polyphenol–protein complexes and the inhibitory effect of such complexes on plant or bacterial protein hydrolysis enzymes, which in turn results in a decrease in CP effectiveness [41]. The AN content of alfalfa was significantly higher than that of sorghum because of the relatively high buffered capacity and CP content of leguminous forage [8]. The content of AN in additive treatment is lower, suggesting that the additives reduce proteolysis and the action of plant enzymes or respiratory enzymes by lowering pH [42].

Changes in the microbial communities of sorghum and alfalfa during silage

The lowest number of strains was observed under the A-LAB treatment. This is because LAB effectively inhibit the growth and reproduction of harmful and stray bacteria through their metabolic activities, thus enabling lactic acid bacteria to rapidly dominate the silage process in a short period and become the dominant strain in the silage process [43]. The results of the present study suggested that inoculation with *Lactobacillus plantarum* and sea buckthorn pomace reduced bacterial alpha diversity in alfalfa samples, and the same results were obtained in the present study [16].

Research has revealed that large amounts of Cyanobacteria are also present in other fresh grasses, such as barley, mulberry leaves [44] and tea [45], which may be due to the presence of soil inclusions in alfalfa. All the silage treatments resulted in a significant increase in Firmicutes because Firmicutes are suitable for growth under anaerobic and low pH conditions [46–47]. which indicates that fermentation was relatively successful, suggesting that MDG may favor the growth of Firmicutes during ensiling.

The dominant genus in sorghum and alfalfa after ensiling was *Lactobacillus*, which indicates better

fermentation, which is in accordance with the findings of Wang et al. [44]. In general, *Clostridiales* were considered harmful bacteria in silage fermentation because they cause high levels of protein degradation, DM loss and production of ammonia and BA and hinder pH reduction; moreover, feeding with silage enriched with *Clostridiales* may lead to reduced feed intake and increased incidence of ketosis in dairy cows [48]. *Kosakonia* has plant growth-promoting properties, and through its metabolic activity, it reduces the contents of AN and volatile chemicals in silage [49]. Excessive levels of these substances in silage can adversely affect feed quality and animal health [50]. Alfalfa and sorghum, when silage alone, contain *Kosakonia* and *Clostridiales*, which indicates that conventional ensiling of sorghum and alfalfa is not easy. *Kosakonia* was detected in all the alfalfa silages (6.74-25.96%), which is in accordance with the results of Wang et al. [51]. This study suggested that *Kosakonia* was isolated mainly from legumes and soybean plants or from alfalfa rhizomes. Interestingly, sorghum and alfalfa silage treated with RP contained significant amounts of *Enterobacter*. The overgrowth and undesirable metabolic activities of *Enterobacter*, particularly their ability to compete with lactic acid bacteria for the production of AA and the conversion of nitrate to nitric oxide, can have detrimental effects on feed quality and animal health and may affect the content and form of AN in silage [52]. These findings suggest that RP may contain high levels of *Enterobacter* and that care should be taken to eliminate *Enterobacter* when RP is used as an additive at a later stage. In our study, compared with the RP treatment, the LAB + RP treatment suppressed *Enterobacter* levels in the alfalfa and sorghum silages. These findings suggest that RP can be coapplied with LAB when it is used at later stages as an additive. The dominant genus in sorghum and alfalfa silage from the other treatments was *Lactobacillus*, suggesting that the addition of LAB, MDG or each should inhibit the growth of *Kosakonia* and *Clostridiales*, further rapidly promoting the dominance of beneficial bacteria (*Lactobacillus*) and thus improving the fermentation quality of sorghum and alfalfa. Our results suggest that MDG and LAB + RP can be used as silage additives.

Lactobacillus plantarum produces LA to rapidly establish an acidic environment, promoting rapid fermentation and inhibiting the increase of different unsafe bacteria, for this reason, stopping the degradation of proteins and sugars in crops [26]. *Lactobacillus homohiochii* is more common in sauce-flavor baijiu and can grow in media supplemented with up to 15% ethanol [53]. *Lactobacillus plantarum*, a beneficial bacterial species, can be effectively enhanced by the combined addition of LAB and RP during anaerobic fermentation. This combination can reduce the abundance of other potentially competing bacteria, thus creating a more favorable environment for the anaerobic fermentation process, which strongly promotes and ensures silage success. *Lactobacillus buchneri* increased in S-MDG, so it was hypothesized that the addition of MDG to pastoral grass forage might increase its aerobic stability. *Lactobacillus panis* is an anaerobic, specialized heterogeneous lactic acid-fermenting lactic acid bacterium belonging to type III *Lactobacillus*, whose fermentation end products are LA, AA, ethanol and CO_2 [54]. *L. panis* is the main producer of LA in the microbiota during Maotai brewing [55]. This explains the greater relative abundance of *L. panis* in this experiment when LAB was applied in combination with MDG. Interestingly, *Lactobacillus acidophilus*, which was added during the experiment, was not detected after ensiling, probably because it is not acid tolerant and was replaced by other acid-tolerant microorganisms.

To reveal and elucidate in greater depth the differences in microbial community composition experienced by the two crops, alfalfa and sorghum, during silage fermentation under different treatment conditions, we specifically used LEfSe, a state-of-the-art bioinformatics tool, to carry out systematic analyses (Fig. 2). *Lactobacillus buchneri* and *Bacillus coagulans* were more abundant in the S-MDG-treated silage. This was due to the large amount of *Bacillus* and a certain percentage of *Lactobacillus* in Moutai Daqu [55]. *Lactobacillus rhamnosus* was more abundant in the S-LAB- and A-LAB-treated groups, which is in accordance with the findings of Guo et al. [56]. *L. rhamnosus* enhances the quality of fermented foods by producing bacteriocins that inhibit harmful microorganisms (e.g., mold and enterobacteria) [57].

Changes in the correlations of microbial communities with the ensiling characteristics of silage

Figure 3(A-B) shows the results of principal coordinate analysis (PCoA) performed at the level of microbial operational taxonomic units (OTUs) in sorghum and alfalfa silage to further assess differences in the distribution of bacterial communities across treatments. The findings revealed distinct variations in the bacterial populations among the groups treated with various additives. Two principal components of sorghum together explained more than 60% of the variation in the distribution of the bacterial community, with PC1 dominating, suggesting the presence of certain specific bacterial taxa that play a decisive role in the composition and changes in the overall bacterial community during sorghum silage fermentation. The contributions of these two principal components were relatively balanced in the alfalfa silage. The effects of different additive treatments on alfalfa silage bacterial communities were more dispersed than those on sorghum silage bacterial communities.

Figure 10 (A-B) shows the results of the canonical correlation analysis (CCA) used to investigate the

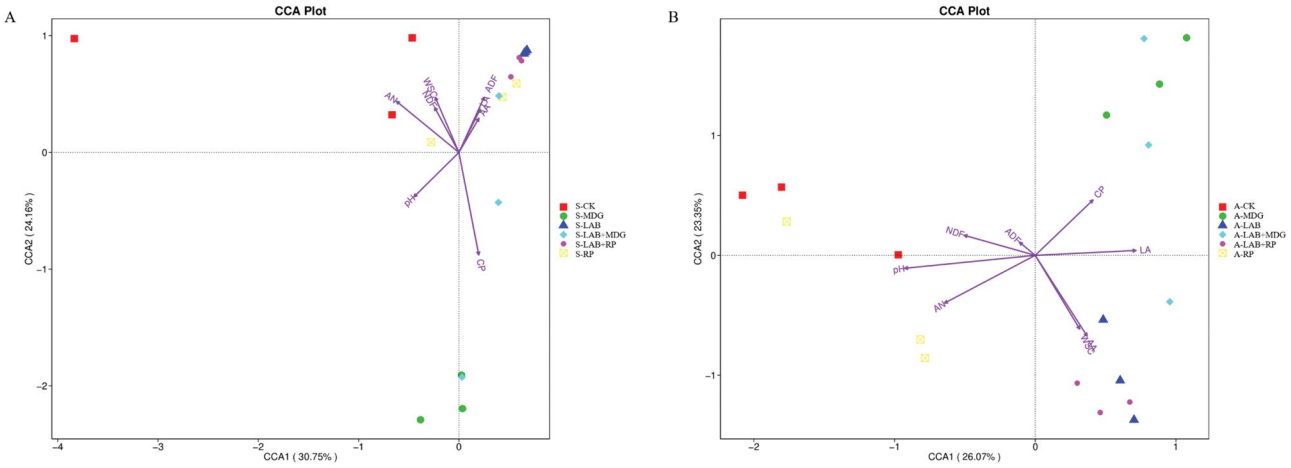

Fig. 10 Canonical correlation analysis (CCA) at the bacterial operational taxonomic unit (OTU) level in sorghum (**A**) and alfalfa (**B**) after 45 days of anaerobic storage. CK, without additives; RP, *Rosa roxburghii* pomace; LAB, *Lactobacillus acidophilus*; LAB + RP, *Rosa roxburghii* pomace combined with *Lactobacillus acidophilus*; LAB + MDG, Moutai distillers' grain combined with *Lactobacillus acidophilus*; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate; LA, lactic acid; AA, acetic acid; AN, ammonia nitrogen

associations between the six treatment groups (CK, MDG, RP, LAB, LAB + MDG, and LAB + RP) and eight environmental parameters (NDF, ADF, CP, WSC, LA, AA, and AN contents and pH) for sorghum and alfalfa. In the prolonged anaerobic preservation environment, all alfalfa plants subjected to different treatments produced high amounts of LAB, a biological process that promotes the production of LA and AA. This series of changes is a normal and expected trend during silage fermentation, reflecting the successful establishment of fermentation conditions and the efficient accumulation of fermentation products. In addition, the CCA results indicated that the addition of RP to both legumes and grasses increased the WSC content, and the combination of *Lactobacillus acidophilus* and RP may have played an important role in increasing LA and AA levels as well as in maintaining CP levels; these consequences are consistent with those of Yang et al. [58].

Enterococcus, unidentified *Rhizobiaceae* and *Enterobacter* species are all gram-negative bacteria, a common group of bacteria, leading to protein hydrolysis and increased AN concentrations due to the high buffering capacity and low WSC content of alfalfa [59]. However, the negative correlation between *Lactobacillus* and AN levels was attributed to the presence of *Lactobacillus*, resulting in increased environmental acidity, which prevents the growth of AN-producing bacteria [60]. Ogunade et al. [61] concluded that microbial activity and plant enzymes work together to produce AN and that the rapid acidification of the overall environment by the plant *L. plantarum* reduces microbial activity, leading to lower AN levels in silage. *Lactococcus lactis* begins lactic acid fermentation during the early phase of ensiling but cannot thrive in acidic conditions because it is sensitive to relatively low pH levels. In contrast, the growth and viability of *Enterobacteriaceae* decline quickly as the pH decreases.

Function prediction and genomic metabolic pathways of the bacterial community in the silages

The carbon sequestration of most bacteria, which uses carbon and energy to oxidize organic components, leads to an increase in "chemoheterotrophy" and "fermentation". Li et al. [62] reported that "nitrogen respiration", "nitrite ammonification" and "nitrite respiration" can lead to sustained AN production. Therefore, "nitrite respiration" and "nitrogen respiration" increased in both materials after ensiling in this experiment, explaining the continued production of AN after ensiling.

The relative abundances of "metabolism" are higher, which suggests that the core regulatory mechanism of the silage fermentation process lies in the activity of bacteria, which successfully convert fermentable substrates into a wide range of metabolites via a series of different metabolic pathways [63].The metabolic pathways that determine fermentation quality at the secondary pathway level are mainly the metabolic pathways for "amino acids", "carbohydrates", "energy", "nucleotides", and "vitamins and cofactors" [64]. Amino acids, as essential nutrients in living organisms, play crucial roles in promoting plant primary metabolism and protein synthesis. Notably, most amino acid metabolic pathways were inhibited by the silage process, most likely due to the low levels of AN in sorghum silage [65]. Furthermore, the silage process also inhibits the metabolism of "inositol phosphate", "propanoate", "glyoxylate and dicarboxylate" and the "citrate cycle" (TCA cycle). This inhibitory effect is closely related to the anoxic environment, as the normal progression of the TCA cycle is dependent on aerobic conditions. In the absence of oxygen, those removed hydrogen

ions cannot successfully enter the respiratory chain for a thorough oxidation reaction [66]. In this study, the relative abundance of the "TCA cycle" was lower in the LAB and LAB + MDG groups of sorghum and alfalfa than in the treatment without additives, suggesting that LA production was greater in these two groups. In conclusion, LAB treatment and LAB + MDG treatment improved forage silage quality by reducing the abundance of the "TCA cycle" and inhibiting "tyrosine metabolism", "tryptophan metabolism", "alanine metabolism" and "lysine degradation".

Conclusion

Adding MDG and RP to alfalfa silage or sorghum silage could improve their nutritional quality and fermentation quality to varying degrees. Comprehensive analyses revealed that the addition of LAB, MDG and a combination of the two could inhibit the growth of *Kosakonia* and *Clostridiales* and rapidly promote the dominance of *Lactobacillus*, thus improving the fermentation quality of sorghum and alfalfa. These findings indicate that MDG can be applied as an additive to other forage silages. If RP is developed as a silage additive, care needs to be taken to control *Enterobacter* production, and coapplication with *Lactobacillus acidophilus* is recommended. After ensiling, the metabolism of "nucleotides" and "carbohydrates" is enhanced, and the metabolism of "amino acids", "energy", and "cofactors and vitamins" decreases. We recommend that farmers apply 25% FW MDG to their production. This study revealed that MDG, LAB + MDG or LAB + RP are good silage additives that improve the quality of silage, thereby increasing the utilization rate of waste and reducing environmental pollution.

Author contributions

Yuanyuan Zhao: Data curation, Formal analysis, Visualization, Writing– original draft. Dianpeng Liu: Investigation. Yulian Chen: Investigation. Yao Lei: Investigation, Resources. Maoya Li: Investigation, Resources. Jiachuhan Wang: Investigation, Resources. Xiangjiang He: Investigation, Resources. Yu Yang: Investigation, Resources. Xinyi Zhang: Investigation, Resources. Shengnan Liu: Investigation. Xiaoqing Zhang: Investigation, Methodology. Qiming Cheng: Conceptualization, methodology, validation, writing– review & editing, supervision, funding acquisition. Chao Chen: Project administration, Funding acquisition.

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Data availability

The datasets generated during the current study are available in the [NCBI] repository, [https://www.ncbi.nlm.nih.gov/, PRJNA1234378].

Declarations

Ethics approval and consent to participate

This research project was conducted in strict accordance with ethical guidelines. We respected and protected the rights and privacy of the participants and ensured the confidentiality of their personal information.

Consent for publication

We, the undersigned, confirm that we have read and approved the manuscript entitled " Regulation of silage fermentation in alfalfa and sorghum by different wastes: Silage characteristics, bacterial community composition and their predicted functional characteristics" and agree to its submission to BMC plant biology. We also confirm that this manuscript has not been published elsewhere and is not under consideration by another journal.

Competing interests

The authors declare no competing interests.

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