

# Investigation of Co-ensiling for the Storage of Grain Stillage and Jerusalem Artichoke Residue from Inulin Extraction

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Jerusalem artichoke residue (JR) was co-ensiled with grain stillage (GS) at various weight mixing ratios (JR only, 4 to 1, 2 to 1, 1.2 to 1, 1 to 1.5, 1 to 2.7, 1 to 7, and GS only) for 10, 30, and 60 d for agricultural biomass storage. Results showed that the middle level of GS to JR ratios, e.g., 1.2 to 1 and 1 to 1.5, achieved the best co-ensiling performance among all studied ratios. The water-soluble carbohydrate (WSC) contents were significantly higher than those of the other treatments ( $p < 0.05$ ), and the lignocellulose contents were significantly lower than those of other treatments ( $p < 0.05$ ). The silages ensiled at the above-mentioned ratios had a higher feed value and biodegradation potential than other ratios. *Lactobacillus* was the dominant bacterial species during the ensiling process, and its relative abundance was significantly correlated with the content of different components, e.g., WSC, crude protein, and starch, as well as fermentation characteristics. Fungal species, e.g., *Kluyveromyces* and *Monascus* were also observed, and the relative abundance of which was positively correlated with different nutritional components. In conclusion, GS and JR can be successfully stored *via* co-ensiling.

**Keywords:** Co-ensiling; Biomass storage; High-throughput sequencing; Fermentation; Microbial community structure

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## INTRODUCTION

The food supply chain of the food industry generates a large amount of food processing waste each year (Teigiserova *et al.* 2019). Landfilling and incineration are the most common food waste treatment approaches, and they have caused serious environmental pollution while wasting a considerable quantity of resources. Given the rich energy content, carbon source, minerals, and nutrients of food processing waste, the valorization of such waste has attracted increasing research interest in terms of both resource recovery and utilization as well as its environmental benefits (Burgos *et al.* 2016). Conversion of such waste into bioenergy, animal feed, and other value-added bioproducts may offer potential technical routes for mitigating environmental pollution and creating extra profit for the food industry. Since food processing waste is characterized by broad diversity, seasonal variability, high biodegradability, and low stability, many studies have been conducted to transform food processing waste into silage for animal feed (Calabrò

and Panzera 2018; Hooker *et al.* 2019). For example, soybean-supplemented apple pomace was used as a substitute for regular commercial feed (*e.g.*, corn and wheat bran), which achieved good fermentation quality (Fang *et al.* 2020). Likewise, the co-ensiling of soybean meal, corn grain, and olive bagasse improved the fermentation characteristics compared to the silage with olive bagasse only (Kasper *et al.* 2019).

As a type of food and energy crop, Jerusalem artichoke has a wide number of applications for food (Díaz *et al.* 2019; Ko *et al.* 2019) and bioenergy production (Lv *et al.* 2019) due to its high yield, high nutritional value, minimal need of fertilizer, and resistance to adverse growth conditions, *e.g.*, plant diseases, low temperature, drought, and saline soil (Long *et al.* 2016; Lv *et al.* 2019). During the processing of Jerusalem artichoke for inulin extraction (Díaz *et al.* 2019) and bioethanol production (Song *et al.* 2017), a large quantity of residues are generated. They quickly putrefy as their high moisture content (MC) and water-soluble carbohydrate (WSC) contents accelerate the spoilage and microbial proliferation, especially in the summer. This necessitates the immediate effective preservation of Jerusalem artichoke residue (JR) to prevent microbial decay. Ensiling may be an economical way to preserve JR for long-term storage and applications while avoiding the potential pollution issues caused by improper disposal of JR.

During the ensiling process, lactic acid bacteria (LAB) convert WSCs into organic acids, which results in a decrease in pH and the inhibition of undesirable microorganisms, *e.g.*, *Clostridium*, *Listeria*, and *Escherichia coli*, thus stabilizing the organic matter for long-term preservation with minimum nutrient loss (McDonald *et al.* 1991). The microbial ecosystem and low pH of the ensiling system favor the long-term storage of food processing byproducts while preserving the nutrients of the silages (Gallagher *et al.* 2018; Rodríguez-Blanco *et al.* 2021). However, the ensiling process can be affected by various factors, *e.g.*, substrate properties, storage conditions, and microbial diversity (Li *et al.* 2019; He *et al.* 2020; Ren *et al.* 2020). Of these factors, the substrate properties are critical in determining the success of ensiling, *e.g.*, inappropriate feedstock compositions, such as insufficient carbohydrate contents and excessive MC, which could cause the ensiling process failure. For instance, high MC of > 70% could promote the activity of spoilage bacteria (*e.g.*, *Clostridium*) which convert lactic acid (LA) to butyric acid and convert amino acids to ammonia, thus impairing the silage quality (He *et al.* 2020). Low MC of < 45% could suppress the overall fermentation process (Li *et al.* 2020). Similar to other highly perishable waste products, (*e.g.*, sugarcane bagasse and sugar beet pulp) improper storage of JR may result in the decomposition of organic matter and energy loss (Ziemiński and Kowalska-Wentel 2015). To prepare feedstocks suitable for ensiling, co-ensiling has been developed by mixing different types of feedstocks at certain ratios to achieve favorable balances in the bacterial community, MC, and nutrient content for the ensiling process, thus producing well-preserved silages (Li *et al.* 2018b; Wang *et al.* 2019a). For example, the co-ensiling of maize straw with vegetable waste achieved desirable silage qualities (Ren *et al.* 2020); citrus pulp that contained highly biodegradable substrates was added to garlic stalk to facilitate the successful ensiling of garlic stalk because it was difficult to ensile garlic stalk alone (Lee *et al.* 2019). The MC of fresh JR is usually too high (greater than 80%) to produce quality silages when it is ensiled alone. Therefore, the co-ensiling of JR with low-MC materials could help achieve proper MC for the production of good-quality silages. Grain stillage (GS) is one of major byproducts of the wine industry, and it contains various nutrients, such as amino acids, protein, starch, fat, vitamins, and minerals (*e.g.*, calcium, phosphorus, potassium, and others) such that GS has high potentials for value-added applications (Olstorpe *et al.* 2010). However, the GS is

perishable and needs to be properly treated and/or utilized in time for resource recovery, while preventing the potential environmental pollution resulted from the misdisposition of the GS. In previous studies, the tubers and tops of Jerusalem artichoke have already been studied for ensiling (Kaya and Caliskan 2010; Koczoń *et al.* 2019). However, little information is available about the treatment of JR from the industrial processing standpoint.

In this study, the fresh JR was co-ensiled with the air-dried GS at different mass ratios, aiming to improving its long-term storage stability as well as preserving organic substances for downstream bioenergy production. The effects of the mass ratio on the silage quality at different ensiling times were evaluated in terms of nutrient components, lignocellulosic compositions, fermentation characteristics, and microbial community structure. This study will provide instructive information for further studies on the storage, conversion, and utilization of JR and other similar food processing byproducts.

## EXPERIMENTAL

### Materials

Air-dried GS with a MC of 15.8% (w/w), a solid waste from the production of China liquor, was collected from Jinhui Liquor Co., Ltd. (Longnan City, Gansu, China). Fresh, wet JR with a MC of 89.3% (w/w), generated during the inulin extraction process, was provided by Xirui Bioengineering Co., Ltd. (Baiyin City, Gansu, China). The chemical compositions of the GS and JR are presented in Table 1.

**Table 1.** Chemical Compositions of Raw Jerusalem Artichoke Residue and Grain Stillage (wt%, dry weight basis)

Chemical Composition	JR	GS
Dry matter (DM)	10.70 ± 0.27	84.17 ± 0.43
Water soluble carbohydrate (WSC)	13.29 ± 0.32	7.75 ± 0.29
Crude protein (CP)	17.72 ± 0.10	16.25 ± 0.08
Starch (ST)	21.08 ± 0.41	26.13 ± 0.08
Acid detergent fiber (ADF)	46.82 ± 1.52	36.92 ± 0.36
Neutral detergent fiber (NDF)	56.48 ± 0.31	54.29 ± 0.35
Acid detergent lignin (ADL)	11.58 ± 0.54	15.02 ± 0.67
Cellulose (CL)	34.55 ± 1.10	21.20 ± 0.39
Hemicellulose (HC)	10.36 ± 1.21	18.07 ± 0.11
Holocellulose (HoC)	44.91 ± 0.28	39.27 ± 0.31
ADF to NDF ratio	0.83	0.68
ADL to NDF ratio	0.21	0.28
ADL to ADF ratio	0.25	0.41
Biodegradation potential (BDP)	3.89	2.62
Relative feed value (RFV)	86.34	103.06

### Co-ensiling of Jerusalem Artichoke Residue and Grain Stillage

To perform co-ensiling, the air-dried GS and wet JR were thoroughly mixed at six different mass ratios (GS to JR), including 4 to 1, 2 to 1, 1.2 to 1, 1 to 1.5, 1 to 2.7, and 1 to 7 to achieve different levels of MC, which were denoted as M3, M4, M5, M6, M7, and M8, respectively (as shown in Table 2). In addition, two single-substrate ensiling controls, *i.e.*, GS or JR alone, were also performed together with the other co-ensiling ratios. Thus, there were 8 different substrates with different GS to JR mass ratios for the ensiling

experiments. Approximately 1000 g of substrate was directly charged into plastic cylinder buckets ( $\varnothing$  1800 mm  $\times$  2600 mm) with a packing density of  $376 \text{ kg}\cdot\text{m}^{-3}$ , followed by the removal of air using a vacuum. A total of 72 plastic buckets (8 substrates  $\times$  3 storage times  $\times$  3 replicates) were tightly sealed and stored in a dark room at ambient temperature ( $25 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ ) for 10, 30, and 60 days, *i.e.*, there were total 24 treatments [8 substrates (different GS to JR mass ratios)  $\times$  3 storage times]. At each sampling time point, the three silage buckets for each treatment were opened and sampled for subsequent analysis.

**Table 2.** Co-ensiling of the As-received Grain Stillage and Jerusalem Artichoke Residue

Treatment	Code	Raw Material (g)		Mass Mixing Ratio (GS to JR)	Moisture Content (%)
		GS	JR		
Grain stillage only	GS	1000	0	100% GS	15.83
Mixed GS and JR	M3	807.07	192.93	4 to 1	30
	M4	670.96	329.04	2 to 1	40
	M5	534.85	465.15	1.2 to 1	50
	M6	398.74	601.26	1 to 1.5	60
	M7	262.63	737.37	1 to 2.7	70
	M8	126.52	873.48	1 to 7	80
JR residue only	JR	0	1000	100% JR	89.30

### Analytical Methods

The dry matter (DM) percentage was measured by drying the samples at a temperature of  $105 \text{ }^\circ\text{C}$  for 48 h in a forced-air oven (Hengke Instrument Co. Ltd, Beijing, China). For further analysis, the dried samples were ground to a particle size of 1 mm with a cutting mill (Jinding Machinery Manufacturing Co. Ltd, Jiangyin, China). The WSC content was determined using the anthrone-sulfuric acid method as previously described by Leyva *et al.* (2008). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined according to the modified Van Soest method (Motte *et al.* 2014). The hemicellulose (HL) content was calculated as the difference between the NDF and ADF contents, while the cellulose (CL) content was determined as the difference between the ADF and ADL contents. The biodegradation potential (BDP) of the silage was estimated as the ratio of holocellulose (HoC) ( $\text{HoC} = \text{HL} + \text{CL}$ ) to lignin content (Ren *et al.* 2020). The total nitrogen (TN) content was determined using a Kjeldahl nitrogen analyzer (K9840, Hanon instruments Co., Ltd, Jinan, China), and the crude protein (CP) content was calculated as the nitrogen content multiplied by 6.25. Starch (ST) analysis was performed by following the method of Hall and Mertens (2008). The relative feed value (RFV) was calculated according to Eq. 1,

$$RFV = \frac{120 \times (88.9 - 0.779 \times ADF)}{1.29 \times NDF} \quad (1)$$

as described by Filik (2020).

To evaluate the fermentation characteristics, the wet silage sample (30 g) was mixed with 270 mL of sterilized distilled water and was homogenized (JJ-2; Guohua Instruments Co. Ltd, Suzhou, China) at 200 rpm for 1 min. The slurry mixture was then filtered through four layers of cheesecloth and qualitative filter paper for the determination of the pH, the organic acid contents, *i.e.*, lactic, formic, acetic, propionic, butyric, and

valeric acids, and the ammonia nitrogen (AN) content. The pH was measured with a glass electrode pH meter (UB-10; Denver Instruments Co. Ltd, Beijing, China). The fermentation metabolites, *i.e.*, ethanol and organic acids, were analyzed with a high performance liquid chromatography (HPLC) (Agilent 1200, Agilent Technology, Waldbronn, Germany) equipped with a refractive index detector, according to the method described by Li *et al.* (2020). Prior to the HPLC analysis, the samples were acidified with H<sub>2</sub>SO<sub>4</sub> (50 wt.%) to decrease the pH to approximately 2 and filtered through 0.22 μm microporous membranes into HPLC vials. The analytical column was a Shodex RSpak KC-811 (300 mm × 8 mm, Showa Denko k.k., Tokyo, Japan). The mobile phase was 3 mM HClO<sub>4</sub> at a flow rate of 1 mL·min<sup>-1</sup>. The separation temperature was 50 °C, and the sample injection volume was 5 μL. The AN content was determined using the phenol-hypochlorite colorimetric method, as described by Broderick and Kang (1980), and expressed as g/kg TN. All of the analyses were conducted in triplicate, and the results were presented on the DM basis unless otherwise specified.

## Microbial Community Analysis

### *Metagenomic DNA extraction*

High-throughput sequencing technology was adopted to monitor the dynamic changes in the microbial community during the ensiling process. For each treatment, representative samples were randomly taken from different layers of the silages and thoroughly mixed. Then, 20 g of these samples were mixed with 180 mL of a sterilized NaCl solution (0.85 wt%) and then centrifuged at 120 rpm for 2 h. The suspension was then filtered through four layers of cheesecloth to obtain the microbial cells used for DNA extraction. Total DNA was extracted using a Water DNA extraction kit (Omega Biotek, Norcross, GA) by following the instructions supplied by the manufacturer, and the DNA quantity and quality were assessed *via* agarose gel electrophoresis. High-molecular-weight DNA samples (a minimal concentration of 20 ng/μL) were used for sequencing.

### *PCR amplification*

Amplification of the V3-V4 hypervariable region of the 16S rRNA was achieved using the following primer pairs: 515F (5'-ACTCCTACGGGAGGCAGCA-3') and 907R (5'-GGACTACH VGG GTWTCTAAT-3'). The fungal ITS1 region amplicon sequencing used the BITS (5'-NNNNNNNNCTACCT GCGGARGGATCA-3) and B58S3 (5'-GAGATCCRTTG YTRAAAGTT-3) universal primers. The amplification of the targeted regions was achieved using the following reagents: 5 μL of Gotaq Green master mix (Promega, Madison, WI), 11.9 μL of DNase-free water, 0.5 μL of MgCl<sub>2</sub> (50 mM), 0.5 μL of deoxynucleotide triphosphates (10 mM), 1 μL of DNA forward and reverse primers (10 μM), and 5 μL of DNA template adjusted for all samples to an average final concentration of 1 ng/μL of total reaction volume. The reaction conditions for the bacterial 16S amplification were as follows: an initial temperature of 95 °C for 3 min, followed by 35 cycles at a temperature of 95 °C for 45 s, a temperature of 50 °C for 60 s, and a temperature of 72 °C for 90 s, and a final extension at a temperature of 72 °C for 10 min. The reaction conditions for the fungal ITS-1 amplification were as follows: an initial temperature of 95 °C for 3 min, followed by 35 cycles at a temperature of 95 °C for 30 s, a temperature of 55 °C for 45 s, and a temperature of 72 °C for 60 s, and a final extension at a temperature of 72 °C for 10 min. After examination *via* gel electrophoresis, the PCR products were denatured with NaOH to generate single-strand DNA fragments and sequenced using an Illumina HiSeq 2500 *via* the paired-end method.

### *High-throughput sequencing of the metagenomic DNA*

To obtain high-quality clean reads, quality filtering on the raw reads was performed under specific filtering conditions. The chimera sequences of the bacteria were removed using the UCHIME algorithm by comparing the sequences with the Silva database, while the chimera sequences of the fungus were removed using the UCHIME algorithm by comparing the sequences with the Unite database. The effective sequences (reads) were counted, and the operational taxonomic units (OTUs) were clustered at a 97% similarity level to perform taxonomic analysis of different samples and obtain the microbial community composition at different levels. Alpha diversity analysis was conducted with QIIME to analyze the species richness (Chao1 and ACE index) and diversity (Shannon and Simpson index). Beta diversity on both the weighted and unweighted UniFrac were calculated using the QIIME software (version 1.7.0). Species with a relative abundance greater than 0.1% were selected for the analysis of the microbial community composition. The correlation thermograms between the fermentation quality and the microbial flora were obtained and analyzed.

### **Statistical Analysis**

The ensiling experiment used a  $3 \times 8$  full factorial design with 3 ensiling times (factor 1) and 8 substrates (with different GS to JR mass ratios) (factor 2). There were 24 treatments with three replicates for each treatment. The statistical model is shown in Eq. 2,

$$Y = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \epsilon_{ij} \quad (2)$$

where Y represents the response variable,  $\mu$  is the overall mean,  $\alpha_i$  is the effect of the ensiling time,  $\beta_j$  is the effect of the substrate,  $(\alpha \times \beta)_{ij}$  is the effect of the interaction between the ensiling time and the substrate, and  $\epsilon_{ij}$  is the residual error (Li *et al.* 2020). The chemical compositions (CL, HL, ST, and WSC), fermentation characteristics (pH, organic acids, and AN), and microbial community diversity after 10 d, 30 d, and 60 d were correlated with the ensiling time and substrate type using the general linear model (GLM) procedure of SPSS (V18.0, SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) with Tukey's multiple comparison test at a significant level of  $\alpha = 0.05$  was used.

## **RESULTS AND DISCUSSION**

### **Characterization of the Raw Grain Stillage and Jerusalem Artichoke Residue**

The raw JR and GS were characterized according to chemical composition. As shown in Table 1, JR had a low DM content (10.7%), which was much lower than the desirable DM (30% to 35%) for silage (He *et al.* 2020). Such a high MC can cause the raw JR to rapidly deteriorate upon exposure to air and could also fail the ensiling process, especially at the high ambient temperatures during summer (He *et al.* 2020). The CP and ST contents of the JR reached 17.7% and 21.1%, respectively, while the JR contained abundant WSCs (13.3%, approximately twice as high as the WSC content in GS), which met the recommended WSC level (greater than 6%) for ensiling processes (Ali *et al.* 2020). In contrast, the raw GS had a much higher DM (84.2%) than JR, but its WSC content (7.75%) was less than the WSC content of the raw JR. The CP (16.2%) and ST (26.1%) contents of raw the GS were similar to those of raw JR. In addition, the raw JR had a

slightly higher structural carbohydrate content (HoC) than the raw GS (45% versus 39%, respectively). The lignification index (expressed as the ratio of ADL to NDF or ADL to ADF) of GS was higher than the lignification index of the JR, which indicated the lower degradability of GS. Based on the chemical compositions of the JR and GS, co-ensiling of these two materials could be a suitable strategy to achieve good-quality silages due to the complementary benefits in the MC and WSC balance: i) the addition of GS reduces the MC of the JR to achieve an appropriate MC for ensiling and avoid the risk of effluent runoff; ii) mixing JR with GS would increase the MC and WSC of GS, thus stimulating the fermentation and hydrolysis of GS during ensiling; and iii) a balanced ratio of MC and WSC for co-ensiling would promote favorable fermentation and produce organic acids which would lower the pH to less than 4.5, resulting in well-preserved silages.

### Dynamic Changes in the Nutrient Components During Ensiling

As important nutrient components, the DM, WSC, CP, and ST are important parameters for silage quality evaluation. Since the raw JR had a lower DM and ST and a higher WSC and CP than the raw GS, the initial DM and ST and WSC and CP contents of the substrates for co-ensiling were reduced and elevated, respectively, with an increasing proportion of JR in the ensiling substrates.

As shown in Table S1, during the ensiling process, the ensiling time, substrate, and the interaction between these two factors exhibited significant effects on the contents of the nutrient components, *i.e.*, the DM, CP, WSC, and ST. The DM content is particularly important for silage fermentation, for LABs need water to grow and reproduce. Due to the relatively high DM content (84.17%) in GS (Table 1), the DM content decreased as the proportion of JR increased (*p*-value less than 0.05). After 60 d of silage, the DM of the M3, M4, and M7 groups increased significantly, while the DM of the M6 and M8 groups decreased significantly. The DM changes of the other groups were not significant. Water-soluble carbohydrates are a vital substrate for microbial metabolism during ensiling, with 60 to 80 g/kg DM being the recommended range to ensure good preservation of silages (Wang *et al.* 2019b; He *et al.* 2020). The WSC content for all substrates decreased considerably in the first 10 d of ensiling due to microbial degradation. Afterwards, it fluctuated slightly until the end of the ensiling process, which could be due to the decomposition of starch and/or structural carbohydrates, *i.e.*, hemicellulose and cellulose, by microbes and/or acids produced additional soluble carbohydrates to compensate for the consumed WSC (Köhler *et al.* 2019).

The overall CP content of most substrates (except for JR alone) decreased significantly, especially during the first 10 d of ensiling. At different time points, the substrates with a higher proportion of JR primarily showed higher CP contents, suggesting that the incorporation of JR was beneficial to the preservation of proteins (Sikora *et al.* 2019).

Starch can hardly be utilized by LAB (except for amylolytic LAB), which primarily functions on monosaccharides, *e.g.*, glucose and fructose (Sikora *et al.* 2019). The ST content variation in the final silage product was mostly related to the initial ST content of the feedstock, and the ST degrading microbes grown during the ensiling process. In the ensiling of GS or JR alone, no obvious pattern in the change in ST content was observed during ensiling. For co-ensiling, higher ST contents were obtained after storage with lower JR proportions, *i.e.*, M3 and M4, during the first 10 d, while the ST content increased during the first 30 d, and then decreased after 60 d for the middle level of JR proportions, *i.e.*, M5, M6, and M7). In addition, only a slight variation in ST was observed in the M8 ratio, which

had the highest JR proportion. Within the same ensiling time, a greater reduction in the ST content was generally observed in the silages with a higher JR proportion.

### Dynamic Changes in the Lignocellulosic Components during Ensiling

The lignocellulosic components considered in this study included ADF, NDF, ADL, CL, and HC. These components have important influences on the silage quality, especially its digestibility. The variation in the structural carbohydrates during storage are shown in Table S2. There was a slight increase in the overall compositions of NDF, ADF, ADL, CL, and HC. Stable ADL and decreased fiber compositions, *i.e.*, NDF, ADF, CL, and HC, were reported by Köhler *et al.* (2019) and Dong *et al.* (2020), who co-ensiled grass with maize and *Broussonetia papyrifera* with perennial ryegrass, respectively. The ADF content in this study was found to gradually decrease in the M5, M6, and M7 samples. However, an increase in the amount of substrates was found in the high and low JR ratio samples, *i.e.*, GS, M3, M4, M8, and JR, which indicated that adjusting the JR to GS ratio was helpful in improving the degradability of silage. The final NDF contents after 60 d of ensiling were greater than the corresponding initial values for most substrates except for M5. For the CL and HC contents, the CL content of the M6 samples was reduced significantly, and the HC content of the M5 group decreased significantly, while the CL and HC contents of the other groups increased by varying degrees after 60 d. These results indicated that the JR to GS ratios of the M5 and M6 samples were more suitable for the degradation of lignocellulosic components during the co-ensiling of JR and GS. Overall, after 30 and 60 d of ensiling, the ADF and NDF contents of all the groups increased to different degrees compared to the raw GS and JR, except for the M5 and M6 samples. With an increase in JR proportion and an elongation of ensiling time, the ADF and NDF contents of the M5 and M6 samples decreased and reached the lowest levels among all ensiling groups, which can be attributed to the acid hydrolysis produced by microorganisms during the fermentation process of the ensilage (Chen *et al.* 2020).

As a primary barrier for the degradation of structural carbohydrates, *i.e.*, CL and HC, a high ADL content can prevent microbes from attacking biodegradable fibers and reducing the digestibility of biomass (Li and Zheng 2018). Overall, the ADL content first decreased and then increased during the 60 d ensiling process. The co-ensiling (M4 through M8 samples) of GS and JR was beneficial to ADL decomposition and efficiently decreased the ADL content compared to its initial value. The RFV represents the digestibility (from a percentage of ADF) and intake potential (from a percentage of NDF) of a feed stuff. As shown in Table S2, the RFV scores of most treatments were assessed as fair (87 to 102) according to the quality grading standard outlined in Firsoni *et al.* (2019). In addition, the RFVs of all the tested groups were higher than the RFVs of quinoa stalks (Filik 2020) and rice straw (Firsoni *et al.* 2019). Additionally, when the mixing ratio was 1.2 to 1 (M5) and 1 to 1.5 (M6), the RFV of the silage was significantly greater than the RFVs of the other treatment groups, which may be due to the appropriate proportion of silage fermentation helping to reduce the content of the various lignocellulose components, thus increasing the RFV of the silage. The biodegradation potential is also an important indicator of biomass degradability before and after the silage process. In this study, the BDP of all treatment groups (except only JR) showed an increasing trend as the ensiling time increased, indicating that undergoing silage for a longer period of time was conducive to the promotion of the degradation performance of silages.

## Dynamic Changes in the Fermentation Characteristics during Ensiling

The pH value is an important parameter for indicating the quality of a silage. A high-quality silage usually has a pH of 3.8 to 4.2, which can effectively inhibit the growth of undesired microorganisms, *e.g.*, *Clostridium butyricum*, *Listeria*, and *E. coli* (McDonald *et al.* 1991). Silages with a pH greater than 4.7 easily decompose and decay. In this study, the pH of the silages of all eight groups of silages rapidly decreased to lower than 3.8 in the first 10 d due to the generation of organic acids, and then the pH continuously increased during the remaining time in storage (Table S3), which could be due to yeasts, molds, and/or other aerobic microorganisms consuming LA and other nutrients for their metabolism (Zeng *et al.* 2020). The pHs of the GS and co-silages of M3 through M7 after 60 d of ensiling were all less than 4.0, which indicated well-preserved silages. The result was similar to a previous report (Mu *et al.* 2020) in which rice straw was co-ensiled with high-moisture amaranth. However, the pH of the M8 and JR silages on day 60 rose to 4.32 and 4.55, respectively (approaching the threshold of putrefaction), which could be ascribed to the high MC derived from the high JR proportion. The high pH was consistent with the low LA contents in the M8 and JR silages, since LA is one of the major organic acids that can efficiently induce a pH drop. Meanwhile, the pH of the silages showed an upwards trend as the proportion of JR in the silages increased over the course of the ensiling period, which could be due to the fact that a high MC can lead to clostridial fermentation and dilute the LAB concentration, thus counteracting the decrease in pH (He *et al.* 2020).

The AN content reflects the degradation of protein and amino acids, *i.e.*, a higher AN content usually suggests higher proteolytic activity and worse hygienic quality of a silage (He *et al.* 2020). In the current study, the AN content in all the silage groups throughout 60 d were lower than the threshold standard (7 g/100 g TN), which was an indicator of successful ensiling (as shown in Table S3). Such low AN contents were possibly related to the rapid reduction in pH to less than 4.0, which is known to greatly inhibit proteolysis. In most cases, the AN content showed an upward trend as the JR proportion increased, which was consistent with the pH variations. This result could be due to the fact that AN has a pH buffering capacity, thus mitigating the pH reduction (Desta *et al.* 2016). As such, the increased AN content of the silages in this study alleviated the pH reduction. These findings indicated that a high proportion of JR in the silages resulted in effective acidification *via* LAB, as well as other beneficial bacteria, which could metabolize and utilize carbohydrates to produce organic acids, *e.g.*, LA and AA. This effect resulted in a reduction in the pH value and as such inhibited the decomposition and utilization of proteins *via* spoilage microorganisms. In addition, the relatively lower AN content in silages with a low proportion of JR suggested that the lower MC of silages could possibly suppress the protein degrading microorganisms by elevating the osmotic pressure.

Lactic acid bacteria are known to be a primary consumer of WSC during the ensiling process by converting WSC to LA, which as a result decreases the silage pH. Therefore, the rapid establishment of LA fermentation is critical to efficient ensiling. In addition, other small-molecule organic compounds can be produced from microbial metabolism during ensiling, *e.g.*, acetic acid (AA), butyric acid (BA), and propionic acid (PA), and their contents can also affect the fermentative characteristics and quality of silages. A high LA content (greater than 3% DM) and a low BA content (less than 2% DM) are usually desired for high-quality silage (Hillion *et al.* 2018). In the present study, the LA content fell to the desired ranges and dominated the organic acids followed by AA. The AA content was found to increase as the proportion of the JR in the silages increased. Specifically, the LA contents of the M6 and M7 silages were greater than the LA contents

of the other silages (Table S3). The LA to AA ratio decreased when the proportion of JR was increased in the silages; however, this ratio was still higher than 3 in most treatments (Table S3). This result indicated that LA fermentation was the dominant microbial metabolism during the ensiling process, which was beneficial in producing good-quality silage (Keshri *et al.* 2018; Xu *et al.* 2018). In addition, a small amount of formic acid (FA) was observed on day 10 and day 30 but disappeared by day 60, except in the silages with JR alone due to its unsuitable silage fermentation environment with a high MC. Due to its high MC and fermentability, agro-industrial organic waste is usually unsuitable for ensiling alone. No PA or BA was found in any silages in this study. This is consistent with the result reported by Mu *et al.* (2020), who revealed that propionic bacteria do not tolerate low pH. According to Hillion *et al.* (2018), good-quality silage should possess a pH less than 4.5, a LA content greater than 3% DM, a BA content greater than 2% of the total volatile fatty acids, and an AN content less than 10% of the TN. Against these criteria, all silages with different substrates in this study were considered well-preserved given their high LA content (3.6% to 9.8% DM), low AN content (less than 5.5% of the TN), and no detectable BA content. Therefore, GS can be a good organic material to be used in co-ensiling with JR to produce quality silages.

### Dynamic Changes in the Bacterial Diversity during Ensiling

During ensiling, both the microbial community composition and diversity can have considerable influence on the silage quality, which was analyzed *via* high-throughput sequencing. For bacteria, both the Chao1 and ACE index, which reflect species richness, decreased in the first 30 d for most silages (except M5) and increased afterwards, except for GS only, in which the Chao1 and ACE index kept decreasing (as shown in Table S4). According to the microbial diversity indicated by the Shannon and Simpson index, the bacterial diversity of the silages with a relatively high proportion of JR, *i.e.*, M5 through M8 and JR only, kept increasing during the entire ensiling process, while the bacterial diversity of the silages with a low JR content, *i.e.*, M3 and M4, decreased in the first 30 d followed by an increase until the end of the ensiling process on day 60 (Table S4). For GS alone, the bacterial diversity decreased throughout the entire ensiling process. These results may suggest that the addition of JR provided more water and carbohydrates to the ensiling system, which benefited bacterial growth and fermentation during the ensiling process.

Figures 1a and 1b show the composition and relative abundance of the bacterial community at phylum and genus levels, respectively. At the phylum level (as shown in Fig. 1a), raw GS primarily contained *Proteobacteria* (62.41%), *Firmicutes* (19.24%), *Bacteroidetes* (9.30%), *Cyanobacteria* (3.86%), and *Actinobacteria* (3.07%). During the ensiling process, the relative abundance of *Proteobacteria* gradually decreased from 53.0% (on day 10) to 50.8% (on day 30) and to 9.4% (on day 60), while *Firmicutes* kept growing. The relative abundance of *Bacteroidetes* first increased to 11.7% on day 10 and to 18.6% on day 30, and then dramatically decreased to 1.36% on day 60. At the end of the ensiling process, *Firmicutes* and *Cyanobacteria* became the dominant species. A similar bacterial community and a shift of dominant species from *Proteobacteria* to *Firmicutes* were also observed in alfalfa ensiling processes (McGarvey *et al.* 2013; Yang *et al.* 2019), due to the proliferation of LAB, which belong to the *Firmicutes* phylum. Raw JR primarily contained *Firmicutes* (97.8%) with a small amount of *Cyanobacteria* (1.15%) (Fig. 1a). During the ensiling process, the relative abundance of *Firmicutes* gradually decreased to 89.4% on day 60, while the relative abundance of *Proteobacteria* and *Cyanobacteria* were elevated to 6.83% and 1.98%, respectively.

For the co-ensiling of GS and JR, the dominant species was observed to be *Firmicutes*, while its relative abundance in different silages varied in different trends (Fig. 1a). With the extension of the ensiling time, the relative abundance of *Firmicutes* further increased in the M3 and M8 samples, while it decreased in the M6 and M7 samples. In the M4 and M5 samples, the relative abundance of *Firmicutes* experienced a decrease during the early phase but increased during the later stage. Overall, a higher relative abundance of *Firmicutes* was observed in response to a higher proportion of JR. *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes* were also detected in co-ensiled silages with different relative abundances. These bacteria, which primarily originated from GS, initially had a higher relative abundance in the M3 and M4 samples compared to the other groups. After 30 d, the relative abundance of these bacteria in the M3 samples decreased along with the increased abundance of *Firmicutes*. The relative abundance of *Cyanobacteria* showed a significant increase in the M4 through M7 samples, which was followed by a decrease to lower than 10% on day 60. The relative abundance of *Proteobacteria* in the M5 through M7 samples and the relative abundance of *Bacteroidetes* in the M4 and M6 samples also increased in the later stage of the ensiling process. In the M8 samples, with the highest proportion of JR for co-ensiling, the relative abundance of these bacteria was always below 1%. Under anaerobic conditions, *Firmicutes* and *Proteobacteria* can effectively degrade fibrous materials in an ensiling system and provide substrates to support microbial growth, thus changing the lignocellulosic components during ensiling.

At the genus level (Fig. 1b), raw GS primarily contained LAB [*Lactobacillus* (11.3%), *Enterococcus*, *Staphylococcus*, and *Streptococcus*] and some non-LAB bacteria, e.g., *Burkholderia* (25.0%), *Acinetobacter* (15.4%), and *Bacteroidales* (4.1%). Raw JR contained LAB [*Lactobacillus* (94.5%)] and a small amount of non-LAB bacteria, e.g., *Bacillus* (3.2%). In the ensiling with GS only, *Lactobacillus* had a relatively low abundance at the beginning (5.5% on day 10 and 0.27% on day 30) but turned into the dominant species by day 60 (87.5%). In addition, other genera were also detected in the GS silages, e.g., *Sphingomonas* (8.1%) and *Bacteroidales* (4.9%) on day 10, and *Bacteroides* (15.7%), *Faecalibacterium* (8.0%), *Citrobacter* (6.0%), and *Lactococcus* (2.44%) on day 30. During the ensiling with JR only, *Lactobacillus* was the dominant bacteria at the genus level throughout all 60 d, with a relative abundance of 98.2%, 89.4%, and 87.5% on day 10, 30, and 60, respectively. In addition, more LABs were found after 60 d, including *Bifidobacterium*, *Enterococcus*, and *Pediococcus*. For non-LAB bacteria, the relative abundance of *Acetobacter* slightly increased from 0.18% on day 10 to 5.1% on day 30 and to 4.3% on day 60.

For co-ensiling, *Lactobacillus* was also the dominant species in all silages (Fig.1b), which was similar to the result reported by Ren *et al.* (2020). After 10 d of ensiling, the relative abundance of *Lactobacillus* increased gradually as the JR content increased in the silages (from M3 to M8). The relative abundance of *Lactobacillus* in the M3 and M4 samples was 46.8% and 56.4%, respectively, which was significantly lower than the relative abundance of *Lactobacillus* in the M5 through M8 samples (88.7% to 95.2%). From day 10 to 30, the relative abundance of *Lactobacillus* in the M3 samples increased to 86.9%, while it decreased in different extents in the M4 to M7 samples. On day 60, the relative abundance of *Lactobacillus* in the M3 and M8 samples still maintained a high level (greater than 90%), while it fell to 70% to 80% in the other silage groups. Similarly, *Lactobacillus* was also the dominant genera in all silages when amaranth was co-ensiled with rice straw (Mu *et al.* 2020). More diverse LABs were found in the M3 through M7 samples, including *Lactobacillus*, *Pediococcus*, *Bifidobacterium*, *Enterococcus*,

*Lactococcus*, *Staphylococcus*, and *Streptococcus*, while only *Lactobacillus* was found as a LAB in the M8 samples. In addition, *Bacillus* and *Acinetobacter* were also found in the M3 through M8 samples. They can consume nutritional components, e.g., protein, and cause deterioration in the silages through oxidation-reduction reactions. However, the relative abundance of these undesired species were all less than 10%, which indicated that these undesirable microbes were suppressed by the LAB, leading to good conditions for long-term preservation (Luo *et al.* 2021).

### Dynamic Changes in the Fungal Diversity during Ensiling

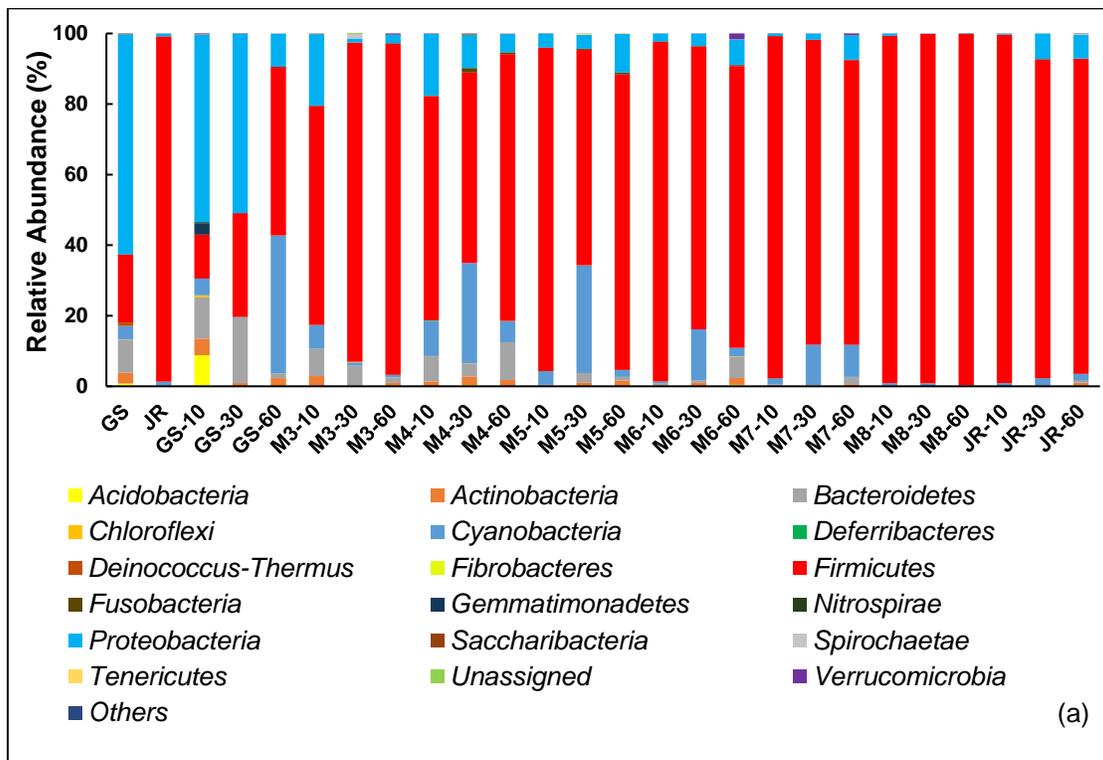
Controlling the MC is critical to the ensiling process. A low MC, e.g., 25% to 35%, can limit the release of leachate and inhibit undesirable microbial activities, e.g., *Clostridia* fermentation. However, fungi can still flourish under low MC conditions (Cheli *et al.* 2013). As a result, the dynamic changes in the fungal diversity were also analyzed during the ensiling process. After ensiling, both the fungal species richness and diversity became higher in most silages with different treatments, which was consistent with the results of the reads and OTUs. It is noteworthy that the M5 samples showed the most dramatic increase in the Chao1 and Shannon index, possibly because the GS to JR mass ratio was appropriate for facilitating fungal growth (Table S5).

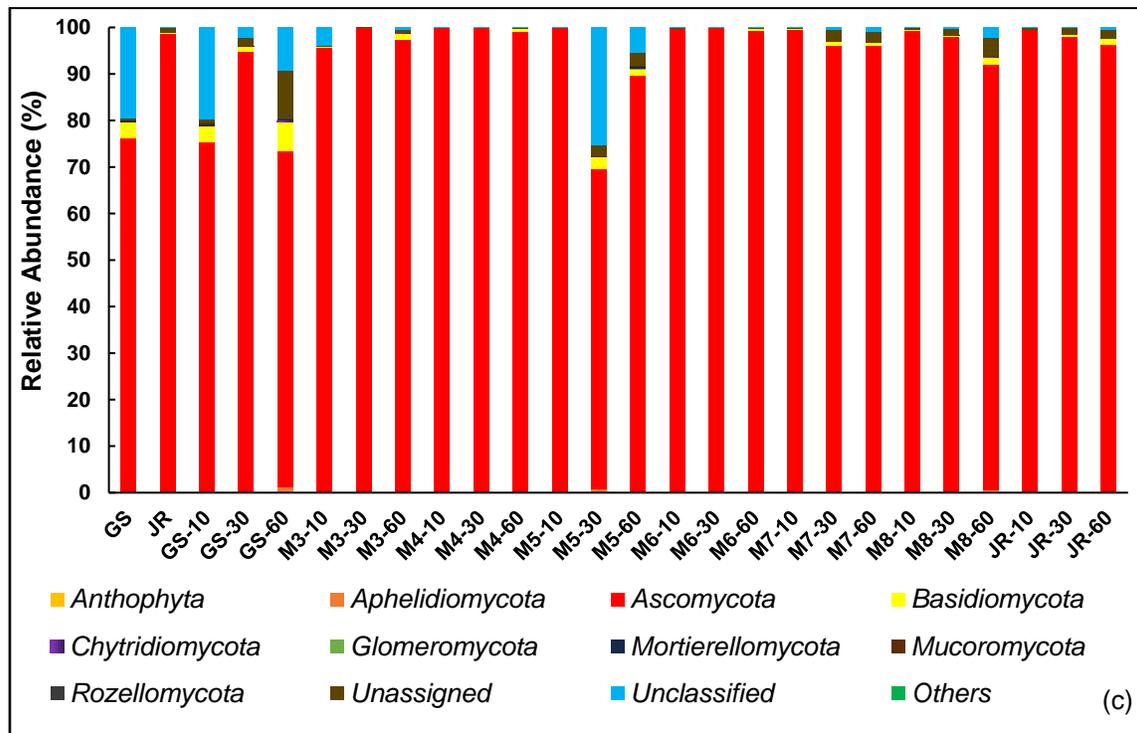
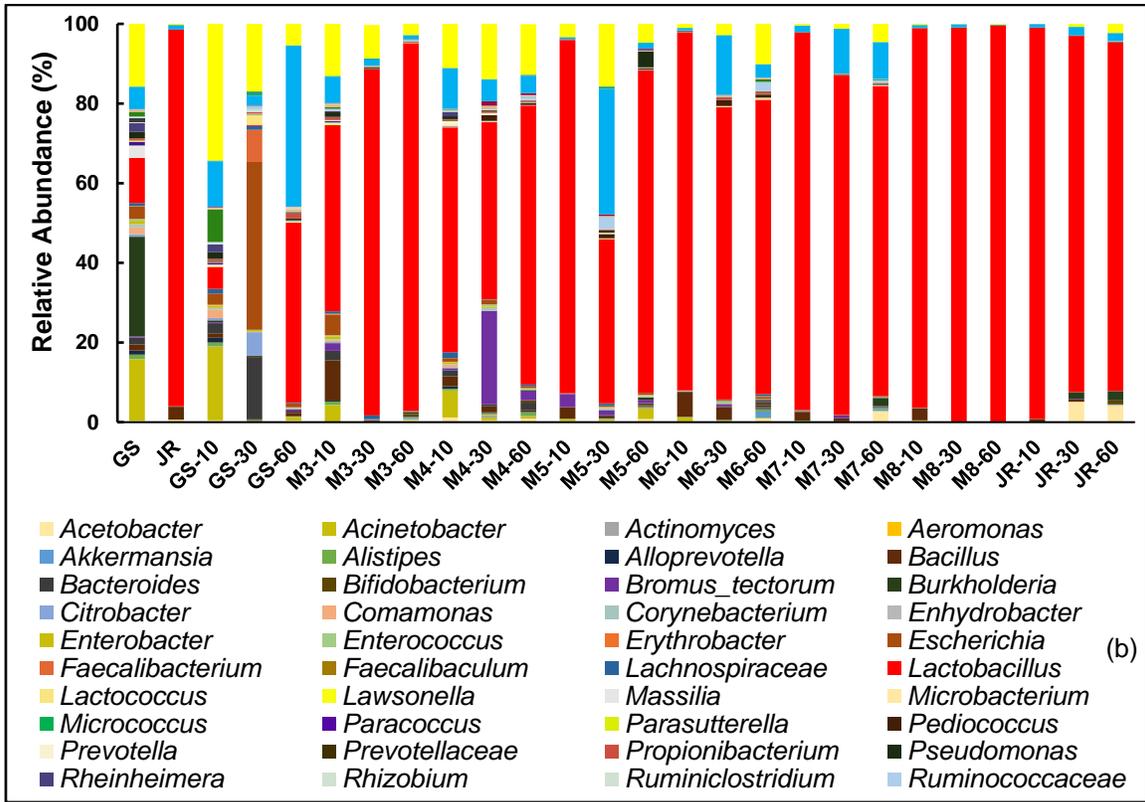
At the phylum level (Fig. 1c), both the raw GS and raw JR primarily contained *Ascomycota*, with a relative abundance of 76.1% and 98.7%, respectively. A small amount of *Basidiomycota* (3.54%) and some other unclassified fungi were also found in the GS. The fungal community did not significantly change after the ensiling of GR and JR alone, with 72.2% *Ascomycota* and 6.2% *Basidiomycota* in GS and 96.3% *Ascomycota* in JR. During the co-ensiling process, *Ascomycota* was still the dominant species with a relative abundance greater than 90% for most silages. However, its relative abundance in the M5 samples particularly decreased to 68.9% on day 30 due to the presence of *Basidiomycota* and *Aphelidiomycota*, while the relative abundance of *Ascomycota* inversely increased to 89.4% on day 60.

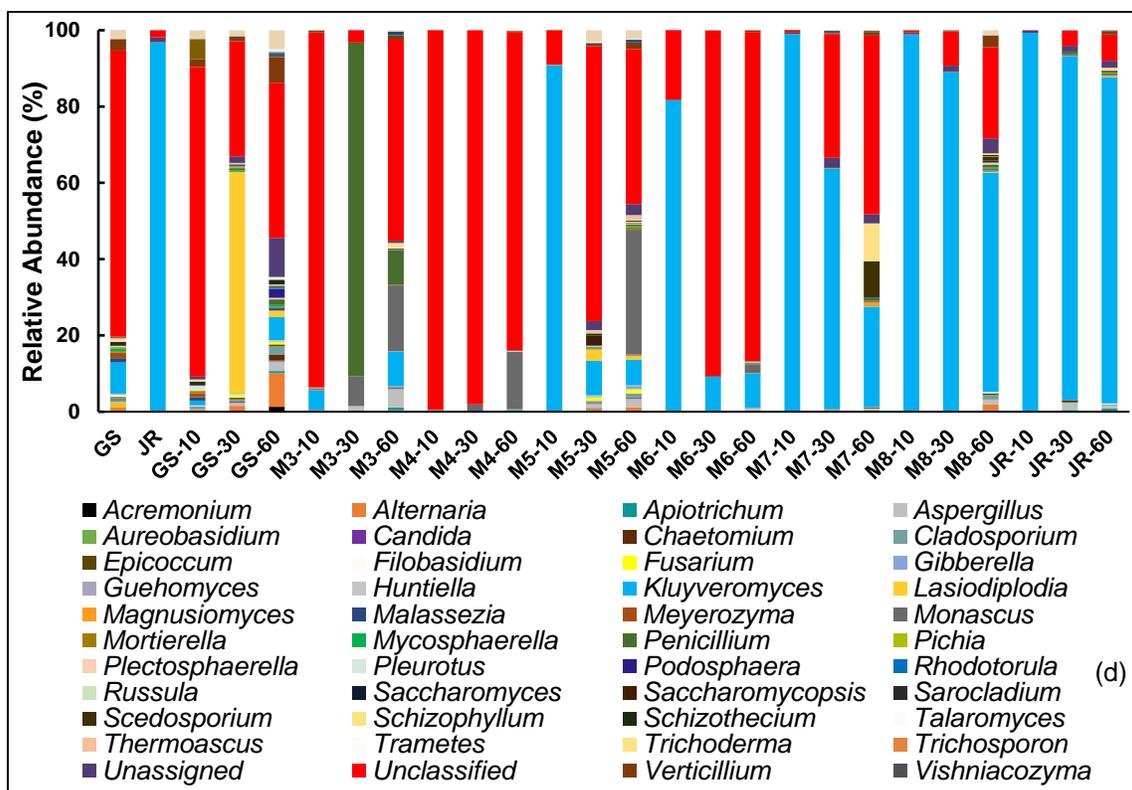
At the genus level (as shown in Fig. 1d), raw GS primarily contained unclassified organism (74.9%), *Kluyveromyces* (8.2%), and *Verticillium* (2.9%), while JR primarily contained *Kluyveromyces* (96.7%) and a small number of unclassified organisms (1.8%). Yeast has been reported to have better survivability in organic acid-rich environments compared to other fungal species, as they are more resistant to a low pH and able to metabolize a wide range of substrates (McDonald *et al.* 1991). During the ensiling process, the yeast-dominated fungal community became more diverse as the pH increased through LA assimilation (Ávila *et al.* 2010). The dominant species in the GS silages changed from unclassified (81.1%) on day 10 to *Lasiodiplodia* (57.90%), and then back to unclassified (30.2%) on day 30. Afterwards, the relative abundance of the unclassified organism increased to 40.7% after 60 d of ensiling, while more *Alternaria* (8.8%), *Verticillium* (6.7%), and *Kluyveromyces* (6.1%) appeared. For the ensiling process with JR only, the dominant species was always *Kluyveromyces* with a relative abundance of 99.3%, 90.2%, and 85.5% on day 10, 30, and 60, respectively. The relative abundance of the unclassified organism increased with time, from 0.12% on day 10 to 3.96% on day 30, and to 6.89% on day 60.

During the co-ensiling process, the dominant fungal genus was also unclassified in the M3 and M4 samples on day 10 with a relative abundance of 93.0% and 99.5%, respectively. An increased proportion of JR turned the dominant species to *Kluyveromyces* in the M5 through M8 samples. From day 10 to 30, the relative abundance of the dominant

fungi all decreased by various extents and even resulted in a change in dominant species. In the M3 samples, the relative abundance of the unclassified organism decreased to 3.2%, and *Penicillium* became the new dominant species, with a relative abundance of 87.5%, while *Monascus* was found for the first time, with a relative abundance of 7.8%. In the M5 and M6 samples, the relative abundance of *Kluyveromyces* decreased to 9.0% and 9.1%, respectively, while the unclassified organism became the new dominant species with a relative abundance of 72.3% and 90.6% on day 30 and 60, respectively. On day 60, the unclassified organism became the dominant fungi in all silages except for the M8 samples, which was consistent with findings from recent studies (Zhou *et al.* 2019). A relatively higher proportion of *Monascus* was also detected in the M3 through M5 samples, and some *Kluyveromyces* was detected in the M3 through M7 sample. The relative abundance of *Kluyveromyces* in the M8 samples further decreased to 57.5%, while the relative abundance of the unclassified organism increased to 23.9%. In addition to those dominant species, *Penicillium* (9.0%) and *Aspergillus* (4.8%) were also found in the M3 samples and *Scedosporium* (9.5%) was found in the M7 samples. In conclusion, the unclassified organism became the dominant fungi in the low JR silages, *i.e.*, GS, M3, M4, M5, and M6, while the dominant fungi was *Kluyveromyces* in the high JR silages, *i.e.*, M7, M8, and JR.







**Fig. 1.** Relative abundance of the microbial community: (a) bacteria at the phylum level; (b) bacteria at the genus level; (c) fungi at the phylum level; and (d) fungi at the genus level (the label, e.g., GS-10, of the x-axis is the substrate name, e.g., GS, followed by the ensiling time in days, e.g., -10)

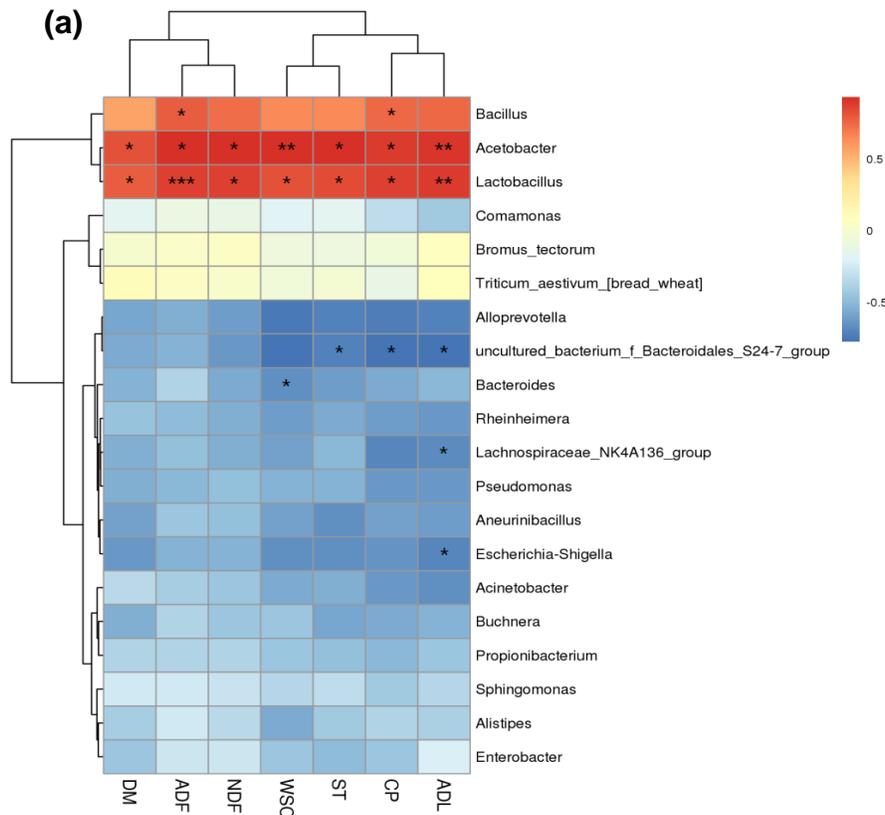
## Correlations between the Fermentation Quality and the Microbial Flora of the Silages

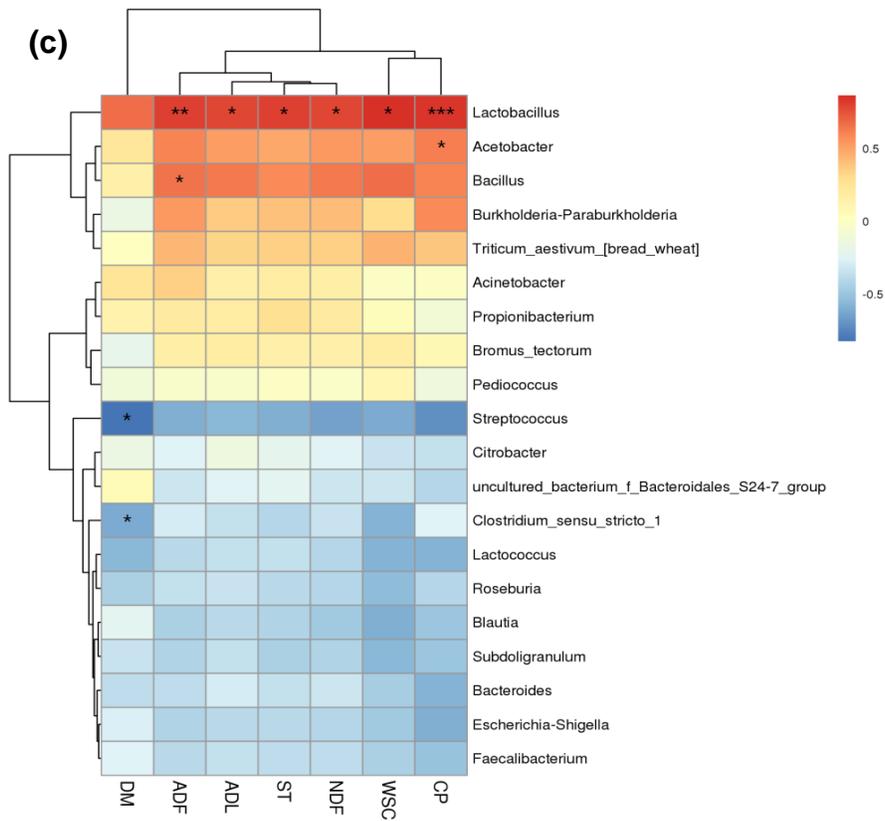
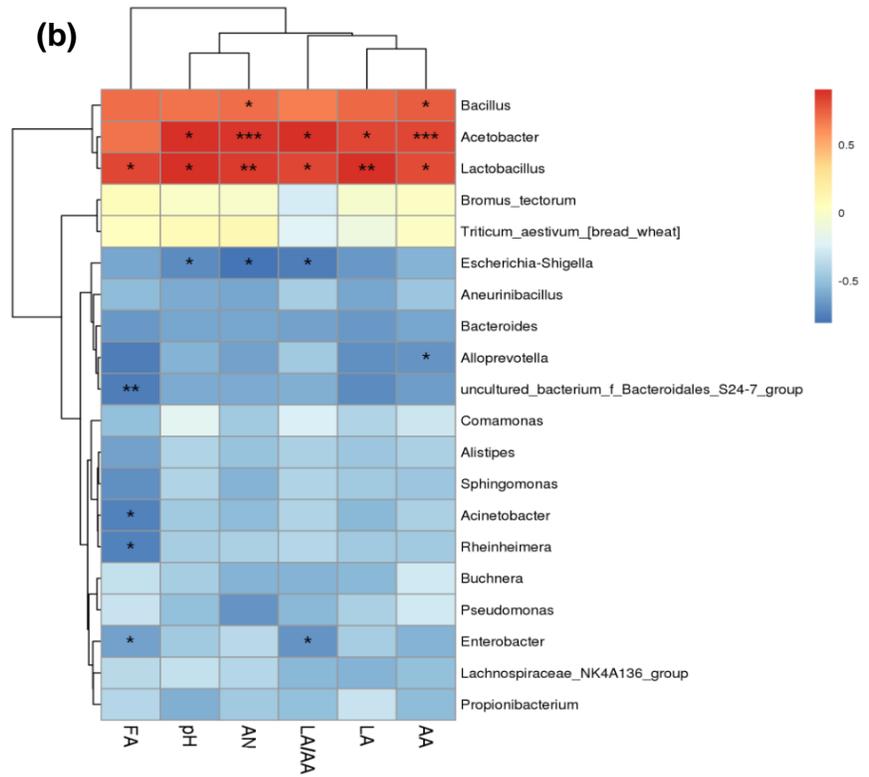
### *Correlations between the fermentation quality and the bacterial community*

The correlations between the fermentation quality and the microbial flora of the silages are shown in Fig. 2. The color gradient bars on the right side of each figure show the correlation coefficients. *Bacillus*, *Acetobacter*, and *Lactobacillus* showed significant positive correlations with the contents of multiple nutritional components, e.g., DM, ADF, NDF, WSC, ST, CP, and ADL on day 10, while only *Lactobacillus* continued to have a significant positive correlation with the content of these nutritional components on day 30 and 60. *Lactobacillus*, *Acetobacter*, and *Bacillus* were responsible for the production of organic acids during the ensiling process (Fig. 2b) on day 10, which reduced the silage pH through the fermentation of WSC. As a result, the activities of undesired microorganisms can be inhibited, e.g., *Enterobacteria* and *Clostridia*, which can degrade proteins. Thus, the organic matter of the feedstock can be preserved (Sikora *et al.* 2019). With the extension of the ensiling time, *Lactobacillus* evolved into the dominant bacteria in the silages at later ensiling stages and produced a large amount of LA to reduce the pH of the silages and preserved nutritional components while inhibiting the growth and reproduction of other bacteria (Ali *et al.* 2020). On the contrary, the relative abundance of *Streptococcus*, *Clostridium*, and *Pediococcus* was negatively correlated with the DM content of the silages, as they consumed the organic matter of the silages during the ensiling process, which led to a reduction in the DM. In addition, negative correlations ( $p$ -value less than

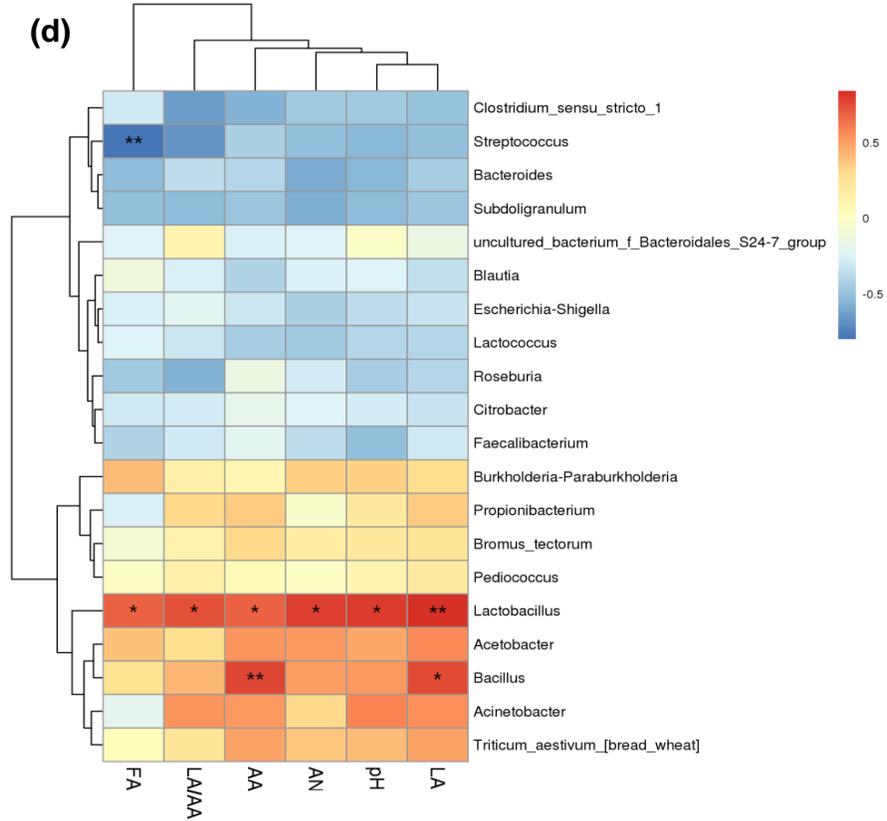
0.05) were found between the WSC content and the relative abundance of the genus *Bacteroides* ( $r = -0.657$ ), *Alistipes* ( $r = -0.730$ ), and Lachnospiraceae ( $r = -0.748$ ). This result indicated that these bacteria might be responsible for the depletion of the WSC.

For the fermentation characteristics, *Lactobacillus* and *Acetobacter* exhibited strong positive correlations with the LA and AA contents on day 10 (as shown in Fig. 2b). The LA and AA contents were positively correlated with *Lactobacillus* and *Bacillus* on day 30 (as shown in Fig. 2d), while only *Lactobacillus* had a significant positive correlation with LA and AA on day 60 (as shown in Fig. 2f). These findings suggested that *Lactobacillus* was the major contributor for producing LA and AA during the late stage of the ensiling process. The FA content showed a high positive correlation with *Lactobacillus* during the entire silage process. On day 10 of the ensiling process, FA was detected in most silages and several bacterial species were found to negatively correlate with the FA content, e.g., *Bacteroidales* ( $r = -0.760$ ), *Acinetobacter* ( $r = -0.751$ ), *Rheinheimera* ( $r = -0.751$ ), and *Enterobacter* ( $r = -0.633$ ). The AN content and pH were positively correlated with *Acetobacter* ( $r = 0.884$  and  $r = 0.891$ , respectively) and *Lactobacillus* ( $r = 0.862$  and  $r = 0.894$ , respectively) on day 10 and were negatively correlated with *Escherichia* ( $r = -0.870$  and  $r = -0.810$ , respectively). However, the AN content and pH were only positively correlated with *Lactobacillus* on day 30 and 60, which could be due to the fact that the low pH suppressed the growth of *Lactobacillus*, which started to proliferate again when the pH increased (Zeng *et al.* 2020).

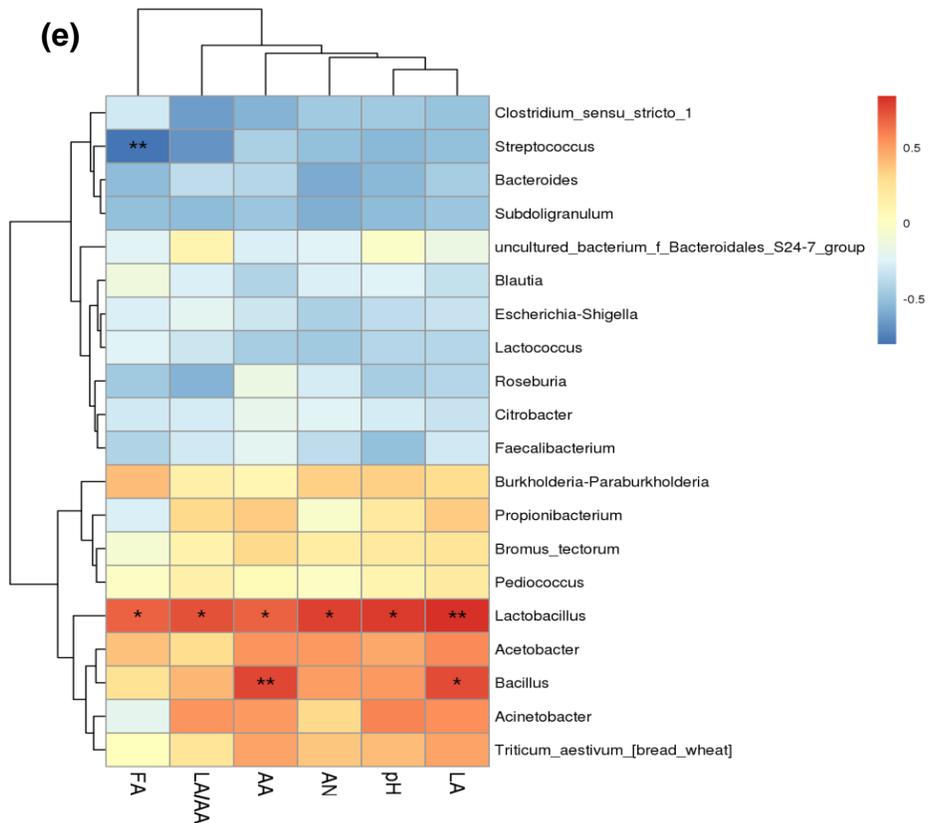


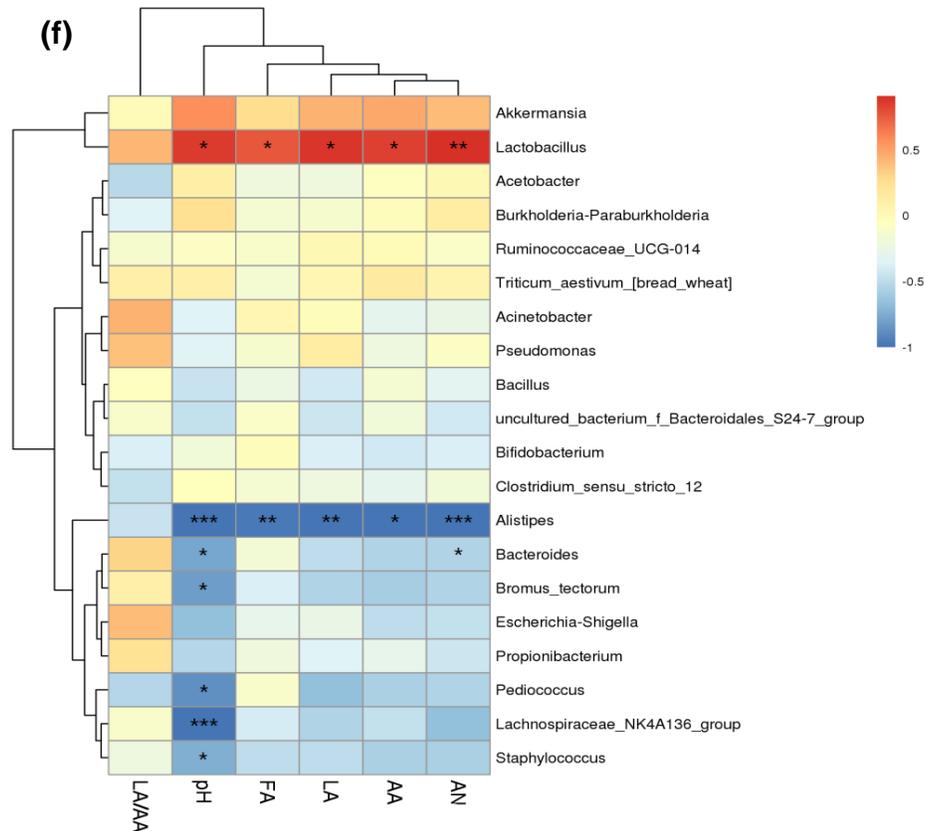


(d)



(e)





**Fig. 2.** Correlations between the bacteria diversity and the fermentation quality: (a) the chemical composition at day 10; (b) the fermentation characteristics at day 10; (c) the chemical composition at day 30; (d) the fermentation characteristics at day 30; (e) the chemical composition at day 60; and (f) the fermentation characteristics at day 60 (\*\*\*) denotes a  $p$ -value less than 0.001; \*\* denotes a  $p$ -value less than 0.01; and \* denotes a  $p$ -value less than 0.05; The color gradient bars on the right side of each figure show the correlation coefficients, e.g., top red means highly positive correlation and bottom blue means highly negative correlation. Note: DM - dry matter; ADF - acid detergent fiber; NDF - neutral detergent fiber; WSC - water-soluble carbohydrates; ST - starch; CP - crude protein; ADL - Acid detergent lignin; FA - formic acid; AN - ammonia nitrogen; LA - lactic acid; AA - acetic acid

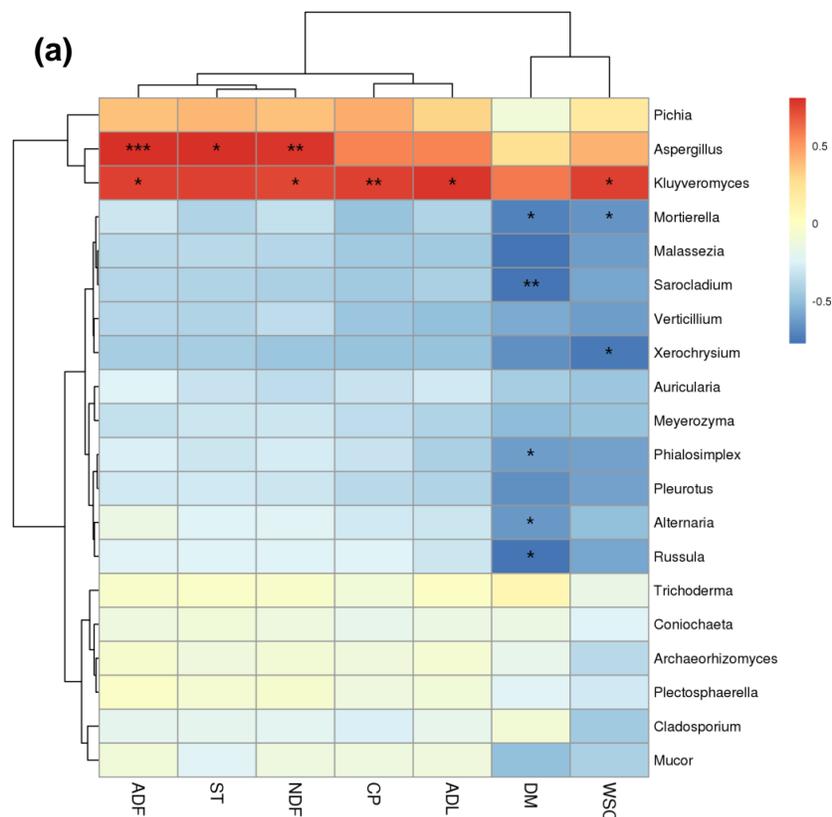
The homo-fermentation of LA was the absolutely dominant process, while some heterofermentative LAB were able to convert LA ( $pK_a = 3.86$ ) into AA ( $pK_a = 4.76$ ), which resulted in a slight increase in pH (Ávila *et al.* 2010). Therefore, the pH of the silages were primarily affected by the type of LAB fermentation. In addition, *Alistipes* was found to negatively correlate with all analyzed fermentation characteristics on day 60, although its relative abundance was low. The final pH of the silages could be affected by multiple species, including *Alistipes*, *Bacteroides*, *Escherichia-Shigella*, *Pediococcus*, *etc.* An increase in the relative abundance of these species can increase the pH of the silage.

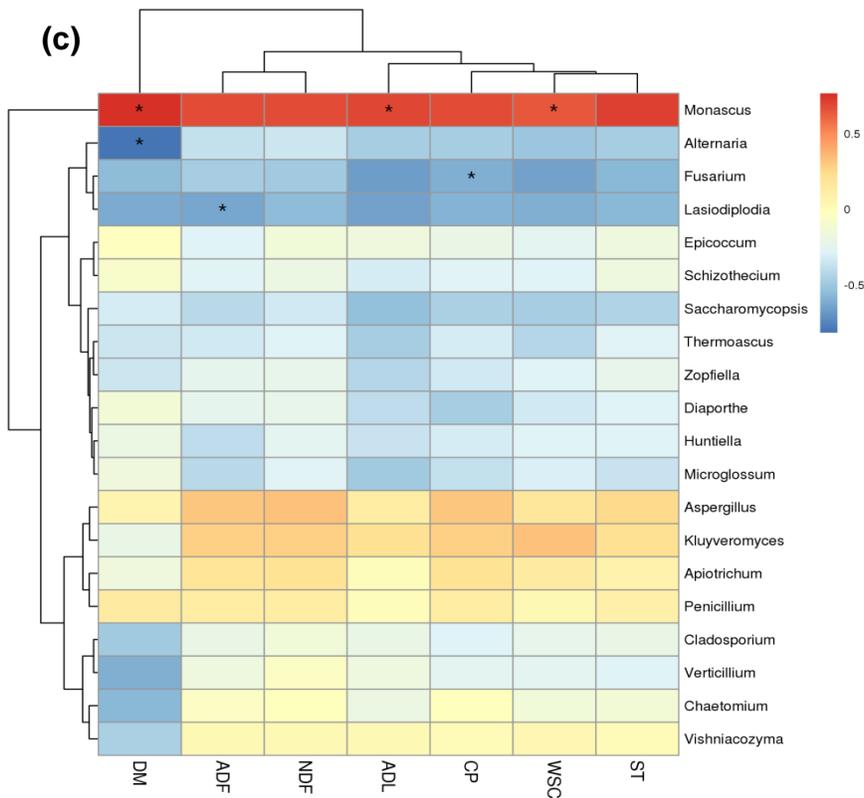
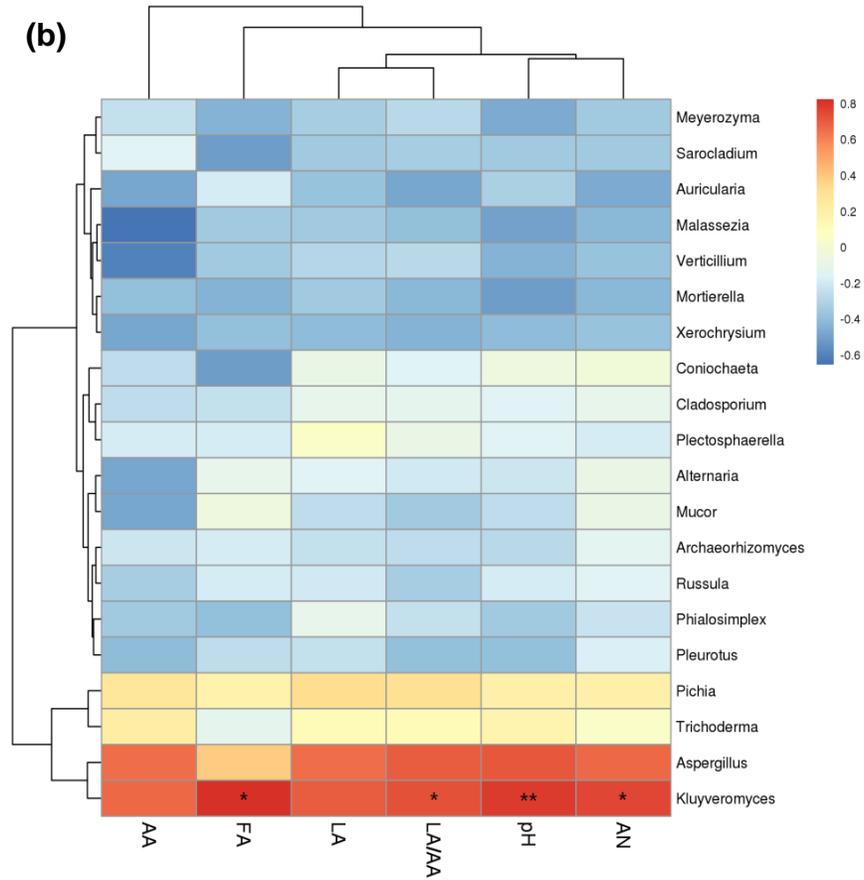
#### *Correlations between the fermentation quality and the fungal community*

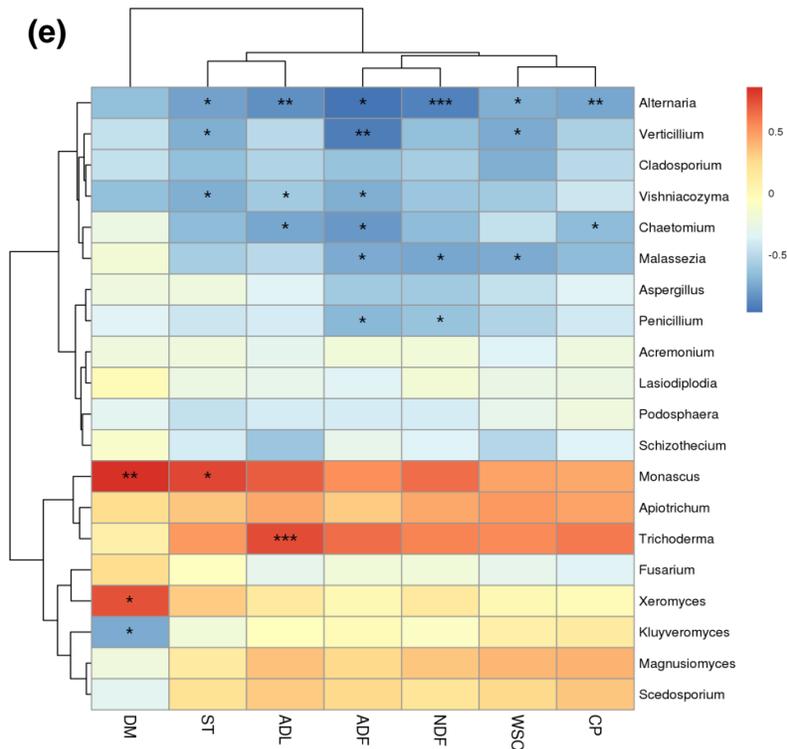
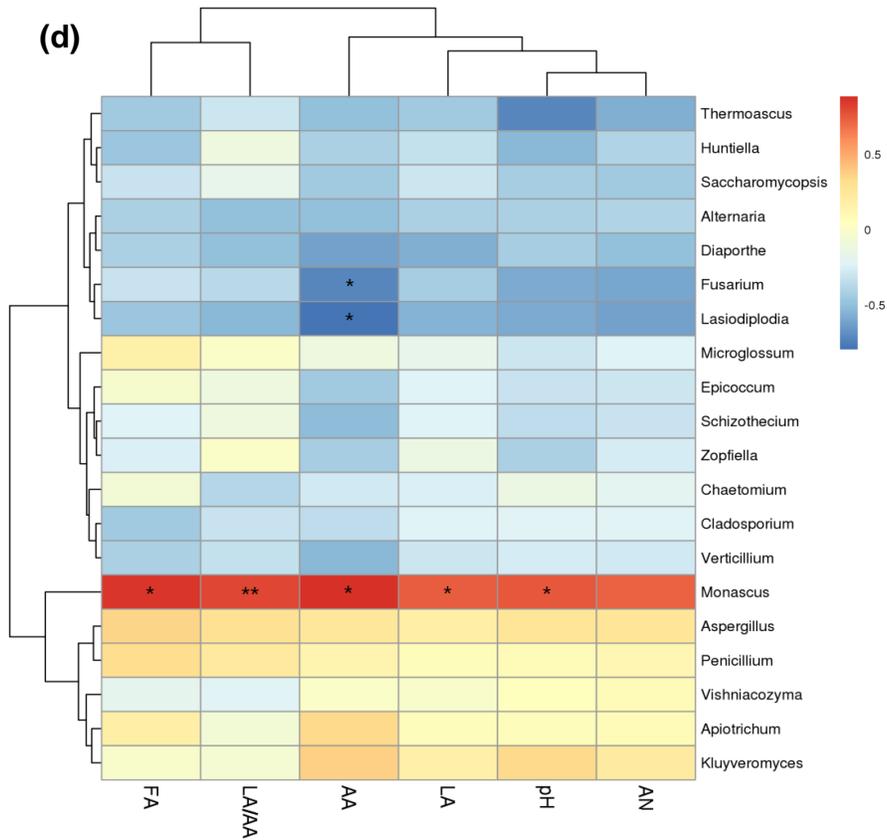
Strong correlations between the fungal species and fibrous components were found in the ensiling process (as shown in Fig. 3a). The color gradient bars on the right side of each figure show the correlation coefficients. On day 10, the contents of multiple components, *i.e.*, ADF, ST, NDF, CP, ADL, and WSC, positively correlated with the

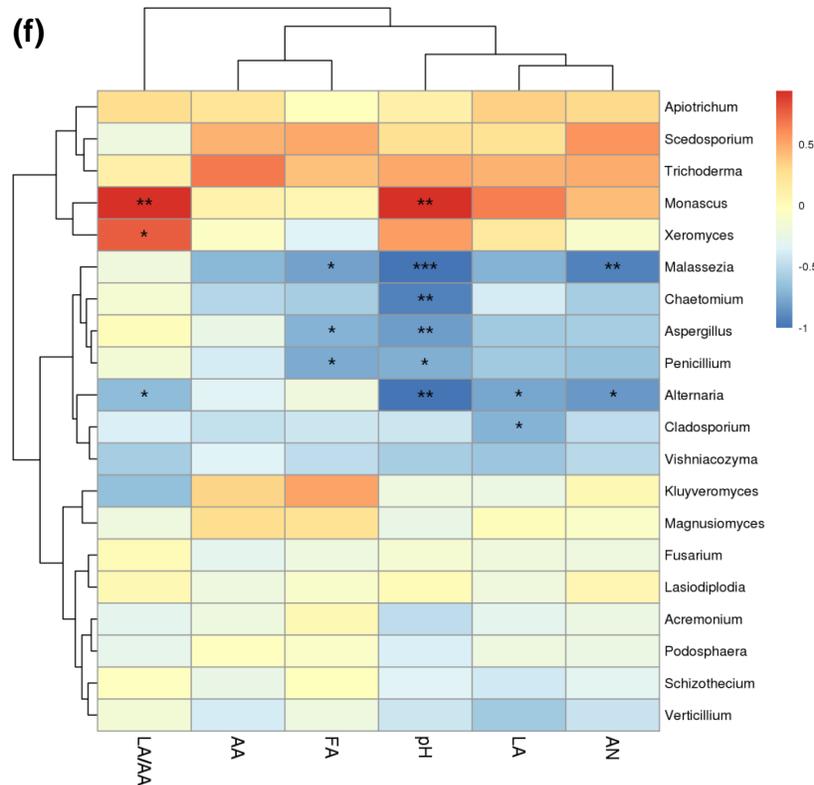
relative abundance of *Aspergillus* and *Kluyveromyces*. As the ensiling process continued, *Monascus*, whose relative abundance increased from 0% to 32.66%, became the most important species in terms of affecting the DM, ADL, WSC, and ST contents. Negative correlations were found between the DM content and the relative abundance of *Mortierella* ( $r = -0.723$ ), *Sarocladium* ( $r = -0.764$ ), *Phialosimplex* ( $r = -0.629$ ), *Alternaria* ( $r = -0.646$ ), and *Russula* ( $r = -0.773$ ), which indicated that these fungi were the major contributors of DM loss. Moreover, the WSC content was negatively correlated with *Mortierella* ( $r = -0.653$ ) and *Xerochrysum* ( $r = -0.743$ ). *Alternaria* was observed to negatively correlate with all analyzed components except for the DM content on day 60, although its relative abundance was low (less than 8.84%).

Highly positive correlations were found between the FA and the relative abundance of the genus *Kluyveromyces* ( $r = 0.824$ ) on day 10, but the relative abundance of *Monascus* positively correlated with the organic acid contents, including FA, AA, and LA on day 30. In addition, on day 60, the FA content was negatively correlated with the relative abundance of *Malassezia* ( $r = -0.799$ ), *Aspergillus* ( $r = -0.722$ ), and *Penicillium* ( $r = -0.763$ ) which were considered the most important colonizing fungi and producers of mycotoxins in silages (Cheli *et al.* 2013). *Kluyveromyces* was observed to positively correlate with the pH and AN content on day 10; in addition, the pH was positively correlated with the relative abundance of *Monascus* on day 30 and 60. In addition, *Malassezia* ( $r = -1.000$ ), *Chaetomium* ( $r = -0.938$ ), *Aspergillus* ( $r = -0.817$ ), *Penicillium* ( $r = -0.830$ ), and *Alternaria* ( $r = -1.000$ ) were found to be negatively correlated with the pH because these fungi could degrade and consume fibrous materials and compete with LAB, and therefore impair the quality of the silages.









**Fig. 3.** Correlations between the fungi diversity and the fermentation quality. (a) the chemical composition at day 10; (b) the fermentation characteristics at day 10; (c) the chemical composition at day 30; (d) the fermentation characteristics at day 30; (e) the chemical composition at day 60; and (f) the fermentation characteristics at day 60 (\*\*\*) denotes a  $p$ -value less than 0.001; \*\* denotes a  $p$ -value less than 0.01; and \* denotes a  $p$ -value less than 0.05; The color gradient bars on the right side of each figure show the correlation coefficients, e.g., top red means highly positive correlation and bottom blue means highly negative correlation. Note: DM - dry matter; ADF - acid detergent fiber; NDF - neutral detergent fiber; WSC - water-soluble carbohydrates; ST - starch; CP - crude protein; ADL - Acid detergent lignin; FA - formic acid; AN - ammonia nitrogen; LA - lactic acid; AA - acetic acid

## CONCLUSIONS

1. The present study illustrated that the co-ensiling of grain stillage (GS) with Jerusalem artichoke residue (JR) could effectively preserve the water-soluble carbohydrates (WSC), crude protein (CP), and starch (ST), as well as other nutrients. The GS to JR mass ratios of 1.2 to 1 and 1 to 1.5 were the best among the studied mixing ratios in terms of the silage quality. Both mass ratios effectively preserved important nutrients, e.g., WSC, CP, and ST, and had a higher relative feed value (RFV) and biodegradation potential (BDP). According to the microbial community analysis, *Lactobacillus* dominated throughout the entire ensilage process and its relative abundance positively correlated with the content of multiple nutritional components, e.g., ADF, WSC, CP, and ST. The relative abundance of multiple fungal species, e.g., *Kluyveromyces* and *Monascus*, was also positively correlated with multiple nutritional components, e.g., DM, ST, and WSC.

2. These results suggested that the co-ensiling of GS with JR could be a successful approach to preserve nutrients and optimize the fermentation qualities of silages. Co-ensiling provides a promising solution for the long-term storage of perishable organic waste in response to the need of timely treatment and sustainable utilization of such wastes. The authors will focus on the internal mechanism of the effects of moisture on the components and microbial changes, and adjust other components (e.g., WSC) to make co-ensiling feasible in future work.

## ACKNOWLEDGMENTS

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## SUPPLEMENTAL

Table S1. Dynamic Changes of Nutrient Components during Ensiling Fermentation (% DM<sup>1</sup>)

Items	Time (Day) <sup>2</sup>	Substrates (with different GS/JR mass ratios) <sup>2</sup>								SEM <sup>3</sup>	p-value <sup>4</sup>		
		GS	M3	M4	M5	M6	M7	M8	JR		T	S	T×S
DM	0	84.17±0.19Ab	70.00±0.01Bb	60.00±0.07Cb	50.00±0.11Da	40.00±0.05Eb	30.00±0.13Fab	20.00±0.11Ga	10.70±0.20Ha	0.106	<0.001	<0.001	<0.001
	10	87.29±0.14Aa	71.57±0.20Bb	63.79±0.09Ca	50.88±0.20Da	45.24±0.09Ea	30.77±0.07Fa	18.96±0.03Ga	10.38±0.01Ha				
	30	87.20±0.01Aa	74.26±0.07Ba	62.28±0.05Ca	50.69±0.04Da	36.37±0.05Ec	29.23±0.05Fb	19.11±0.02Ga	10.39±0.07Ha				
	60	83.83±0.21Ab	74.72±0.03Ba	63.70±0.07Ca	49.53±0.03Da	34.59±0.08Ec	31.35±0.06Fa	16.85±0.03Gb	10.34±0.05Ha				
	Time mean	85.62A	72.64B	62.44C	50.28D	39.05E	30.34F	18.73G	10.45H				
WSC	0	7.75±0.12Ha	8.82±0.07Ga	9.57±0.01Fa	10.33±0.05Ea	11.08±0.03Da	11.84±0.02Ca	12.59±0.01Ba	13.29±0.14Aa	0.045	<0.001	<0.001	<0.001
	10	5.89±0.30Db	7.31±0.11Bb	6.56±0.16Cb	8.30±0.03Ab	7.02±0.08Bd	5.19±0.07Ed	5.77±0.12Dc	8.19±0.08Ab				
	30	4.88±0.15Ec	5.58±0.14Dc	6.58±0.11Cb	10.32±0.09Aa	8.24±0.02Bc	10.49±0.05Ab	6.12±0.07Dbc	4.14±0.09Fc				
	60	6.22±0.08Eb	6.24±0.11Ec	4.38±0.19Fc	8.58±0.10Bb	9.26±0.13Ab	7.04±0.03Dc	6.87±0.07DEb	7.89±0.09Cb				
	Time mean	6.19F	6.99E	6.77E	9.38A	8.90B	8.64BC	7.84D	8.38C				
CP	0	16.25±0.12Ha	16.53±0.09Ga	16.73±0.02Fa	16.93±0.14Ea	17.13±0.12Da	17.33±0.11Ca	17.53±0.16Ba	17.72±0.07Ad	0.016	<0.001	<0.001	<0.001
	10	14.71±0.15Eb	14.59±0.09Ed	14.22±0.03Fc	14.97±0.05Dd	15.38±0.12Cd	15.01±0.07Dc	15.62±0.16Bc	18.41±0.15Ac				
	30	14.80±0.16Eb	15.73±0.02Db	15.55±0.10Db	15.88±0.12CDb	16.13±0.16BCb	13.38±0.04Fd	16.23±0.06Bb	18.68A±0.07b				
	60	14.64±0.12Fb	14.98±0.06Ec	14.38±0.08Gc	15.16±0.05Dc	15.66±0.06Cc	15.75±0.11Cb	16.35±0.12Bb	19.76±0.02Aa				
	Time mean	15.10G	15.46E	15.22F	15.74D	16.08C	15.37E	16.43B	18.64A				

ST	0	26.13±0.01Aa	25.16±0.10Bd	24.47±0.17Cc	23.78±0.08Dc	23.09±0.02Ec	22.41±0.02Fb	21.72±0.06Ga	21.08±0.11Hb	0.046	<0.001	<0.001	<0.001
	10	25.42±0.30Cb	26.08±0.08Bc	27.19±0.11Ab	23.56±0.12Dc	23.69±0.13Dc	21.93±0.22Fb	20.97±0.06Gb	22.45±0.11Ea				
	30	26.77±0.21Ca	27.26±0.14Bb	27.63±0.14Ab	27.87±0.15Aa	26.43±0.21Da	24.16±0.19Ea	20.73±0.10Fb	13.97±0.06Gd				
	60	24.34±0.18Dc	31.85±0.04Aa	28.39±0.07Ba	26.00±0.08Cb	25.47±0.26Cb	17.07±0.15Fc	20.68±0.21Eb	17.58±0.11Fc				
	Time mean	25.67C	27.59A	26.92B	25.30CD	24.67D	21.39E	21.03E	18.77F				

Note:<sup>1</sup>DM-dry matter, GS-grain stillage, JR-*J. artichoke* residue, CP-crude protein, WSC-water soluble carbohydrates, and ST-starch.

<sup>2</sup>Different capital letters in the same row show significant difference among different substrates with the same ensiling time at  $p_{critical}=0.05$  while different lowercase letters in the same column show significant difference among different ensiling time for the same substrate at  $p_{critical}=0.05$ . The values are the mean±standard deviation of three replicates.

<sup>3</sup>SEM-standard error of means.

<sup>4</sup>T-ensiling time, S-substrate, and TxS-the interaction between ensiling time and substrate.

**Table S2.** Dynamic Changes of Lignocellulosic Components during Ensiling (% DM<sup>1</sup>)

Items	Time (Day)	Substrates (with different GS/JR mass ratios) <sup>2</sup>								SEM <sup>3</sup>	p-value <sup>4</sup>		
		GS	M3	M4	M5	M6	M7	M8	JR		T	S	T×S
ADF	0	36.92±0.31 Hd	38.83±0.2 8Gc	40.18±0.2 0Fc	41.52±0.3 2Ea	42.87±0.0 9Da	44.22±0.1 1Ca	45.57±0.2 9Bc	46.82±0.2 1Ab	0.105	<0.00 1	<0.001	<0.001
	10	41.46±0.58 Db	41.87±1.7 8Db	42.94±0.6 0Cb	40.14±0.2 5Ea	41.62±1.1 6Dab	44.30±0.3 7Ba	44.19±0.5 5Bc	46.22±0.7 6Ab				
	30	39.56±0.97 Ec	40.58±0.8 1DEb	43.48±1.1 6Bab	41.31±1.2 9CDa	42.79±1.0 7BCa	43.63±0.5 8Bab	48.53±0.1 8Ab	48.19±1.0 2Aa				
	60	43.43±0.51 Ca	43.22±1.1 0Ca	45.29±1.1 2Ba	41.07±1.0 7Da	40.36±0.2 2Db	43.09±0.3 6Cb	50.50±1.0 5Aa	49.77±0.7 2Aa				
	Time mean	40.34D	41.13CD	42.97BC	41.01CD	41.91C	43.81B	47.20A	47.75A				
NDF	0	54.29±1.08 Hc	54.71±1.1 9Gb	55.01±0.3 4Fc	55.31±0.5 5Ea	55.61±1.0 8Dab	55.90±1.0 0Cb	56.20±0.2 1Bb	56.48±0.3 0Ab	0.124	<0.00 1	<0.001	<0.001
	10	59.61±1.03 Bb	60.43±2.0 9Ba	64.20±0.8 1Aa	52.75±0.9 4Db	54.48±1.0 0Cdb	54.48±1.0 5Cdb	56.17±0.3 8Cb	59.20±0.6 7Ba				
	30	53.67±1.62 CDc	55.10±1.2 4Cb	57.17±0.4 1Bb	53.77±0.7 6Cdb	55.48±0.5 6BCab	57.13±1.0 7Ba	59.09±1.2 7Aa	52.47±0.5 3Dc				
	60	62.55±0.91 Aa	62.60±1.4 9Aa	63.39±1.2 3Aa	52.81±1.2 8Db	56.71±1.0 8Ca	58.08±0.6 6BCa	59.84±0.5 1Ba	58.04±0.6 9BCa				
	Time mean	57.53C	58.21B	59.94A	53.66F	55.57E	56.40D	57.83C	56.55D				
ADL	0	15.02±0.21 Aa	14.36±0.3 8Ba	13.89±1.7 1Ca	13.42±0.9 0Db	12.95±0.4 4Eab	12.48±0.2 9Fc	12.02±0.3 6Gb	11.58±0.4 2Hab	0.098	<0.00 1	<0.001	<0.001
	10	11.80±0.71 Dc	11.67±1.0 6Db	13.00±0.7 9CDa	16.30±0.8 7Ba	13.65±0.9 4Ca	19.21±0.9 7Aa	15.72±1.2 8Ba	13.13±0.9 8CDa				
	30	11.45±1.43 BCc	12.84±0.8 1ABb	11.08±0.2 2Cb	10.79±0.8 3Cc	11.64±0.9 4BCb	14.26±0.4 4Ab	14.14±0.4 0Aa	10.43±0.6 8Cb				
	60	13.73±0.68 Bb	15.68±0.7 1Aa	13.68±0.4 6Ba	11.97±0.7 4Cc	12.32±0.6 3Cab	12.13±0.8 8Cc	10.54±0.6 2Db	12.62±0.9 0BCa				
	Time mean	13.00CD	13.64B	12.91CD	13.12CD	12.64D	14.52A	13.11CD	11.94E				

CL	0	21.20±0.23 Hc	23.78±0.4 1Gc	25.59±0.5 5Fc	27.41±0.2 9Eb	29.23±0.8 3Da	31.04±0.4 7Ca	32.86±0.4 4Bb	34.55±0.1 0Ab	0.065	<0.00 1	<0.001	<0.001
	10	28.96±0.71 Ba	29.50±0.8 4Ba	29.24±0.2 5Bb	23.13±0.6 3Ec	27.27±0.5 9Cb	24.40±0.6 0Dc	27.78±0.9 7Cc	32.39±0.3 7Ac				
	30	27.42±0.50 Fb	27.04±0.0 3Fb	31.70±0.9 5Ca	29.82±0.6 0Da	30.45±0.1 4Da	28.67±0.1 8Eb	33.69±0.2 6Bb	37.06±0.6 8Aa				
	60	29.00±0.36 Da	26.85±0.4 7Eb	30.91±0.6 6Ca	28.41±0.3 9Db	27.34±0.4 9Eb	30.27±0.5 2Ca	39.26±0.5 5Aa	36.45±0.2 4Ba				
	Time mean	26.65D	26.79D	29.36C	27.19D	28.57CD	28.60CD	33.40B	35.11A				
HC	0	18.07±0.09 Aa	16.58±0.0 1Bb	15.53±0.0 5Cc	14.48±0.2 1Da	13.43±0.2 3Eb	12.38±0.1 1Fc	11.34±0.1 9Gab	10.36±0.3 4Hb	0.079	<0.00 1	<0.001	<0.001
	10	18.85±0.60 Ba	19.26±0.5 9Ba	21.96±0.5 1Aa	13.31±0.7 1Cab	13.56±0.3 4Cb	10.87±0.6 9Dd	12.68±0.2 7Ca	13.68±0.4 9Ca				
	30	14.81±0.69 ABb	15.22±0.8 9Ab	14.39±0.7 7Bc	13.16±0.9 2Cab	13.39±0.5 3Cb	14.20±0.8 7Bb	11.26±1.0 8Dab	4.98±0.71 Ec				
	60	19.83±0.78 ABa	20.07±0.9 1Aa	18.80±0.2 7Bb	12.44±0.3 7Eb	17.05±0.8 6Ca	15.68±0.3 9Da	10.04±0.5 5Fb	8.97±0.40 Gb				
	Time mean	17.89A	17.78A	17.67A	13.35C	14.36B	13.28C	11.33D	9.50E				
HoC	0	39.27±0.09 Hd	40.36±0.2 7Gd	41.13±0.2 4Fd	41.89±0.3 4Eab	42.66±0.7 2Db	43.43±0.3 1Cb	44.20±0.2 8Bb	44.91±0.2 0Ab	0.054	<0.00 1	<0.001	<0.001
	10	47.81±0.45 Cb	48.76±0.2 5Ba	51.20±0.3 3Aa	36.44±0.1 7Fc	40.83±0.2 6Ec	35.27±0.1 0Gc	40.46±0.0 7Ec	46.07±0.3 3Da				
	30	42.23±0.54 Dc	42.26±0.8 7Dc	46.09±0.2 3Ac	42.98±0.3 2CDa	43.84±0.4 3Ca	42.87±0.6 9CDb	44.95±0.8 4Bb	42.04±0.1 6Dc				
	60	48.82±0.44 Ba	46.92±0.5 9Cb	49.71±0.4 0Ab	40.84±0.0 4Fb	44.39±0.6 6Ea	45.95±0.3 4Da	49.30±0.1 7ABa	45.42±0.2 8Db				
	Time mean	44.53B	44.58B	47.03A	40.54E	42.93C	41.88D	44.73B	44.61B				
RFV	0	103.05±1.1 4Aa	99.72±0.7 8Ba	97.40±2.3 5Ca	95.12±1.1 8Dc	92.85±3.0 9Ed	90.61±3.1 4Fb	88.39±2.3 7Gb	86.35±1.2 1Hb	0.295	<0.00 1	<0.001	<0.001
	10	88.36± 2.15Dc	86.76± 5.04Ec	80.36± 1.60Fc	101.66± 2.14Aa	96.49± 3.27Ba	92.89± 1.46Ca	90.21± 1.28Da	83.12± 1.77Ec				

	30	100.75± 4.32Ab	96.75± 3.05Bb	89.56± 1.89Cb	98.14± 2.90Ab	93.19± 2.03Bc	89.42± 2.08Cb	80.47± 1.94Dc	91.06± 2.17Ca				
	60	81.91± 1.60Dd	82.08± 0.76Dd	78.72± 2.80Dd	100.30± 3.85Aa	94.26± 2.06Bb	88.63± 1.41Cc	77.05± 1.93Ed	80.36± 1.78Dd				
	Time mean	93.51C	91.33D	86.51E	98.81A	94.20B	90.39D	84.03F	85.22F				
BDP	0	2.62±0.14H b	2.81±0.28 Gc	2.96±0.35 Fc	3.12±0.18 Eb	3.29±0.09 Db	3.48±0.14 Ca	3.68±0.37 Bb	3.89±0.21 Aab	0.022	<0.00 1	<0.001	<0.001
	10	4.06±0.28A a	4.20±0.37 Aa	3.95±0.22 Aa	2.24±0.12 DEc	3.00±0.21 Cc	1.84±0.09 Ec	2.59±0.27 CDd	3.52±0.28 Bb				
	30	3.73±0.49A Ba	3.30±0.21 BCb	4.16±0.07 Aa	4.00±0.32 Aa	3.78±0.73 ABa	3.01±0.13 Cb	3.18±0.17 Cc	4.04±0.27 Aa				
	60	3.56±0.17B a	3.00±0.13 Cc	3.63±0.09 Bb	3.42±0.21 Bb	3.61±0.18 Ba	3.80±0.31 Ba	4.69±0.27 Aa	3.61±0.24 Bab				
	Time mean	3.49C	3.33D	3.68AB	3.20E	3.42CD	3.03F	3.54B	3.77A				
<p>Note:<sup>1</sup>DM-dry matter, GS-grain stillage, JR-<i>J. artichoke</i> residue, NDF-neutral detergent fibre, ADF-acid detergent fibre, ADL-acid detergent lignin, CL-cellulose, HC-hemicellulose, HoC-holocellulose, RFV-relative feed value and BDP-biodegradation Potential.</p> <p><sup>2</sup>Different capital letters in the same row show significant difference among different substrates with the same ensiling time at <math>p_{critical}=0.05</math> level. Different lowercase letters in the same column show significant difference among different ensiling time for the same substrate at <math>p_{critical}=0.05</math> level. The values are the mean±standard deviation of three replicates.</p> <p><sup>3</sup>SEM-standard error of means.</p> <p><sup>4</sup>T-ensiling time, S-substrate, and T×S-the interaction between ensiling time and substrate.</p>													

**Table S3.** Dynamic Changes of pH, Organic Acids (% DM<sup>1</sup>), and Ammonia-N Contents during Ensiling

Items	Time (Day)	Substrates (with different GS/JR mass ratios) <sup>2</sup>								Substrate mean	SEM <sup>3</sup>	p-value <sup>4</sup>		
		GS	M3	M4	M5	M6	M7	M8	JR			T	S	TxS
pH	0	3.47 ± 0.01Hc	3.73 ± 0.11Gb	3.90 ± 0.01Fa	4.06 ± 0.22Ea	4.25 ± 0.13Da	4.42 ± 0.01Ca	4.61 ± 0.18Ba	4.77 ± 0.01Aa	4.15a	0.001	<0.001	<0.001	<0.001
	10	3.47 ± 0.01Gc	3.49 ± 0.01Fd	3.53 ± 0.01Fd	3.56 ± 0.00Dd	3.66 ± 0.01Ed	3.54 ± 0.01Cd	3.76 ± 0.00Bd	3.78 ± 0.02Ad	3.60d				
	30	3.70 ± 0.01Fb	3.66 ± 0.01Hc	3.69 ± 0.01Gc	3.73 ± 0.01Ec	3.78 ± 0.01Dc	3.89 ± 0.01Bc	3.99 ± 0.01Ac	3.81 ± 0.03Cc	3.78c				
	60	3.83 ± 0.01Ga	3.82 ± 0.01Fa	3.81 ± 0.01Fb	3.86 ± 0.01Db	3.84 ± 0.01Eb	3.97 ± 0.01Cb	4.32 ± 0.01Bb	4.55 ± 0.01Ab	4.00b				
	Time mean	3.62H	3.68G	3.73F	3.80E	3.88D	3.96C	4.17B	4.23A	—				
LA	0	7.44 ± 0.21Aa	6.12 ± 0.01Bb	5.23 ± 0.02Cb	4.53 ± 0.01Dd	3.47 ± 0.11Ec	2.61 ± 0.03Fc	1.65 ± 0.01Gd	0.82 ± 0.01Hd	3.98d	0.015	<0.001	<0.001	<0.001
	10	5.43 ± 0.01Dd	8.11 ± 0.20Aa	5.93 ± 0.09Cb	6.08 ± 0.04Cb	3.18 ± 0.04Ec	7.52 ± 0.02Ba	5.91 ± 0.03Cb	6.10 ± 0.08Ca	6.03b				
	30	5.69 ± 0.04Dc	6.16 ± 0.05Cb	8.73 ± 0.02Aa	9.03 ± 0.03Aa	8.75 ± 0.13Ab	7.58 ± 0.18Ba	7.40 ± 0.14Ba	5.57 ± 0.09Db	7.36a				
	60	5.74 ± 0.02Cb	4.98 ± 0.06Ec	4.56 ± 0.03Fc	5.21 ± 0.00Dc	9.82 ± 0.02Aa	7.30 ± 0.03Bb	4.77 ± 0.11Ec	3.65 ± 0.01Gc	5.41c				
	Time mean	6.08E	6.34A	6.12D	6.21C	6.31A	6.26B	4.23F	4.04F	—				
AA	0	0.52 ± 0.17Aa	0.42 ± 0.02Bb	0.35 ± 0.01Cb	0.28 ± 0.12Dc	0.21 ± 0.01Ed	0.14 ± 0.03Fc	0.07 ± 0.01Gd	—	0.25d	0.005	<0.001	<0.001	<0.001
	10	0.28 ± 0.01Ed	0.54 ± 0.02Ca	0.51 ± 0.03Ca	0.45 ± 0.01Db	0.26 ± 0.06Ec	0.71 ± 0.04Bb	0.51 ± 0.00Cc	1.02 ± 0.02Ac	0.53c				
	30	0.39 ± 0.01Fc	0.34 ± 0.01Fc	0.54 ± 0.00Ea	0.64 ± 0.05Da	0.66 ± 0.03Db	0.70 ± 0.02Cb	1.71 ± 0.01Ab	1.36 ± 0.03Bb	0.77b				
	60	0.43 ± 0.00Db	0.30 ± 0.10Ec	0.35 ± 0.02Deb	0.44 ± 0.02Db	1.30 ± 0.01Ca	1.39 ± 0.02Ca	1.97 ± 0.03Ba	2.80 ± 0.01Aa	1.47a				
	Time mean	0.41F	0.34F	0.44E	0.45E	0.61D	0.73C	1.77A	1.30B	—				

AN (g/kg TN)	0	2.55± 0.01Ac	2.44± 0.03Bb	2.37± 0.01Cd	2.31± 0.10Dc	2.23± 0.04Ec	2.16± 0.10Fb	2.08± 0.01Gb	2.01± 0.02Hb	2.27d	0.008	<0.001	<0.001	<0.001
	10	3.30±0. 02Ca	2.23±0. 04Ec	2.84±0.0 3Dc	3.56±0. 07Ba	1.62±0. 03Gd	4.20±0. 01Aa	2.94±0. 05Db	1.91±0. 10Fc	2.83c				
	30	2.72±0. 06Eb	3.18±0. 07Da	3.71±0.0 4Ca	3.19±0. 02Db	3.23±0. 01Db	4.32±0. 03Ba	5.18±0. 05Aa	2.21±0. 05Fb	3.47b				
	60	3.33±0. 07Ca	2.21±0. 05Dc	3.20±0.0 2Cb	3.02±0. 05Cb	4.05±0. 07Ba	4.22±0. 10Ba	5.11±0. 05Aa	5.38±0. 04Aa	3.82a				
	Time mean	2.98D	2.52F	3.03C	3.02C	2.79E	3.73B	3.83A	2.88D	—				
LA/AA	0	15.15± 4.60Ab	12.10± 0.04Bd	10.10± 0.02Cd	8.75± 0.33Dc	6.06± 0.01Ed	4.09± 0.03Fc	1.89± 0.04Gc	—	7.27d	0.135	<0.001	<0.001	<0.001
	10	19.39± 0.00Aa	15.02± 0.00Bc	11.63±0. 01Dc	13.51±0 .01Ca	12.23±0 .01Db	10.59± 0.01Fa	11.59± 0.00Ea	5.98±0. 00Ga	12.49a				
	30	14.59± 0.00Cc	18.12± 0.00Aa	16.17±0. 00Ba	14.11±0 .03Ca	13.26±0 .00Da	10.83± 0.00Ea	4.33±0. 02Fb	4.10±0. 00Fb	11.94b				
	60	13.35± 0.00Bc	16.60± 0.03Ab	13.03±0. 01Bb	11.84±0 .01Cb	7.55±0. 00Dc	5.25±0. 00Eb	2.42±0. 00Fb	1.30±0. 00Gc	8.92c				
	Time mean	15.62B	15.46A	12.73C	12.05C	9.77D	7.69E	5.06F	2.84G	—				

Note:<sup>1</sup>DM-dry matter, GS-grain stillage, JR-*J. artichoke* residue, AA-acetic acid, FA-formic acid, LA-lactic acid, ND-not detected.

<sup>2</sup>Different capital letters in the same row show significant difference among different substrates with the same ensiling time at  $p_{critical}=0.05$  level. Different lowercase letters in the same column show significant difference among different ensiling time for the same substrate at  $p_{critical}=0.05$  level.

The values are the mean±standard deviation of three replicates.

<sup>3</sup>SEM-standard error of means.

<sup>4</sup>T-ensiling time, S-substrate, and T×S-the interaction between ensiling time and substrate.

**Table S4.** Alpha Diversity of Bacteria during Ensiling

Treatment	Time (day)	Reads	OTU	ACE	Chao1	Simpson	Shannon	Coverage
GS	10	59299	307	309.40	311.67	0.04	4.53	0.9997
	30	80023	262	271.27	274.16	0.19	2.89	0.9997
	60	80019	169	178.27	182.20	0.18	2.63	0.9998
M3	10	79927	324	325.36	325.50	0.12	3.33	0.9999
	30	80200	213	234.87	233.78	0.26	2.21	0.9995
	60	80151	284	303.67	329.88	0.19	2.30	0.9994
M4	10	55596	287	291.87	293.07	0.10	3.25	0.9997
	30	80156	282	284.46	289.00	0.16	3.01	0.9999
	60	79828	305	310.03	308.67	0.10	3.29	0.9998
M5	10	79949	188	212.90	217.29	0.59	1.09	0.9994
	30	80042	328	333.32	334.18	0.12	3.28	0.9998
	60	80175	251	257.50	266.30	0.18	2.58	0.9997
M6	10	79915	208	221.87	228.71	0.48	1.27	0.9995
	30	62696	195	198.33	198.44	0.33	1.89	0.9998
	60	72356	300	304.78	307.09	0.09	3.37	0.9997
M7	10	80128	179	255.30	263.77	0.46	1.17	0.9989
	30	80058	163	196.29	196.68	0.20	1.91	0.9994
	60	79841	299	310.91	310.21	0.22	2.38	0.9996
M8	10	80273	169	256.19	252.28	0.57	1.08	0.9989
	30	80226	63	85.87	84.00	0.24	1.83	0.9997
	60	79968	135	274.46	232.65	0.15	2.08	0.999
JR	10	80143	54	493.94	286.50	0.24	1.63	0.9995
	30	80096	117	146.00	134.88	0.14	2.29	0.9995
	60	80403	234	285.68	283.76	0.18	2.32	0.9991

**Table S5.** Alpha Diversity of Fungi during Ensiling

Treatment	Time (day)	Reads	OTU	ACE	Chao1	Simpson	Shannon	Coverage
GS	10	76870	83	108.81	90.00	0.14	2.50	0.9999
	30	79715	200	201.64	201.67	0.38	1.82	0.9999
	60	79271	182	187.01	182.60	0.04	4.09	1
M3	10	59237	67	68.76	69.50	0.69	0.85	0.9999
	30	79599	15	32.39	30.00	0.77	0.50	0.9999
	60	80084	80	86.57	81.50	0.30	1.83	0.9999
M4	10	79872	24	30.47	31.00	0.98	0.06	0.9999
	30	80014	13	29.57	23.00	0.93	0.19	0.9999
	60	80173	88	90.93	90.63	0.71	0.61	0.9999
M5	10	79576	33	40.68	36.50	0.83	0.33	0.9999
	30	79935	264	266.22	266.10	0.16	3.24	0.9999
	60	80065	157	176.94	175.00	0.19	2.59	0.9999
M6	10	79896	42	48.65	47.14	0.70	0.51	0.9999
	30	79969	57	72.16	65.75	0.82	0.35	0.9998
	60	79801	71	76.62	78.00	0.61	0.92	0.9999
M7	10	79736	38	60.76	47.75	0.98	0.08	0.9998
	30	80233	77	81.17	86.33	0.50	1.00	0.9999
	60	79995	78	81.33	80.00	0.25	1.85	0.9999
M8	10	80019	36	44.41	45.00	0.98	0.08	0.9999
	30	79936	80	88.09	84.71	0.80	0.48	0.9998
	60	80220	158	162.75	161.75	0.36	2.07	0.9999
JR	10	80108	28	41.11	37.43	0.99	0.05	0.9998
	30	79931	58	66.38	65.86	0.82	0.54	0.9999
	60	79802	73	74.29	73.33	0.73	0.84	1