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ORIGINAL ARTICLE

Fermentation dynamics and diversity of bacterial community in four typical woody forages

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Abstract

Woody forage is a new feeding resource used worldwide, and silage is the main long-term storage, mediated by micro-organisms present during their processing. The objectives of our work were to evaluate the fermentation dynamics and to characterize the bacterial community of our typical woody forages. We selected four typical woody forages: paper mulberry (*Broussonetia papyrifera*), mulberry (*Morus alba* L.), moringa tree (*Moringa oleifera*), and *Anthocephalus chinensis* (*Neolamarckia cadamba*). The materials were ensiled and sampled after ensiling 1, 3, 5, 7, 15, 30, and 60 days. Our results indicate that woody forages have good forage properties with relatively high crude protein content and low neutral detergent fiber and acid detergent fiber content. However, the water soluble carbohydrate content in paper mulberry was considerably low (18.67 g kg^{-1}), which makes ensiling difficult. The lactic acid and acetic acid contents in each of the four materials were reduced after 3 days of ensiling and increased again after 30 days of ensiling, with the exception of *Anthocephalus chinensis*. *Anthocephalus chinensis* and moringa tree were well-preserved after 7 and 60 days of ensiling, respectively, with low pH and ammonia nitrogen content. *Cyanobacteria* was predominant in moringa tree and *Anthocephalus chinensis* before ensiling, and *Lactobacillus* became dominant after 15 days of ensiling. *Enterobacter* dominated the paper mulberry and mulberry during fermentation process and accelerated their poor silage quality. Therefore, the conformity of bacterial community succession with ensiling parameters guaranteed the final quality of woody forage silages, and this might aid in controlling the manufacturing process.

Keywords Woody forage · Silage quality · Dynamics · Bacterial community

Introduction

Developing new forage resources is a priority to answer the increasing demand for animal products in numerous countries, including China. Woody forage has recently been proposed as a new type of livestock feed (Hejcman et al. 2016), as woody forage is characterized by high yields and high crude protein (CP). In our study, four typical woody forages were chosen as materials, and the introduction of them was described as follows. Paper mulberry (*Broussonetia papyrifera*) is a deciduous tree and is grown throughout China, including temperate, tropical, and subtropical regions. The CP content of paper mulberry leaves has been reported to range between 18 and 24%, exhibiting great potential for development as an unconventional resource (Xiong 2010). Mulberry (*Morus alba* L.) is also a deciduous tree with CP and water soluble carbohydrate (WSC) contents and annual fresh yield (60–90 t/hm^2) greater than alfalfa (Huang et al. 2006). Moringa tree (*Moringa oleifera*) may be another successful forage and can be harvested at 45-day intervals, yielding a total biomass of 35 tons of dry matter (DM) per hectare (Cohen et al. 2016), and *Anthocephalus chinensis* (*Neolamarckia cadamba*) may also be a successful forage (Deng et al. 2011). Importantly, several seminal studies have indicated that woody forage mixed with grasses or crops could be fed to animals and may successfully improve the livestock growth rate and production performance (Li 2010; Osmari et al. 2011; Cohen et al. 2016).

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The principal harvest season of woody forage is June to August—periods with high temperatures and precipitation. These environmental conditions lead to high forage moisture, and each of our selected woody forages may reach nearly 80%. Because of this high moisture, ensiling may be more suitable for preserving woody forages, in contrast with traditional hay. Ensiling is a forage preservation method commonly used for moist forage crops with satisfactory conservation of nutrients (Xue et al. 2015). Silage quality depends on many factors, the most important being epiphytic microbial community, water soluble carbohydrate (WSC), buffering capacity (BC), and DM content (Zhang et al. 2017). Li et al. (2017) also reported that polyphenol played an important role in improving silage quality. During the ensiling process, different micro-organisms populate the materials, differentially altering silage quality. Using plate count methods and qPCR, Konig et al. (2017) evaluated the number of bacterial colonies present and identified bacterial communities to genera by denaturing gradient gel electrophoresis (Zhang et al. 2016) or 16S rRNA gene sequencing and found ensiling influenced the relative abundance of many bacterial communities. Li et al. (2015) studied the bacterial community mixed with microalgae and revealed that the similarly microalgae additions to silage influenced bacterial community structure of Manyflower silvergrass. Kraut et al. (2016) assessed the microbiota of large-scale silages produced in a bunker silo and found that the location of silage influenced microbial communities, as well. Taxonomic diversity was lower in silage located in the center or edges, while silage in corners of the bunker exhibited highly diverse microbiota, with low abundances of lactic acid bacteria (LAB) and high levels of *Enterobacteriaceae*. However, limited research has been conducted assessing fermentation and microbial community characteristics during woody forage ensiling. Therefore, we ensiled four woody forage species and sampled following 1, 3, 5, 7, 15, 30, and 60 days to assess fermentation dynamics. To provide primordial basis for successful preservation of woody forages, we evaluated microbial communities throughout ensiling using both plate count methods and high-throughput sequencing technology.

Materials and methods

Silage materials and ensiling

Paper mulberry and mulberry were harvested at the experimental base of Luoyang Academy of Agriculture and Forestry Science in Henan of China (34.39 N, 112.12 E, elevation 250 m, annual mean temperature 15 °C, average annual precipitation 603 mm) on June 24, 2016, during the rainy and hot season. Woody biomass was wilted for 14 h following harvest. Moringa tree and *Anthocephalus chinensis* were harvested from the experimental base of South China Agricultural University in Guangzhou (23.14 N, 113.32 E, elevation 11 m, annual mean temperature 22.2 °C, average annual precipitation 1623–1899 mm) on August 11, 2016, and the biomass was not wilted. Each of the four woody species was a 2-year-old plant, with a height of approximately 2–2.5 m. We used orchard shears to collect woody twigs. The materials were chopped into 2–3 cm pieces using a hand hay cutter, and 150 g was packed into plastic bags (20 × 30 cm). Then, air was removed using a vacuum sealer (DZ-280/2SE, Furuide machinery Co., Ltd., Shandong, China). Between each collection, new experimental gloves were used to prevent bacterial contamination between samples. Twenty-one aliquots of silage were collected for each woody species. The plastic bags were stored at room temperature until further analysis.

Chemical characteristic

Pre-ensiled materials were sampled to determine the baseline chemical composition, and three replicate silages of the four species were opened and sampled at 1, 3, 5, 7, 15, 30, and 60 days after ensiling. Ten grams of each silage sample was homogenized in a blender with 90 ml distilled water for 1 min and filtered through four layers of cheesecloth and three layers of filter paper as described by Zhang et al. (2015), and ammonia nitrogen (NH_3-N), organic acid content, and pH were assessed. pH was assessed using a FiveEasy 20K; Mettler-Toledo International Inc., Greifensee, Switzerland. The organic acid content included lactic acid, acetic acid, propionic acid, and butyric acid and was measured by HPLC (column, Shodex RS Pak KC-811; Showa Denko K.K., Kawasaki, Japan; detector, DAD, 210 nm, SPD-20A; Shimadzu Co., Ltd., Kyoto, Japan; eluent, 3 mmol l^{-1} $HClO_4$, 10 ml min^{-1}; temperature, 50 °C). Dry matter (DM) of residue material was determined by oven-drying at 65 °C for 48 h, ground to pass through a 1-mm screen, and stored at room temperature for later analysis. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured according to Van Soest et al. (1991) using an ANKOM 2000 Fiber Analyzer. For NDF measurements, a heat-tolerant analyzed enzyme and sodium sulfite were also included. Acid detergent lignin (ADL) was determined using 72% H_2SO_4 digestion ADF followed by incineration: the fraction lost on incineration approximated ADL. WSC content was determined using the anthrone method. CP content was calculated by multiplying total nitrogen (TN) by 6.25, while the TN was determined according to Kjeldahl procedure. The buffering capacity (BC) of the silage raw materials was measured by suspending 1 g of sample in 100 ml of distilled water for 30 min, followed by titration to pH 4.0 with lactic acid (0.1 mol l^{-1}). NH_3–N was analyzed with the phenol-sodium hypochlorite method.

Microbial analyses with plate count method

Ten grams of pre-ensiled forages and silages was sampled and immediately stored at − 80 °C. An additional 10 g was blended with 90 ml of sterilized water and serially diluted in sterilized distilled water from 10^{-1} to 10^{-5}. The number of LAB was measured by the plate count method using de Man, Rogosa, and Sharpe (MRS) agar incubated at 30 °C for 48 h in an anaerobic box (TE-HER Hard Anareobox, ANX-3; Hirasawa Ltd., Tokyo, Japan). Yeast was counted on rose bengal medium agar after incubation at 28 °C for 48 h, and the coliform bacteria were counted on eosin-methylene blue agar after incubation at 37 °C for 48 h. All microbial data were $\log_{10}$-transformed for fresh matter (FM) calculations. All media were obtained from Beijing Aoboxing BIO-TECH Co., Ltd., Beijing, China.

Illumina HiSeq sequencing of complex bacterial populations

The samples stored at − 80 °C were sent to Majorbio Bio-Pham Technology Co., Ltd. (Shanghai, China). Pre-ensiled materials and silage samples were added to a 10 mmol l^{-1} of sterilized phosphate-buffered saline (pH 7.4), and DNA extraction was performed. The V4-V5 region of the bacteria 16S ribosomal RNA gene was amplified by PCR (95 °C for 2 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR program included initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. To minimize PCR bias, PCR reactions for each sample were conducted in triplicate, and mixtures of three PCR products were used for DNA concentration determination and sequencing (Li et al. 2015). The DNA samples were paired-end sequenced (2 × 250) on an Illumina MiSeq platform. For quality-control purposes, any sequences that contained mismatches and ambiguous reads in primers were removed.

Operational units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU115) 16S rRNA database using a confidence threshold of 70%.

Calculations and statistical analysis

Data for silage fermentation and chemical composition were analyzed by two-way analysis of variance to evaluate the effects of woody forage species (W), ensiling days (D), and their interaction (D × W). The means were then compared for significance by Duncan's multiple range method. All statistical analyses were performed using the general linear model procedure of SAS 9.0 (SAS Institute, Cary, NC, USA, 2002). Significance was declared at $P < 0.05$ unless otherwise noted. The alpha-diversities of the samples included Shannon index, Chao richness estimator, Good's coverage, relative abundance of bacteria at the genus level, principle component analysis (PCA), and Spearman correlation heat maps. Heat maps were calculated online by the i-sanger platform (version, http://www.i-sanger.com/). The base output of Spearman correlation heat map is a distance matrix representing the correlation coefficient between each micro-organism in the community and environmental factor variable.

Results

Chemical and microbial characteristics before ensiling in four typical woody forages

The dry matter (DM) in paper mulberry and mulberry was greater than moringa tree or *Anthocephalus chinensis* (Table 1), because the paper mulberry and mulberry were wilted for 14 h. The pH of *Anthocephalus chinensis* silage was 5.1, lower than the pH values of the other three materials. The WSC content in paper mulberry was considerably lower than any of the other materials (Table 1). Buffering capacity (BC) strongly influences ease of ensiling, with lower BC values indicating ensiling is easier. In our study, the BC value of paper mulberry (94.15) was higher than the other materials, while *Anthocephalus chinensis* was the lowest (29.25) (Table 1).

Fermentation quality of our selected woody forages

pH, lactic acid, acetic acid, propionic acid, butyric acid, and NH_3–N were all affected by both woody forage species (W) and ensiling days (D), and the interaction between W and D was significant ($P < 0.001$) (S. Table 1). While pH in all four woody forage silages decreased after 60 days of ensiling, only moringa tree and *Anthocephalus chinensis* had a pH of less than 4.00. The lactic acid content did not exhibit a consistent trend across days of ensiling. Acetic acid contents in paper mulberry and mulberry was much greater compared to moringa tree or *Anthocephalus chinensis*, and decreased acetic acid is also an indicator of enhanced silage quality. During ensiling, the propionic acid and butyric acid concentrations changed little for paper mulberry, mulberry, or *Anthocephalus chinensis*. Propionic acid was not detectable in moringa tree throughout ensiling, and butyric acid was only detectible for the first 7 days of ensiling. Generally, the NH_3–

Table 1 Chemical and micro-organism composition of four typical woody forages prior to ensiling

Items	Paper mulberry	Mulberry	Moringa tree	*Anthocephalus chinensis*	SEM	*P* value
Dry matter (g kg^{-1})	340.2[b]	347.2[a]	248.1[c]	276.1[d]	13.73	< 0.0001
pH	6.74[a]	6.59[a]	5.80[b]	5.10[c]	0.10	< 0.0001
Water-soluble carbohydrates (g kg^{-1} DM)	18.67[c]	73.68[a]	56.40[b]	50.80[b]	2.83	< 0.0001
Crude protein (g kg^{-1} DM)	230.5[b]	224.9[b]	252.2[a]	180.3[c]	3.44	< 0.0001
Neutral detergent fiber (g kg^{-1} DM)	284.9	242.1	258.8	303.4	19.76	0.2088
Acid detergent fiber (g kg^{-1}DM)	183.4[b]	148.5[b]	150.8[b]	226.8[a]	10.34	0.0022
Acid detergent lignin (g kg^{-1} DM)	64.16[b]	30.41[d]	44.90[c]	130.9[a]	3.54	< 0.0001
Lactic acid bacteria (log cfu g^{-1} FM)	5.47[a]	5.73[a]	3.65[b]	ND	0.24	< 0.0001
Coliform bacteria (log cfu g^{-1} FM)	5.44[ab]	5.62[a]	4.92[b]	ND	0.19	< 0.0001
Yeasts (log cfu g^{-1} FM)	5.47[b]	7.07[a]	4.89[c]	ND	0.16	< 0.0001
Buffering capacity (g LA kg^{-1} DM)	94.15[a]	66.87[b]	50.67[c]	29.25[d]	3.23	< 0.0001

DM, dry matter; *LA*, lactic acid; *ND*, not detected. Means in the same row ([a–d]) with different superscript letters differ significantly from each other ($P < 0.05$)

N concentration increased with time for each of our four woody forages. However, the NH_3–N concentration of paper mulberry was considerably higher, compared to the other forages.

Microbial community of our selected woody forages

Change in bacterial community structure was calculated at the genus level with a threshold set at 1.0% during ensiling. The number of sequences was standardized relative to the minimum number of 28,108 sequences obtained from a single sample. Each of the four pre-ensiling materials had marked differences in bacterial community structure (Fig. 1). *Pantoea* (30.66%), *Cyanobacteria* (34.40%), and *Enterobacter* (12.24%) were the predominant bacteria present in paper mulberry material before ensiling; *Sphingomonas* (14.20%), *Pseudomonas* (21.02%), *Pantoea* (15.76%), and *Enterobacter* (8.98%) were the predominant bacteria present in mulberry material; *Cyanobactria* (95.16%) predominated moringa tree; *Cyanobactria* (36.93%) and *Methylobacterium* (9.69%) were dominant in *Anthocephalus chinensis* (Fig. 1). During ensiling, the proportion of *Enterobacter*, *Enterococcus*, *Lactococcus*, *Pediococcus*, and *Lactobacillus* increased, while the proportion of *Pantoea* and *Cyanobacteria* decreased to < 0.2% in paper mulberry silage. In mulberry, *Pseudomonas*, *Pantoea*, and *Sphingomonas* species decreased during ensiling, while *Enterobacter* (55.24%), *Lactobacillus* (28.80%), and *Enterococcus* (6.92%) dominated after 60 days of ensiling. *Cyanobacteria* decreased from 95.16 to 0.43% after 15 days of ensiling in moringa tree, with *Enterobacter* (22.68%) and *Lactobacillus* (70.33%) dominating the microbiota after 60 days ensiling. In *Anthocephalus chinensis*, *Cyanobacteria* and *Methylobacteria* decreased to less than 2% after ensiling, while *Lactobacillus* increased from 0.74 to 84.86%.

The dominant epiphytic LAB in paper mulberry, mulberry, moringa tree, and *Anthocephalus chinensis* were *Lactococcus* (68.00%), *Enterococcus* (82.67%), *Lactobacillus* (75%), and *Lactobacillus* (55.08%), respectively (Fig. 2). During ensiling, *Lactococcus* was replaced by *Enterococcus* and *Pediococcus* in paper mulberry, while *Lactobacillus* dominated all other materials.

Relationship between chemical characteristics and microbial community

The Spearman correlation heat maps of each material were determined at the genus level (S. Fig. 1). The results showed a significantly negative correlation between *Lactobacillus* and *Pediococcus* and pH in paper mulberry and moringa tree, which revealed that the *Lactobacillus* or *Pediococcus* determined the silage quality in these two materials. We found a significantly positive correlation of *Enterobacter* and *Methylobacterium* with pH, but also a positive correlation with lactic acid concentration in *Anthocephalus chinensis*. *Lactococcus* positively correlated with WSC in paper mulberry and mulberry; this may be due to the relatively high count of *Lactococcus* at the beginning of ensiling. The Spearman correlation heat map of the total material was determined at the genus level (S. Fig. 2). The pH was tightly ($P < 0.001$) negatively correlated with *Lactobacillus* (− 0.699), but positively correlated with *Citrobacter* (0.568), *Cronobacter* (0.623), *Enterococcus* (0.564), *Lactococcus* (0.574), and *Pantoea* (0.755). A significantly negative correlation was observed between WSC content and *Citrobacter*, *Enterobacter*, *Enterococcus*, *Lactococcus* and *Pediococcus*. However, positive correlations occurred between lactic acid and acetic acid and these bacterial species.

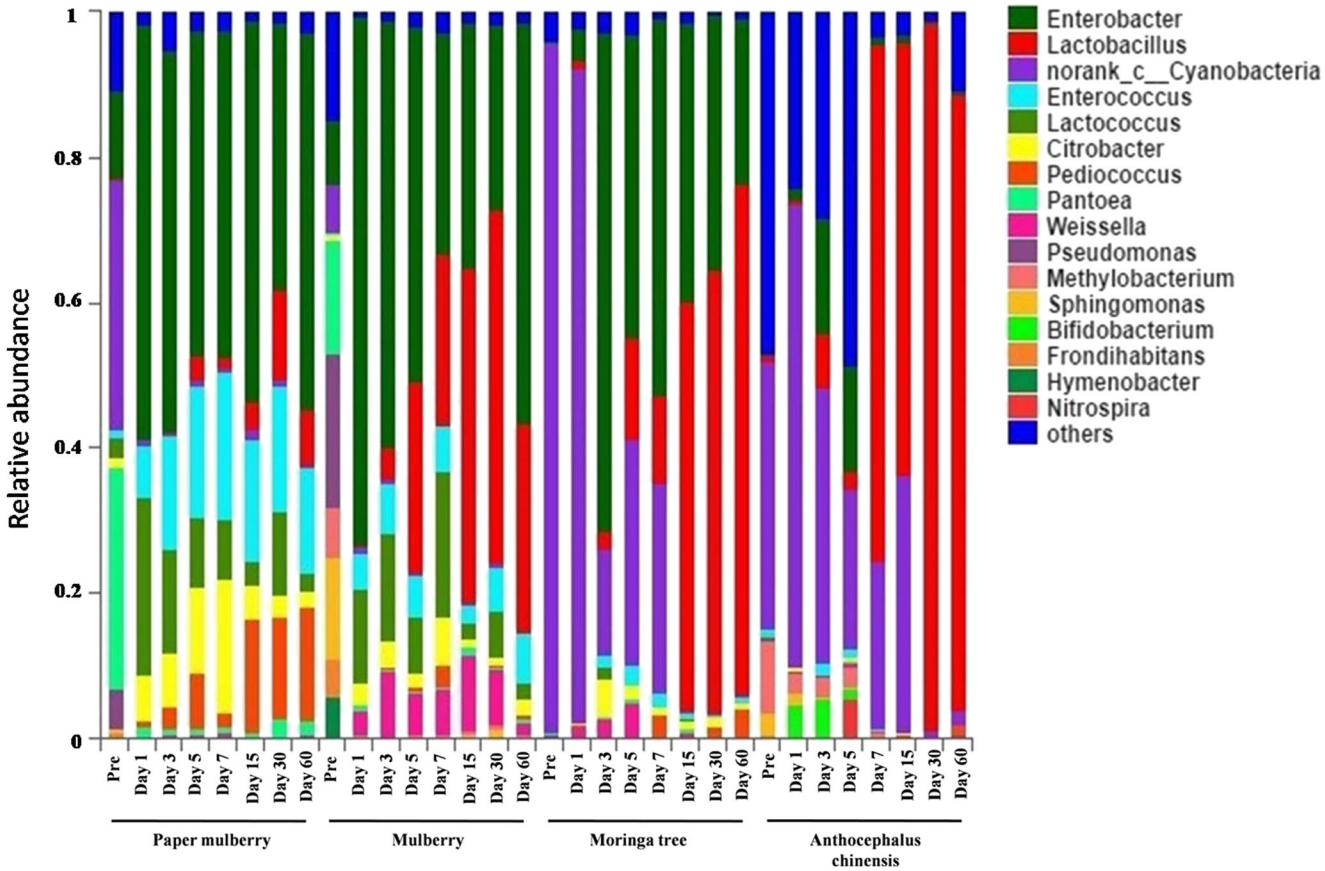

Fig. 1 Bacteria relative abundance of dominant-defined genera in the ensiling process of the paper mulberry, mulberry, moringa tree, and *Anthocephalus chinensis*. Silages were opened after 1, 3, 5, 7, 15, 30, and 60 days

Discussion

Our results indicate woody biomass may be a useful forage and can be stored through ensiling. In our study, all four woody species had relatively high content of CP and low NDF and ADF content. The CP contents of each of the four woody species (between 180.3 and 252.2 g kg^{-1} DM) were higher than CP typical of alfalfa (160–230 g kg^{-1} DM). Similarly, the NDF (242.1–303.4 g kg^{-1} DM) and ADF (148.5–226.8 g kg^{-1} DM) concentrations of the woody species assessed in this study were lower than the typical values reported for alfalfa (NDF, 374.3–400.3 g kg^{-1} DM; ADF, 290.5–312.8 g kg^{-1} DM) (Silva et al. 2016; Tao et al. 2017). Therefore, the woody species of this study may have improved forage properties, compared to alfalfa.

While all four woody species were considered acceptable for silage quality and storage, the silage quality of *Anthocephalus chinensis* and moringa tree were superior to those of paper mulberry and mulberry. For example, *Anthocephalus chinensis* was maintained and able to be successfully stored after 7 days, and moringa tree was well-preserved and able to be stored after 60 days of ensiling, as a low pH (< 4.2) and beneficial bacterial community (mainly *Lactobacillus*) was achieved at these times. Alternatively, paper mulberry and mulberry silages produced lower quality silage, exhibiting high pH and harmful microbial community structure (mainly *Enterobacter*) throughout the 60 days of this study. Based on the bacterial community and chemical analysis in our study, the natural fermentation of paper mulberry and mulberry led to poor silage quality.

Before ensiling, it is important to consider the BC value and WSC concentration to predict the quality of fermentation. Previous research indicates a relatively high BC value may be difficult to ensile. For example, the BC value of maize, a forage notably easy to ensile (McGarvey et al. 2013), is 23 g LA kg^{-1} DM (Herrmann et al. 2015). Alfalfa, one of the most difficult crops to ensile (McAlliser et al. 1998), has a BC value ranging from 66 to 78 g LA kg^{-1} DM (Tao et al. 2017; Wang et al. 2009; Wang et al. 2017). In our study, the BC value of paper mulberry (94.15 g LA kg^{-1} DM) was considerably greater than the other woody forages, while mulberry (66.87) and moringa tree (50.67) exhibited moderate levels, and *Anthocephalus chinensis* had a low BC content (29.25). The low BC content in *Anthocephalus chinensis* is likely due to its low pH (5.10), which is presumably caused by elevated concentrations of chlorogenic acid, cadambinic acid, cadambine acid, quinovic acid, n-hexadecanoic acid, and octadecanoic acid in the leaf tissue (Arti and Pradeep 2016).

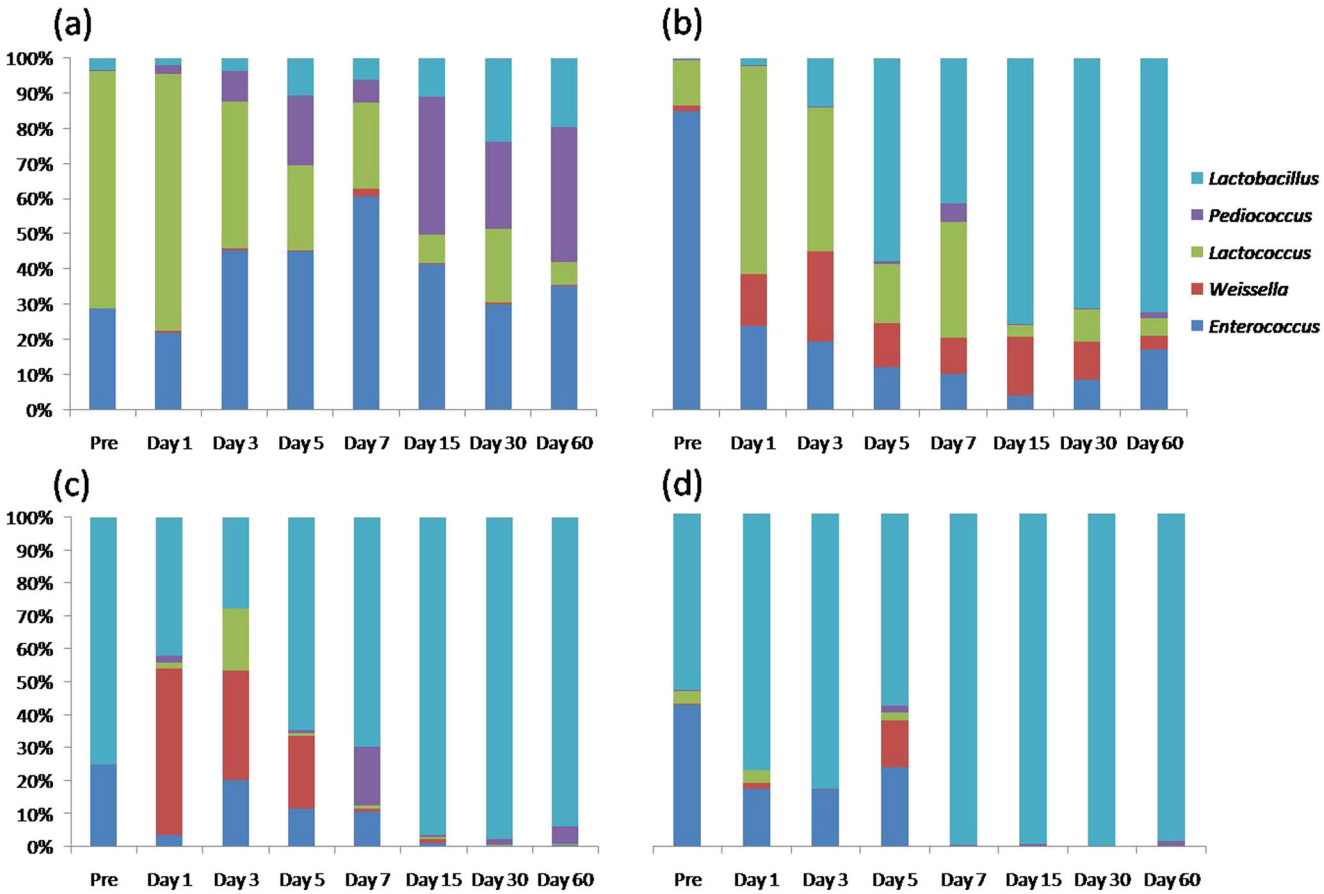

Fig. 2 Lactic acid bacteria relative abundance in paper mulberry (**a**), mulberry (**b**), moringa tree (**c**), and *Anthocephalus chinensis* (**d**) in the different silages according to fermentation time at the genus level. Silages were opened after 1, 3, 5, 7, 15, 30, and 60 days

According to these BC values, *Anthocephalus chinensis* may be the easiest to ensile, of the four woody species assessed in our study. Likewise, WSC, with a recommended level of 60 to 70 g kg^{-1} DM to achieve well-preserved conservation (Zhang et al. 2016), is much greater than that observed in paper mulberry (18.67 g kg^{-1}DM). Therefore, a combined assessment of BC value, and WSC content, indicates *Anthocephalus chinensis* will be the easiest to ensile, and paper mulberry maybe difficult to ensile, across all four woody species assessed in our study.

During ensiling, members of *Enterobacter* were discovered in paper mulberry and mulberry; these bacteria can adapt to a wide range of temperature and moisture conditions and can convert lactic acid to acetic acid (Gan et al. 2017). This may explain the high concentrations of acetic acid found in paper mulberry and mulberry silage in the early days of ensiling and correspond to the second stage of fermentation. Yang et al. (2011) reported that the fermentation process in silage can be classified into four primary stages, and in the second short stage, coli-type bacteria and other fungi produce acetic acid. Then, the subsequent increase in acetic acid concentration after ensiling 15 days in paper mulberry and mulberry maybe because LAB failed to use WSC to produce sufficient lactic acid (Yang et al. 2016). When insufficient lactic acid is produced, resulting silage is unable to inhibit the growth of acetic acid-producing bacteria, allowing for acetic acid fermentation to occur.

Different lactic acid content variations have been reported in previous studies, and lactic acid content in silage may be affected by the species of crop, lactic acid-producing LAB, lactic acid-utilizing yeast, or *Enterobacter* (Zhang et al. 2016). In our study, lactic acid-utilizing yeasts were present only during the first 3 and 5 days in mulberry and paper mulberry, respectively, and were not detected at any time in moringa tree and *Anthocephalus chinensis* (S. Table 2), while *Enterobacter* was detected in paper mulberry, mulberry, and moringa tree during ensiling. On the other hand, the lactic acid concentration increased during the first 3 days of ensiling in paper mulberry, mulberry, and *Anthocephalus chinensis*, while moringa tree increased during the first 7 days. Thus, the observed decrease in lactic acid may be due to the presence of *Enterobacter*, rather than lactic acid-utilizing yeast.

NH_3–N is produced through CP degradation, and therefore, the NH_3–N concentration is a reliable indicator of proteolysis in silage, with well-preserved silage displaying less than

100 g kg^{-1} TN (Zhang et al. 2015). Dong et al. (2017) reported that low pH could suppress *Enterobacteria*, *Clostridia*, and other micro-organisms, thereby decreasing concentrations of NH_3–N with concomitant loss in consumption of WSC. In our study, considerable levels of *Enterobacter* were observed in paper mulberry, mulberry, and moringa tree, and NH_3–N concentrations increased linearly during ensiling in these three materials. However, the final NH_3–N content in *Anthocephalus chinensis* (2.01 g kg^{-1} TN) was the lowest of all four woody species, likely due to the deactivation of proteases induced by the low pH (Li et al. 2017), and the inhibition of *Enterobacter*. Meanwhile, the WSC content in moringa tree and *Anthocephalus chinensis* decreased insignificantly after ensiling and was greater than that of paper mulberry and mulberry, throughout the 60 days of this study.

Epiphytic LAB naturally present on forage are responsible for fermentation during ensiling (Waroon et al. 2016), and the ratio of lactic acid-producing cocci and rods was also important. Cai et al. (1998) reported that when lactic acid-producing cocci (e.g., heterofermentative *Weissella* and *Leuconostocs* species and homofermentative *Pediococci*, *Lactococci*, and *Enterococci* species) were overabundant, fermentation became insufficient. In contrast, lactic acid-producing rods (*Lactobacilli*) promoted sustained lactic acid production and sufficient fermentation. In our study, LAB counts in paper mulberry and mulberry were greater than 10^5 cfu/g FM, while those in moringa tree and *Anthocephalus chinensis* were relatively low (10^4 cfu/g FM). These values are inconsistent with our observed silage quality. This may because the dominant LAB was *Lactococcus* and *Enterococcus* in paper mulberry, *Enterococcus* in mulberry, while moringa tree and *Anthocephalus chinensis* was *Lactobacillus* prior to ensiling. Therefore, the silage quality in paper mulberry and mulberry were characterized by greater LAB counts, but relatively greater abundance of less beneficial bacteria resulted in lower fermentation quality than moringa tree and *Anthocephalus chinensis*.

To further understand the microbial communities during the fermentation process, the relative abundance of bacterial communities at genus level was determined. Among these genera, *Enterobacter*, *Lactobacillus*, and *Cyanobacteria* were the dominant bacterial communities. The presence of *Enterobacter* has been reported to be responsible for increased pH, which triggers a proliferation of other aerobic micro-organisms (Zhang et al. 2015). *Cyanobacteria* are photosynthetic bacteria that are common in all freshwater systems (Crush et al. 2008), and the moringa tree and *Anthocephalus chinensis* were harvested in a region with high precipitation (Guangzhou, China), possibly resulting in greater abundances of *Cyanobacteria* prior to ensiling. Moreover, *Cyanobacteria* can produce microcystin, which is a potential inhibitor of key regulatory enzymes in both animals and plants. This can lead to an overwhelming of the antioxidant capacity, followed by cell death (McElhiney et al. 2001). Fortunately, our study indicates ensiling can decrease the abundance of *Cyanobacteria*, thus reducing these adverse effects. Pantoea can reduce NH_3–N concentrations (Ogunade et al. 2018) and was found in paper mulberry prior to and after ensiling. However, the NH_3–N content was inconsistent with results reported by Ogunade et al. (2018), and more research is needed to illuminate the roles of these microbes during ensiling.

Many parameters assessed in our study indicate moringa tree and *Anthocephalus chinensis* are superior species for silage, compared to paper mulberry and mulberry. While several parameters indicate paper mulberry and mulberry may produce poor silage, these were primary due to high pH and harmful microbial community structure (mainly *Enterobacter*).

Conclusion

In summary, this study revealed variations in bacterial communities in different stages of woody forage silages. Our results confirmed that *Enterobacter* members were the most dominant order in paper mulberry and mulberry, whereas *Lactobacillus* phylotypes prevailed in moringa tree and *Anthocephalus chinensis* after ensiling. *Anthocephalus chinensis* was maintained and able to be successfully stored after 7 days, and moringa tree was well-preserved and able to be stored after 60 days of ensiling, as a low pH (< 4.2) and beneficial bacterial community (mainly *Lactobacillus*) was achieved at these times. Alternatively, paper mulberry and mulberry silages produced lower quality silage, exhibiting high pH and harmful microbial community structure (mainly *Enterobacter*) throughout the 60 days of this study. The results also demonstrated that woody species (paper mulberry, mulberry, moringa tree, and *Anthocephalus chinensis*) have a relatively high CP content and low NDF and ADF content, indicating their successful use as high-quality forage, while more studies should be conducted to improve the silage quality of paper mulberry and mulberry.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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