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Effect of *Lactobacillus plantarum* Inoculation on Chemical Composition, Fermentation, and Bacterial Community Composition of Ensiled Sweet Corn Whole Plant or Stover

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Abstract: Sweet corn is a feed resource with a high content of water-soluble carbohydrates (WSC) available for ruminant production. This study was conducted to investigate the effect of inoculation with *Lactobacillus plantarum* on fermentation and nutritional quality of sweet corn silage. Sweet corn whole plant (WP) and sweet corn stover (CS) were ensiled in mini silos with or without inoculation of *L. plantarum*. Proximate composition and fermentation variables, and composition of the bacterial community, were evaluated before ensiling and at the end of the first, second, and third month after ensiling. There was fiber degradation in CS silage after three months of ensilage, but not in WP silage. Inoculation of WP silage, but not of CS silage, with *L. plantarum*, increased starch content. The relative abundance of genus *Lactobacillus* was increased by inoculation with *L. plantarum* by 14.2% and 82.2% in WP and CS silage, respectively. Inoculation with *L. plantarum* was not necessary to achieve adequate fermentation of either WP or CS silage, as the abundance of native lactic acid bacteria in both materials seemed suitable for adequate fermentation. That said, increased starch content in WP resulting from inoculation with *L. plantarum* can increase the nutritive value of WP for ruminants.

Keywords: silage; *Lactobacillus plantarum*; sweet corn; fermentation; bacterial community

1. Introduction

Mutant genes present in sweet corn prevent the formation of starch from reducing sugars and sucrose during the milk-ripe stage [1]. As a result, sweet corn can have 10 to 20 times as much water-soluble carbohydrate (WSC) content than regular corn [2]. Sweet corn kernel is mainly consumed as human food [3]. At present, about 1.4 million hectares of sweet corn are grown annually worldwide [4]. Cultivation of sweet corn has also been increasing in China in recent years [5]. Its growth area has increased from less than 10,000 hectares in 2000 to 400,000 hectares in 2018. In particular, about 70% sweet corn in China is grown in subtropical areas [5], where the temperature is optimal for sugar accumulation in kernels [6] and other parts of the plant [7].

Sweet corn stover (CS) is obtained after harvesting the ears of whole plant corn (WP), representing about 75% of total dry matter of the plant [8]. Sweet corn stover is thus a resource available in large amounts, which can be used in ruminant feeding. The Chinese Guangdong province alone, for instance, produces about 10-million tons of sweet corn

stover annually [5]. Using whole sweet corn plant as an animal feed is also of interest to increase the amount of plant biomass harvested [9]. Furthermore, harvesting the whole sweet corn plant for feed including kernels also enhances its nutritive value compared to stover alone due to its high content of rapidly fermentable carbohydrates [10], which is important for animal categories with high nutrients requirements such as high-yielding dairy cows in early lactation [11].

Both sweet corn stover and whole plant can be ensiled and fed to ruminants. It is therefore important to study the ensiling processes of sweet corn stover and whole plant, and to seek ways to improve fermentation and nutritive value of the resulting silages. Ensiling plant substrates with excess moisture can result in the loss nutrients such as WSC, hemicellulose, and cellulose [12]. Sweet corn is typically higher in moisture than regular corn [3]. Kurle et al. [13] reported that sweet corn was more susceptible to spoilage than regular corn during the ensiling process. The unwanted fermentation in sweet corn silages include activity from *Clostridium* and yeast, which might be caused by excessive WSC [14]. An alternative to improve the ensiling process of sweet corn and avoid nutrient losses is to enhance the amount and activity of lactic acid bacteria (LAB) through the inoculation of *Lactobacillus plantarum* as a live added lactic acid bacterium [15,16]. To our knowledge, the effects of inoculation with LAB on fermentation and the bacterial community composition of ensiled sweet corn have not been investigated. We hypothesized that inoculation with *L. plantarum* would improve fermentation and nutritional quality of CS and WP silages, as reflected in the evolution of pH, organic compounds, proximate composition, and the bacterial community composition. Our objective was to investigate the effects of inoculating *L. plantarum* on the fermentation and microbial composition of ensiled CS and WP as silage substrates available in subtropical China for ruminant feeding.

2. Materials and Methods

2.1. Forage Harvest, Ensiling Conditions, and Sampling

Sweet corn cultivar Huatian 1 WP and CS were harvested on the same day (20 days after silking) at a stubble height of 15 to 20 cm, and chopped with a ZC-6 ensilage cutter (Yiyang Agriculture Machinery Factory, Qufu, China) to about 3 cm. Stover was harvested after first harvesting apart the ears. We randomly selected 24 10-kilogram portions of each material for ensiling in individual mini-silos. Half of the portions of each forage were sprayed with a 50 mL suspension of 10^7 CFU/mL *Lactobacillus plantarum* (Yaxin biotec, Taiwan, China) at a dosage of 10^5 CFU/g of fresh forage, as recommended by the manufacturer (Inoculation, I+), while the other half of the portions received an equal volume of distilled water (Control, I−). The substrates were packed and compressed manually into polyvinyl chloride mini-silos (5 L, Yifang Plastic Mfg. Co., Ltd., Dongguan, China), and hermetically sealed with a screw top. About 4 kg fresh forages were filled in mini silos. A total of 48 silos, with 12 silos for each combination of substrate and presence or absence of inoculant, were stored at ambient temperature (ranging from 27 to 38 °C). Four fresh samples of chopped CS and WP obtained before inoculation and ensilage were stored at −80 °C. These initial forage samples were then pooled per each ensilaged substrate for subsequent analysis of proximate composition and bacterial community initially present, and designated as samples from 0 month after ensiling. At the end of the first, second, and third months after ensiling, 4 mini-silos per each of the four combinations of ensiled substrates and inoculation were opened, the upper 2 cm layer was discarded, and the remaining substrate mixed thoroughly. About 100 g sub-samples were obtained through quartering and crushed for two minutes into 4–8 mm particles with a high-speed grinding machine (Xichu Co., Shanghai, China). Twenty-five grams of crushed samples were macerated in 225 mL distilled water with a stomacher and shaken at 150 rpm and 4 °C for 24 h. Water extracts were filtered using two layers of Whatman filter paper (pore size of 11 µm, Xinhua Co., Hangzhou, China). The filtered liquid was acidified with 25% (v/v) meta-phosphoric acid and frozen at −20 °C for subsequent analysis of concentrations

of ammonium (NH_4^+), lactic acid, and volatile fatty acids. Dry matter loss could not be calculated as the initial mass of silage was not recorded.

2.2. pH Determination

pH was measured according to the recommended procedure by Bernardes et al. [17]. In brief, A second 25 g portion of crushed samples were macerated in 225 mL distilled water and blended for 30 s, and pH determined with a portable electrode pH meter (Starter 300, Ohaus Instruments Co., Ltd., Shanghai, China).

2.3. Determination of Ammonia Nitrogen, Lactic Acid, Volatile Fatty Acids, and Ethanol

Samples were thawed at room temperature and centrifuged at $15,000 \times g$ and 4°C for 10 min. The supernatant was filtered through a $0.22\ \mu\text{m}$ membrane into a glass vial for organic acids and NH_4^+ analysis. The concentrations of total and individual volatile fatty acids (VFA) and ethanol were analyzed following the procedures described by Playne [18]. Lactic acid was analyzed by high performance liquid chromatography (Agilent HPLC 1290, Agilent Inc., Santa Clara, CA, USA), as described by Zhou et al. [14]. Ammonium concentration was determined using the phenol-hypochlorite procedure according to Chen et al. (2016).

2.4. Proximate Composition

Proximate composition of substrates before ensiling, and of silages after 1, 2, or 3 months of ensilage was analyzed. Contents of dry matter (method 934.01), total ash (method 942.05), ether extract (method 7.045), and crude protein (method 990.03) were determined according to AOAC [19]. The NDF and ADF contents were determined with a Fibretherm analyzer (C. Gerhar, Königswinter, Germany) with a heat-stable amylase and expressed inclusive of residual ash following the procedures of Van Soest et al. [20]. The starch content was determined after pre-extraction with 80% ethanol through measuring glucose released from starch hydrolysis with an α -amylase (Sigma Chemical Co., St. Louis, MO, USA) according to Karthner et al. [21]. Contents of WSC were measured according to the anthraquinone colorimetric reaction with furfural or hydroxymethyl furfural, which were produced by dehydration reaction with concentrated sulfuric acid according to Zahiroddini et al. [22].

2.5. DNA Extraction, qPCR, and Pyrosequencing

Thirty-gram portions of crushed samples were frozen at -80°C , lyophilized, and ground to about 1 mm. Two hundred and fifty-milligram samples were used for DNA extraction using the QIAamp fast DNA stool mini kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. The concentration and purity of total DNA were measured using a ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA). All microbial DNA samples were diluted to $10\ \text{ng}/\mu\text{L}$ for qPCR analysis.

Total bacterial abundance was quantified by qPCR as copies of 16S rRNA genes in a LightCyclerTM 480 (Roche Molecular Systems, Inc., Pleasanton, CA, USA). Forward (F) and reverse primers (R) were: 5'-CGGCAACGAGCGCAACCC -3' (F) and 5'-CCATTGTAGCACGTGTGTAGCC (R) [23]. Ten nanogram DNA samples were analyzed in triplicate in $10\ \mu\text{L}$ amplification reactions consisting of $1 \times$ qPCR SuperMix (Transgen, Beijing, China) and $0.1\ \mu\text{M}$ each primer (Sangon Biotech, Shanghai, China). Five-gradient dilutions of plasmid DNA including target 16S rRNA genes achieved in our previous work [24] were used for standard curves. The qPCR procedure consisted of an initial cycle at 94°C for 30 s, 40 cycles of 94°C for 5 s, 60°C for 15 s, and 72°C for 10 s [23]. Final absolute copies of target 16S rRNA genes were estimated by relating the *Ct* value to standard curves and were expressed as copies/g of lyophilized samples (\log_{10} transformed).

The relative abundance of bacterial genera in fresh forages and silages was assessed by amplicon pyrosequencing. The 16s rRNA gene V3–V4 hypervariable region of bacterial ge-

nomic DNA was used for PCR amplification with the primers 5'-CCTAYGGGRBGCASCAG-3' (341F) and 5'-GGACTACNNGGGTATCTAAT-3' (806R) [25]. PCR reactions were performed in triplicate in a 20 µL mixture containing 0.8 µL of each primer (10 µM), 10 ng of template DNA, 2 µL 2.5 mM dNTPs, 0.4 µL of FastPfu primer (Transgen, Beijing, China), and 4 µL 5 × FastPfu Buffer (Transgen, Beijing, China). The thermal cycling programming was performed as follows: 3 min for an initial denaturation step at 95 °C, 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were excised from 2% agarose gels and purified using a QIAquick Gel extraction kit (Qiagen, Hilden, Germany). Amplicons from each reaction mixture were quantified fluorometrically, normalized and pooled at equimolar ratios based on the concentration of each amplicon. Amplicons were sequenced with the Illumina MiSeq PE300 platform at the Novagen Technology, Beijing, China. Quality control of the sequence reads was performed using MOTHUR v.1.35 [26] following the protocol described by Kozich et al. [27]. The high-quality reads were clustered into operational taxonomic units (OTUs) at a 97% similarity and representative sequences were defined by abundance of each OTU. Taxonomy analysis was conducted using the RDP classifier v.11.1 with a minimum support threshold of 80% [28] against the SILVA database v.128 [29].

2.6. Statistical Analyses

The initial models for all response variables included the three main effects and double interactions:

$$\text{Response}_{ijk} = \text{substrate}_i + \text{inoculation}_j + \text{month}_k + \text{substrate} \times \text{month}_{ik} + \text{substrate} \times \text{inoculation}_{ij} + \text{month} \times \text{inoculation}_{jk} + \text{error}_{ijk}$$

Triple interactions were not included because they were not central to the hypothesis being tested and in order to diminish the possibility of overfitting models by adding an extra parameter.

When significant ($p < 0.05$) interactions were present, one main effect was compared across each level of the interacting effect. Non-significant ($p \geq 0.05$) interactions were eliminated and the reduced models were re-fitted. All regressions were fitted using the *lm()* function of R 3.4 software (R Core Team, <https://www.R-project.org/> (accessed on 11 December 2018)).

3. Results

3.1. Proximate Composition of Forages and Silages

At harvest, proximate composition of WP was: 30.9% DM, and on a DM basis: 7.80% CP, 43.8% NDF, 28.7% ADF, 30.7% starch, 15.3% WSC, and 2.26% EE. Proximate composition of CS at harvest was: 23.1% DM, and on a DM basis: 7.23% CP, 66.8% NDF, 42.6% ADF, 8.08% starch, 13.4% WSC, and 1.70% EE.

Contents of DM, OM, and starch were higher ($p < 0.001$, Figure 1), while NDF and ADF were lower ($p < 0.001$) in WP than in CS silage. There was an interaction between month after ensiling and inoculation on DM and OM contents ($p \leq 0.01$), with higher DM and OM contents in inoculated compared with non-inoculated silage at the end of the third month after ensiling ($p < 0.001$), and no differences at the end of the first and second months after ensiling ($p \geq 0.08$). Substrate and month after ensiling interacted on NDF and ADF contents ($p < 0.001$), with NDF and ADF of CS silage decreasing in the third month ($p < 0.001$), whereas there were no changes in WP silage ($p \geq 0.11$). There were interactions between substrate and inoculation on starch and CP content ($p \leq 0.04$). Inoculation decreased starch content in CS silage ($p = 0.004$) but increased starch content in WP silage ($p = 0.001$). Content of CP was increased by inoculation in WP silage ($p = 0.015$) but not in CS silage ($p = 0.86$). Content of EE decreased after 1 month of ensiling in CS silage only ($p < 0.001$; interaction $S \times M$, $p < 0.001$). All dual interactions between substrate, month, and inoculation on WSC content of silage were significant ($p < 0.001$). There was a

tendency for WSC contents of both substrates to decrease in the first and the third months after ensiling ($p \leq 0.08$). Inoculation increased WSC in CS silage ($p < 0.001$) but not in WP silage ($p = 0.45$). With both substrates, inoculation increased WSC content at the end of first and third months after ensiling ($p \leq 0.04$), but not by the end of the second month after ensiling ($p = 0.16$).

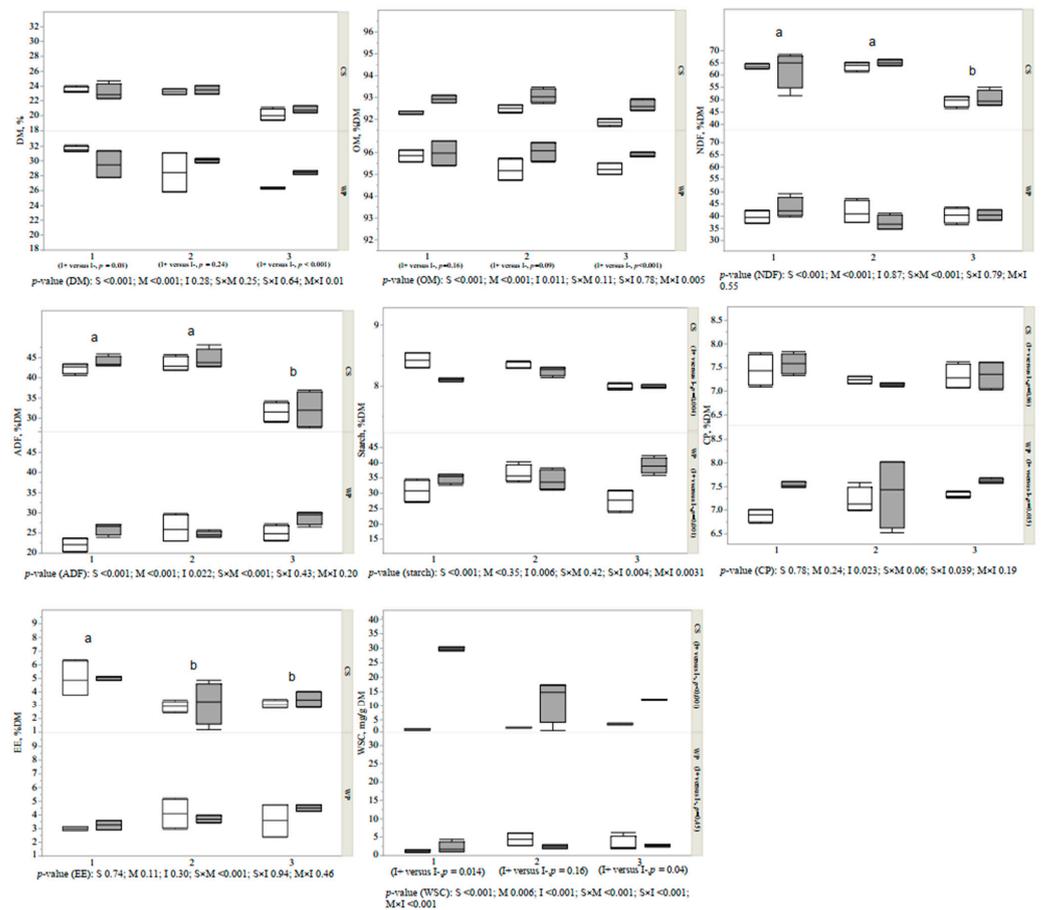


Figure 1. Effect of substrate, time after ensiling, and inoculation of *Lactobacillus plantarum* on chemical composition of silage ($n = 4$). CS, sweet corn stover; WP, sweet corn whole plant. Sampling time points (months after ensiling) are scattered along the x axis. Boxes filled in gray indicate inoculation with *Lactobacillus plantarum*. Horizontal lines inside the boxes indicate the median, upper and lower lines of boxes indicate upper and lower quartiles, respectively, and whiskers indicate the maximum and the minimum. p -values for the following effects are provided in legends below the graph: M, Months after ensiling; S, substrate; I-, not inoculated; I+, inoculated; S \times M; interaction between substrate and month; S \times I; interaction between substrate and inoculation; M \times I; interaction between month and inoculation. DM, dry matter; OM, organic matter (100% minus ash content); NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; EE, ether extract; WSC, water soluble carbohydrates. ab Unlike-lower case letters within a substrate differ ($p < 0.05$).

3.2. Fermentation Variables

There was an interaction between substrate and inoculation on pH ($p < 0.001$, Figure 2), with pH being decreased by inoculation in CS silage ($p = 0.016$) but not in WP silage ($p = 0.38$). There was a tendency for a decrease in NH_4^+ concentration in inoculated silages at the third month ensiling ($p = 0.07$; interaction M \times I, $p = 0.07$). Lactic acid concentration was not influenced by inoculation ($p = 0.28$), regardless of substrate (interaction S \times I, $p = 0.22$). Lactic acid concentration of WP silage increased in the third month after ensiling ($p = 0.003$), whereas it did not change in CS silage ($p \geq 0.38$; interaction S \times M). Ethanol concentration tended to increase in the third month of ensilage in inoculated silages ($p = 0.004$; interaction

M × I, $p = 0.07$), and in WP silage ($p = 0.10$; interaction S × M, $p = 0.09$). There were interactions between substrate and month after ensiling on total VFA, acetic acid, and propionic acid concentrations ($p \leq 0.028$). Total VFA and acetic acid concentration in CS silage increased in the second month after ensiling and decreased in the third month ($p < 0.001$), whilst in WP silage total VFA and acetic acid concentration increased at the end of the third month after ensiling compared with the end of the first month after ensiling ($p < 0.001$). Month after ensiling and inoculation also interacted on acetic acid concentration ($p < 0.001$), with lower acetic acid concentration in inoculated silages at the end of the first and third month after ensiling ($p \leq 0.034$), but not at the end of the second month after ensiling ($p = 0.83$). Butyrate concentration was not affected by substrate, inoculation, month, or their interactions ($p \geq 0.36$).

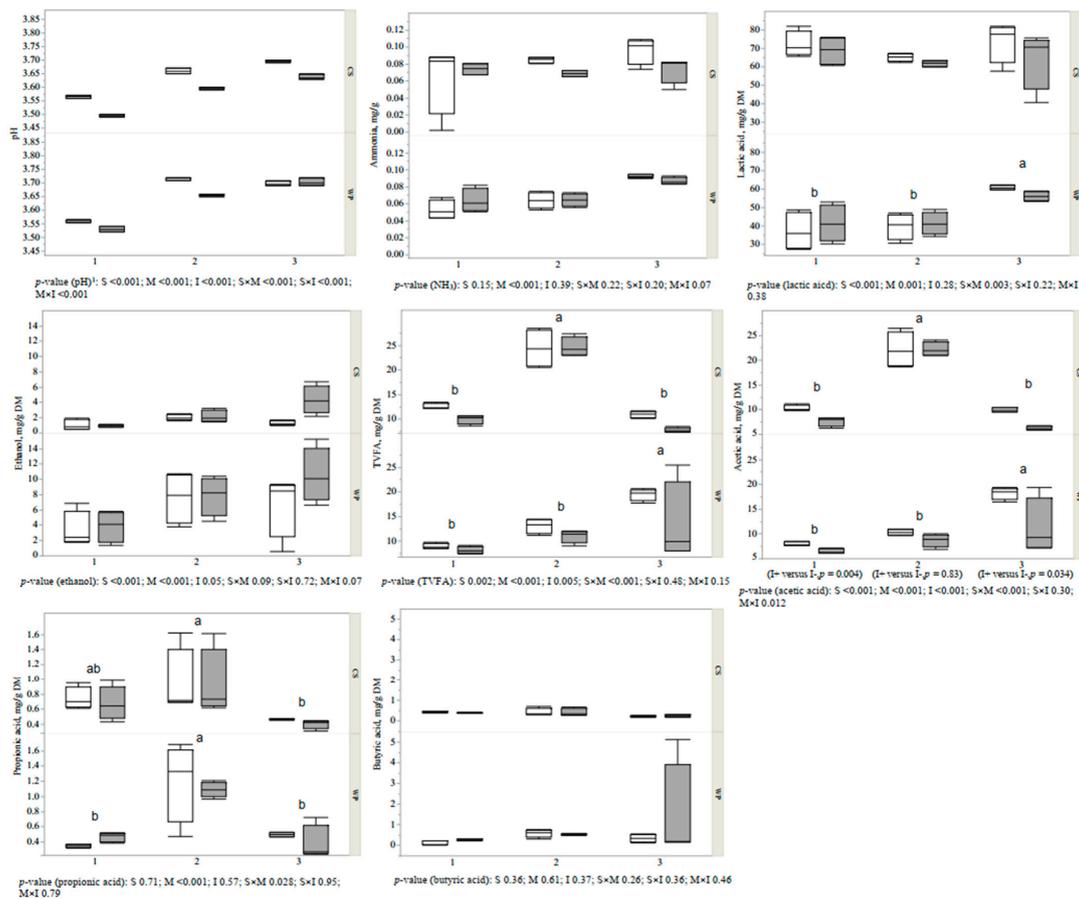


Figure 2. Effects of substrate, time after ensiling, and inoculation of *Lactobacillus plantarum* on fermentation variables of silage ($n = 4$). CS, sweet corn stover; WP, sweet corn whole plant. Sampling time points (months after ensiling) are scattered along the x axis. Boxes filled in gray indicate inoculation with *Lactobacillus plantarum*. Horizontal lines inside the boxes indicate the median, upper and lower lines of boxes indicate upper and lower quartiles, respectively, and whiskers indicate the maximum and the minimum. p -values for the following effects are provided in legends below the graph: M, Months after ensiling; S, substrate; I−, not inoculated; I+, inoculated; S × M; interaction between substrate and month; S × I; interaction between substrate and inoculation; M × I; interaction between month and inoculation. ^{ab} Unlike-lower case letters within a substrate differ ($p < 0.05$).

3.3. Microbial Community Composition

There were no interactions with regards to total bacterial 16S rRNA gene copies ($p \geq 0.12$, Figure 3). Total bacterial 16S rRNA gene copies were higher at the end of the first month after ensiling than at the end of second and third month after ensiling ($p < 0.001$).

There was no effect of inoculating with *L. plantarum* on total bacterial 16S rRNA gene copies ($p = 0.83$).

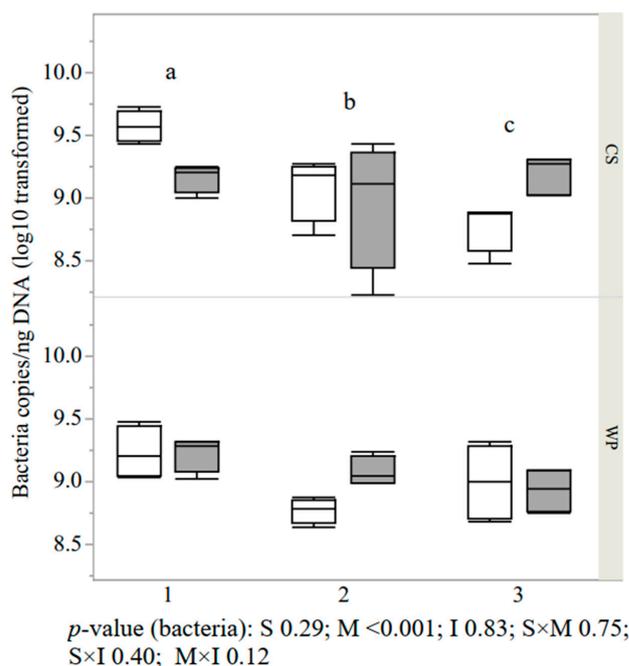


Figure 3. Effects of substrate, time after ensiling, and inoculation of *Lactobacillus plantarum* on total bacterial 16S rRNA gene copies in silage ($n = 4$). Substrates are shown in different panels. CS, sweet corn stover; WP, sweet corn whole plant. Sampling time points (months after ensiling) are scattered along the x axis. Inoculation with *Lactobacillus plantarum* is indicated with gray filling in the box plots. p -values for the following effects are provided in legends below the graph: M, Months after ensiling; S, substrate; I, inoculation; S × M, interaction between substrate and month; S × I; interaction between substrate and inoculation; M × I; interaction between month and inoculation. ^{abc} Unlike letters indicates differences ($p < 0.05$) among time points after ensiling.

Bacterial genera dominating fresh CS and WP forages before ensiling were *Pseudomonas*, *Klebsiella*, *Pantoea*, *Enterobacter*, and *Lactococcus* (Figure 4). *Lactobacillus* accounted for only 0.4% of total bacterial amplicons in both CS and WP before ensiling.

The Shannon bacterial diversity index at the genus level was higher in WP silage than in CS silage ($p < 0.001$, Figure 5). Inoculation resulted in lower Shannon index regardless of the ensiling substrate or month ($p = 0.004$; interaction S × I, $p = 0.12$; interaction M × I, $p = 0.13$). The Shannon diversity index of *Lactobacillus* OTUs was consistently lower in the inoculated silages ($p \leq 0.003$).

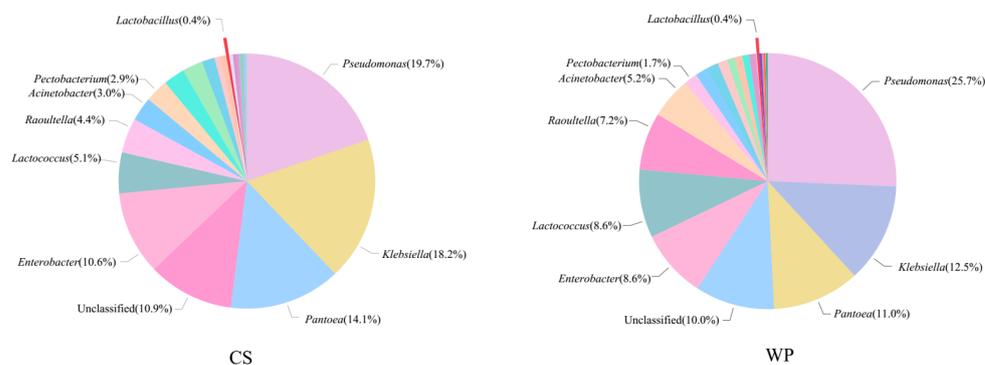


Figure 4. Bacterial communities at genus level of sweet corn stover (CS) and whole plant (WP) before ensiling. Top 9 genera and *Lactobacillus* are labeled and their relative abundance is shown in brackets.

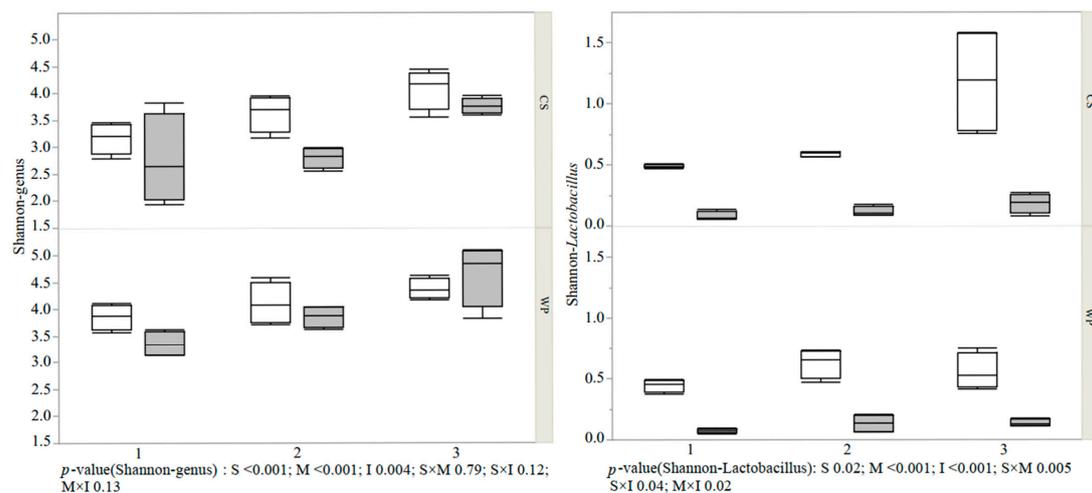


Figure 5. Effects of substrate, ensiling time, and inoculation of *Lactobacillus plantarum* on the Shannon index of bacterial diversity at the genus level and of the *Lactobacillus* genus diversity at OTU level ($n = 4$). Substrates are shown in panels. CS, sweet corn stover; WP, sweet corn whole plant. Sampling time points (months after ensiling) are scattered along the x axis. Inoculation with *Lactobacillus plantarum* is indicated with gray filling in the box plots. p values for the following effects are provided in legends below the graph: M, Months after ensiling; S, substrate; I, inoculation; $S \times M$; interaction between substrate and month; $S \times I$; interaction between substrate and inoculation; $M \times I$; interaction between month and inoculation.

Inoculation resulted in higher abundance of genus *Lactobacillus* in WP silage ($p = 0.01$) but not in CS silage ($p = 0.63$; interaction $S \times I$, $p < 0.001$; Figure 6). Inoculation increased the abundance of *Lactobacillus* at the end of first month after ensiling ($p = 0.014$), and did not affect it at the second and third month of ensilage ($p \geq 0.11$; interaction $M \times I$, $p = 0.09$). *Pseudomonas* increased after ensiling and was at its highest abundance at the end of the third month after ensiling in CS silage ($p < 0.001$), whilst it reached its highest abundance in month 2 after ensiling in WP silage ($p = 0.03$; interaction $S \times M$, $p = 0.005$). Inoculation increased *Pseudomonas* in CS silage ($p = 0.05$) but not in WP silage ($p = 0.41$; interaction $S \times I$, $p = 0.04$). Abundance of *Klebsiella* was higher in WP silage than in CS silage ($p < 0.001$), but was not influenced by inoculation regardless of ensiling month ($p \geq 0.19$; interaction $M \times I$, $p = 0.28$). Inoculation did not affect *Klebsiella* in CS silage ($p = 0.74$) but decreased *Klebsiella* in WP silage ($p = 0.004$; interaction $S \times I$, $p = 0.06$). Inoculation had no effect on *Enterobacter* in CS silage ($p = 0.57$) but decreased *Enterobacter* in WP silage ($p = 0.01$; interaction $S \times I$, $p = 0.01$). Inoculation decreased *Enterobacter* at the end of the first and second month after ensiling ($p \leq 0.045$), but had no effect on *Enterobacter* at the end of the third month after ensiling ($p = 0.65$; interaction $M \times I$, $p = 0.01$). *Acinetobacter* reached its highest abundance at the end of the second month after ensiling in CS silage ($p < 0.001$) but not in WP silage ($p = 0.57$; interaction $S \times M$, $p < 0.001$). *Pantoea* reached its highest abundance at the end of the first month after ensiling in CS silage ($p = 0.05$), and at the end of the third month after ensiling in WP silage ($p < 0.001$; interaction $S \times M$, $p < 0.001$). Inoculation decreased *Pantoea* in CS silage ($p < 0.001$) but not in WP silage ($p = 0.11$; interaction $S \times I$, $p = 0.002$). Inoculation decreased *Pantoea* at the end of the first month after ensiling ($p = 0.05$), but had no influence thereafter ($p \geq 0.32$; interaction $M \times I$, $p < 0.001$). Inoculation tended to increase the abundance of *Pectobacterium* at the end of the second and third month after ensiling ($p \leq 0.06$), but not in month 1 after ensiling ($p = 0.21$; interaction $M \times I$, $p = 0.03$). Inoculation decreased *Tartumella* at the end of the first month after ensiling ($p = 0.04$), but had no influence thereafter ($p \geq 0.15$; interaction $M \times I$, $p = 0.01$).

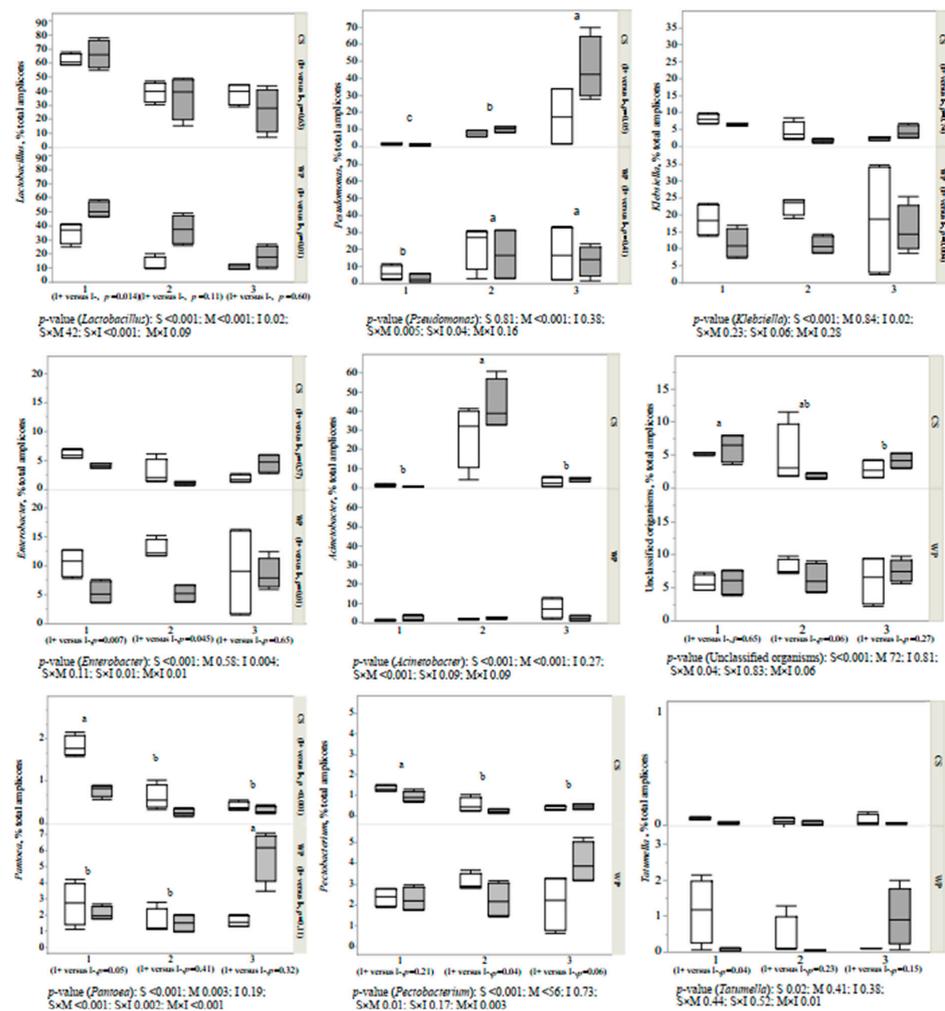


Figure 6. Effects of substrate, ensiling time, and inoculation of *Lactobacillus plantarum* on bacterial relative abundance (>0.5%) in silage (n = 4). Substrates are shown in panels. CS, sweet corn stover; WP, sweet corn whole plant. Sampling time points (months after ensiling) are scattered along the x axis. Inoculation with *Lactobacillus plantarum* is indicated with gray filling in the box plots. *p* values for the following effects are provided in legends below the graph: M, Months after ensiling; S, substrate; I–, not inoculated; I+, inoculated; S × M; interaction between substrate and month; S × I; interaction between substrate and inoculation; M × I; interaction between month and inoculation. ^{abc} Unlike-lower case letters within a substrate panel differ (*p* < 0.05).

4. Discussion

High lactic acid production, low pH, and low butyric acid concentration are key indicators of successful ensiling [11]. Lactic acid production is crucial for rapidly decreasing silage pH, and thus inhibiting deleterious epiphytic proteolytic microorganisms such as clostridia and molds [30]. The concentration of lactic acid in CS silages in our study (about 40 mg/g DM) was comparable with that reported for CS silages of sweet corn [14] and regular corn [31]. As far as WP silage with sweet corn in our study, lactic acid content was about two times higher than WP silage of regular corn [32]. The pH of all the treatments evaluated in this experiment fluctuated within 3.54 and 3.68, which can be defined as adequate [11]. Butyric acid is an indicator of spoilage in silage, as butyric acid producers such as genus *Clostridium* can degrade lactic acid and thus increase the growth of spoilage microbes [33]. In the meta-analysis conducted by Blajman et al. [34], the mean concentration of butyric acid in corn silages was of 1.1 mg/g DM, which is higher than what we found in both substrates at all time points, except the WP silage at the end of the third month. Therefore, butyric acid concentration was small, and genus *Clostridium* were not detected in

any of the silages. As inoculation with *L. plantarum* did not increase lactic acid concentration, it seems that there were enough numbers of native LAB attached to plants at harvest to produce enough lactic acid in the non-inoculated silages, although in WP silage there was a tendency to increase the abundance of the genus *Lactobacillus* with inoculation of *L. plantarum*. In summary, both substrates seemed to be successfully ensiled without inoculation, based on key indicator variables such as pH, lactic acid, and butyric acid concentrations, and the abundance of genus *Clostridium*.

The initial LAB communities present in the plant material to be ensiled are important to the adequacy of fermentation and silage quality (McDonald, 1991; Lin et al., 1992b; Yang et al., 2010). There is lack of knowledge about how many lactic acid bacteria are required to start fermentation successfully. In most studies, LAB were inoculated at levels between 10^5 and 10^7 CFU/g [34]. The initial relative abundance of the *Lactobacillus* genus in fresh corn forages in our study (0.41%) was comparable with previous reports [35,36]. After ensiling and with oxygen depletion, strict anaerobes begin to dominate the bacterial community [37]. In our study, genus *Lactobacillus* reached a relative abundance of 53.7% in silages within month 1 after ensiling, which is similar to what was reported by Xu et al. [31] but lower than what was reported by Keshri et al. [36] and Drouin et al. [32], who reported genus *Lactobacillus* to account for >80% of total bacteria in corn silage. Corn silage is characterized by a succession of heterofermentative LAB such as *Pediococci*, *Leuconostoc*, and *Streptococci* during long storage periods [32,38]. However, this heterofermentative LAB succession was not detected in our study. Factors such as relatively high moisture [13] and NH_4^+ content in sweet corn compared to regular corn could contribute to explain the relatively low LAB abundance [39–41].

In some studies, inoculation with LAB such as *L. plantarum* [36,42,43] and *L. buchneri* [44] was reported to increase lactic acid concentration in corn silage. However, other studies found no effect of inoculation of corn silage with homofermentative LAB on lactic acid concentration [36,45–47]. In our study, even though the abundance of *Lactobacillus* was comparatively low relative to some previous studies, inoculation with *L. plantarum* seemed to have increased its dominance within the genus *Lactobacillus*, as indicated by the substantial decrease in the diversity of *Lactobacillus* OTUs in the inoculated silages. However, production of lactic acid was unaffected by inoculation with *L. plantarum*. The dominant LAB can affect silage fermentation end-products [48]. Inoculation with heterofermentative LAB can decrease lactic acid but increase acetic acid concentration [34]. In the present study, inoculation with *L. plantarum* decreased acetic acid concentration, in agreement with previous reports [34,44,46]. On the other hand, others have reported that acetic acid was not affected by inoculation of *L. plantarum* [36,42]. The concentrations of ethanol and acetic acid in sweet corn stover silage was comparable with a previous report [14]. Acetic acid inhibits yeast metabolism of lactic acid [49].

In agreement with previous findings [36,44], the major yeast product ethanol was unaffected by inoculating *L. plantarum* in either CS or WP silage, suggesting that inoculation with *L. plantarum* had no effect on yeast growth during anaerobic storage.

In both fresh WP and CS before ensiling, there was considerable abundance of aerobic and facultative anaerobic bacteria, such as genus *Pseudomonas*, genus *Klebsiella*, genus *Enterobacter*, genus *Pantoea*, genus *Lactococcus*, and genus *Raoultella*, but limited abundance of genus *Lactobacillus*. In a previous study conducted in Israel, the dominant bacterial genera in fresh corn whole plant were *Acinetobacter*, *Klebsiella*, *Weissella*, and *Sphingomonas* [36]. In three different locations in Iran, the epiphytic bacterial communities in fresh corn whole plant forage were dominated by the family Leuconostocaceae and genera *Luconostoc* and *Weissella* [35]. In the United States, the most abundant genera in fresh samples of whole plant corn were *Flavobacterium* and *Chryseobacterium*, belonging to the *Flavobacteriales* order [32]. Altogether, those three studies and ours indicate the existence of substantial variation among different regions in the world in the epiphytic bacterial community in fresh corn crops. Possibly, the succession of LAB could also be influenced by initial composition of epiphytic bacterial communities.

Fermentation can be promoted by adding 7% sugars to WSC-deficient silage substrates such as wheat straw [50]. This level of WSC produces good quality grass silages, as also indicated by Amer et al. [51]. The WSC content of sweet corn was much higher than this level, which likely also contribute to adequate production of lactic acid (>40 mg/g). Although residual WSC can, on the one hand, induce the growth of undesirable microbes such as lactate-assimilating yeasts [34], they reflect a more efficient silage fermentation [44,52]. Interestingly, inoculating *L. plantarum* in WP silage preserved greater content of WSC as well as CP and starch compared to non-inoculated silages. Inoculating LAB in high moisture substrate for ensilage can allow for a more desirable fermentation [53]. The inoculation of WP silage with *L. plantarum* might be beneficial to preserve starch content, as indicated by a 14% increase in starch content caused by inoculating *L. plantarum* in WP silage. In the present study, WP and CS were harvested from the same field and on the same day, thus the only agronomical difference in the initial material for ensilage was the presence or absence of corn ears. It is possible that the density of attached native LAB was lower in corn ears than in CS, and that thus removing corn ears from WP increased the density of native *Lactobacillus* in the resulting CS. In agreement with this possibility, Lynch et al. [54] reported that native LAB attached to CS was higher in comparison with corn ears. Inoculating corn ears with two strains of *L. plantarum* did not affect starch content [54]. However, unlike our results, inoculation of corn ear silage with *L. plantarum* did not affect or even decrease LAB counts compared to the non-inoculated control [54].

Fiber seemed to be degraded in CS silage in the third month after ensiling, as the NDF content of CS silage was 22% lower after the third month of ensilage compared to the second month. On the other hand, fiber seemed to be unaffected in WP silage. After WSC are depleted by fermentation during ensiling, fiber may be partially digested and fermented as an alternative substrate [50]. It is possible that the increase observed in acetic acid concentration in CS silage at the end of the second month after ensiling could be a result of fermentation of fiber. The idea of fiber serving as a source of fermentable sugars, however, is difficult to reconcile with the fact that NDF and ADF contents in WP silage were unaffected, even numerically, considering that WSC were lower in CS silage than in WP silage.

Members in genus *Acinetobacter* are aerobic bacteria reported to be present in incompletely tight silage bunkers [55] or during aerobic exposure [56]. Genus *Acinetobacter* increased at the end of the second month after ensiling in CS silage, which might imply incomplete anaerobic conditions. Incomplete anaerobic conditions can also allow for yeast proliferation and ethanol production, in agreement with the observed increase in ethanol in the third month after ensiling in CS silages. We speculate that fiber might have been degraded in microaerobic pockets during the third month after ensiling, as suggested by the simultaneous decrease in fiber content in the CS silage and increase in *Acinetobacter* abundance. It has been reported that heterofermentative lactic acid bacteria produces the most acetate during the process of long-term preservation [32]. However, that was not our case. Our results showed fiber degradation and increased acetic acid in CS silage during the third month after ensiling, indicating the acetic acid may have been produced as a result of fibrolytic activity. *Acinetobacter* has been shown to degrade cellulose and hemicellulose to monomers such as glucose and xylose [57,58]; if that was the case, the presumably microaerophilic environment may have still allowed for some acetic acid formation. Genus *Pseudomonas*, another aerobic bacterium found in silage in the current study, can use acetic acid as a carbon source [59]. The increase in genus *Pseudomonas* during the ensilage process might help explain the decline in acetic acid content during the third month of ensilage.

5. Conclusions

Successful ensiling could be achieved in both CS and WP silage with or without inoculation of *L. plantarum*. It is thought that the lack of major effects of inoculation with *L. plantarum* was due to the presence of enough native LAB in the ensiled corn plants. However, greater preservation of starch in WP silage could be achieved by inoculating

L. plantarum. Inoculation with *L. plantarum* could then help improving the nutritional quality of WP silage for ruminants with high energy requirements, such as dairy cows in early lactation. The effects of inoculation with *L. plantarum* on the aerobic stability of these two ensiled substrates remain to be investigated.

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