Effect of Different Regions and Ensiling Periods on Fermentation Quality and the Bacterial Community of Whole-Plant Maize Silage

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This study aimed to explore the changes in the microbial community on the silage material surface and during the ensiling process of whole-plant maize in different regions. Whole-plant maize silages were sampled in Ziyun, Guanling, and Weinning counties within warm and humid climate areas in southern China. Silages were sampled at 0, 2, 5, 10, 20, and 45 days during ensiling. The nutritional components, fermentation properties, and microbiomes were examined to evaluate the influence of sampling area and fermentation time on the quality of silage. The results showed that the pH values of all silages significantly decreased (<4.2 at ensiling day 2) during fermentation and all silages achieved satisfactory fermentation at 45 days. Butyric acid was not detected during ensiling, and the contents of acetic acid and ammonia nitrogen in the final silages were below 6 g/kg DM and 50 g/kg total nitrogen, respectively. Weissella was the dominant epiphytic bacteria of raw material in Ziyun and Weinning, while Lactobacillus was prevalent in Guanling. Lactobacillus dominated the ensiling process, and its abundance significantly increased with increasing fermentation time in the three groups. Lactobacillus was negatively correlated with pH of all silages (\( p < 0.05 \)) and positively correlated with lactic acid, propionic acid and acetic acid (\( p < 0.05 \)). Furthermore, the bacterial community was significantly correlated with environmental factors. Altitude had a highly positive correlation with the abundance of Stenotrophomonas, Chryseobacterium, and Massilia (\( p < 0.01 \)), while precipitation was negatively correlated with these bacteria. The humidity and average temperature significantly influenced the Lactobacillus and Weissella abundances of fresh whole-plant maize. During the ensiling process, the silages from three regions had similar bacterial dynamic changes, and the Lactobacillus formed and maintained good fermentation characteristics in whole-plant maize silage.

Keywords: whole-plant maize, silage, fermentation quality, microbial community, environmental factor

INTRODUCTION

Ensiling is a common method for preserving fresh forage and contributes to an uninterrupted supply of forage feedstuff to ruminant animals (Pahlow et al., 2003). Lactic acid bacteria (LAB) are the most important beneficial bacteria in the process of ensiling fermentation (Zhu et al., 2010), which results in organic acids with water-soluble carbohydrate (WSC) as fermentation substrate under anaerobic conditions to reduce pH value for achieving the purpose of long-term preservation of silage (Keshri et al., 2018). It is well established that LAB regularly relate
to silage fermentation belong to the genera Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, and Weissella (Pang et al., 2011; Ni et al., 2018; Yang et al., 2019). The quality of silage mainly depends on the composition and abundance of microbial communities during ensiling (Kung et al., 2018; Zi et al., 2021a). Whole-plant maize silage has a better absorption rate and higher nutritional value than other silages, and is the most common feed for ruminants worldwide (Khan et al., 2015; Zhang et al., 2019). Various silage microbial compositions have been found in different forages (Guo et al., 2018; Xu et al., 2019; Yuan et al., 2019). In addition, for maize ensiling, previous studies have reported that Lactobacillus was the dominant contributors during ensiling and subsequent exposure to air (Lin et al., 1992; Zhou et al., 2016; Hu et al., 2018). Generally, the composition of microorganisms changes greatly before and after ensiling (Sepehri and Sarrafzadeh, 2019). Therefore, it is of great significance to further study the microbial diversity changes in silage for understanding the whole fermentation process and finding the root cause of fermentation quality changes.

As previously mentioned, uncontrollable climatic conditions affect silage fermentation, microorganisms and aerobic stability (Bernardes et al., 2018; Guan et al., 2018). Ensiling at high temperatures reduces both the lactic acid (LA) concentration and aerobic stability (Ashbell et al., 2002). Moreover, it has been reported that temperature affects the bacterial diversity and fermentation quality of silage (Wang et al., 2019; Li et al., 2021). Epiphytic bacterial communities in fresh forage depends mainly on geographical location, climate, and cutting (Guo et al., 2018; Yang et al., 2019). Cai et al. (1999) studied the microorganisms attached to the surface of corn, sorghum, alfalfa and Italian ryegrass collected from the same place and found that there were very few Pediococcus attached to the surface of sorghum and ryegrass and few Lactobacillus attached to the surface of alfalfa. The community of epiphytic bacteria in corn material has been shown to be affected by rainfall and humidity, and the microbial community during ensiling has been shown to be affected by temperature (Guan et al., 2018).

The composition of the epiphytic community is an important factor of silage quality and changes in the microbial community during fermentation (Gharechahi et al., 2017). Nevertheless, the effect of the environment on the epiphytic community of whole-plant maize silage has rarely been reported. We hypothesized that the succession characteristics of whole-plant maize silage are different in different areas. Therefore, this study aimed to evaluate the correlation between bacterial communities and sampling area environmental factors (altitude, precipitation, temperature, and humidity) and then to examine the dynamic changes in microbial community diversity and fermentation quality during the ensiling of whole-plant maize.

MATERIALS AND METHODS

Study Sites and Sample Collection

Whole-plant maize was planted in Ziyun County (Z) (106°10′E, 25°37′N, altitude 840 m), Guanling County (G) (105°24′E, 25°57′N, altitude 1350 m), and Weining County (W) (104°16′E, 26°55′N, altitude 2230 m). The monthly changes of temperature, precipitation and humidity in the three regions in 2019 are shown in Figure 1. The variety of maize was Qingfeng No. 4. The samples were planted on February 25, 2019; March 28, 2019; and April 2, 2019 in Ziyun, Guanling, and Weining, respectively. Samples were collected at the dough stage according to local tradition on July 31, 2019; September 12, 2019; and September 18, 2019. Instantly, the materials were collected and transported in ice boxes and stored at ~80°C before use. Another part of the collected materials was chopped to approximately 2 cm by a hand paper cutter. After mixing thoroughly, the material was vacuum-packed into plastic bags. Each bag contained approximately 500 g of fresh matter. A total of 54 bags (three treatments × six ensiling durations × three replicates) were prepared and stored at normal temperature (25–30°C), and the fermentation time of all samples was approximately 45 days. Samples were used to determine the chemical composition, fermentation quality and microbial community on days 0, 2, 5, 10, 20, and 45.

Chemical and Fermentation Profile Analyses

Specimens were determined by drying the samples in a forced air oven at 65°C for 72 h and passed through a 1.0 mm sieve before the chemical assay. The contents of dry matter (DM) and crude protein (CP) were measured according to previously published study (AOAC, 2000). The WSC content was determined by colorimetric after-reaction with anthrone reagent (Turula et al., 2010). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using a previously established method (Van Soest et al., 1991). To measure fermentation quality, 20 g samples were suspended in 180 mL of distilled water overnight at 4°C and filtered through four layers of cheesecloth. Then, the filtrates were used to determine the pH value and ammonia-N (NH₃-N) and organic acid contents. The pH, lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA), ammonia-N (AN), and total nitrogen (TN) concentrations were measured as previously established (Li M. et al., 2019).

Bacterial Community Analysis

The DNA extraction was operated using the HiPure Soil DNA extraction kit (Magen, Guangzhou, China) according to the manufacturer’s instructions. The 16S rDNA V5-V7 region of the ribosomal RNA gene were amplified by PCR (94°C for 2 min, followed by 30 cycles at 98°C for 10 s, 62°C for 30 s, and 68°C for 30 s and a final extension at 68°C for 5 min) with primers 799F (AACMGGATTAGATACCCCKG) and 1193R (ACGTTCATCCCCCATTTC) (Logue et al., 2016). Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions and quantified using ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, United States). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina platform according to the standard protocols.
Tag assembly was carried out using filtered reads according to the following principles: the overlap between paired-end reads should be more than 10-bp and have less than a 2% mismatch. The unique tags were obtained by removal of redundant tags using MOTHUR software (Schloss et al., 2009). The effective tags were clustered into operational taxonomic units (OTUs) of ≥97% similarity using UPARSE (version 9.2.64) pipeline. Diversity metrics were determined using the core-diversity plug-in within QIIME (Callahan et al., 2016). The microbial diversity within an individual sample was assessed using the following alpha diversity indices: the Chao1 richness estimator and Shannon diversity index. Beta diversity was analyzed to assess the structural variation of microbiota across specimens, and then principal component analysis (PCA) was conducted (Vázquez-Baeza et al., 2013). The relative abundances of different bacterial communities at the phylum and genus levels were also analyzed. Pearson correlation coefficient between environmental factors and species was calculated in R project psych package (version 1.8.4). The heat map function of the R software and genus information for the Whole-plant silages were used to generate a heat map. Environmental factors during corn growth and fermentation quality after silage were selected for Spearman correlation analysis with bacterial community. The data were analyzed using the free online platform of Omicshare tools.

**Statistical Analyses**

The statistical analyses of two-way ANOVA were performed using SPSS 20.0. Duncan’s HSD test was employed to determine the differences in the treatment means, where significant differences were declared at $P < 0.05$, and the data are expressed as the mean and the standard error of the mean (SEM).

**RESULTS**

**Chemical Analysis of Whole-Plant Maize Ensiling**

The changes in nutritional components during different regions of ensiling are shown in Table 1. The DM contents of all maize raw materials ranged from 268.6 to 303.3 g/kg, while the highest DM content was 303.3 g/kg in the W group ($P < 0.05$), and the lowest DM content was 268.6 g/kg in the G group ($P < 0.05$). The content of DM in each group decreased with increasing fermentation time and was in the order of $W > Z > G$ samples at any stage of fermentation. The content of CP in the Z and G groups was lower than that in the W group ($P < 0.05$), while that in the Z group was lower than that in the G and W groups on day 45 ($P < 0.05$). The WSC content of each group decreased significantly throughout the fermentation process. The contents of NDF and ADF in the Z group were significantly higher than those in the other groups at any stage of fermentation, and they showed a similar decreasing trend with the extension of fermentation time. Moreover, the contents of NDF and ADF in the G group were lower than those in the other groups on day 45 ($P < 0.05$). The ensiling time (D) and treatment (T) significantly affected the chemical composition ($P < 0.001$).
The results also showed a significant interaction between D and T for the contents of DM, WSC, NDF, and ADF (P < 0.001).

The Fermentation Property of Whole-Plant Maize During Ensiling

Table 2 illustrates the fermentation quality of whole-plant maize silage in different regions. The pH value of each group decreased significantly on the second day of fermentation (P < 0.05), all of which were below 4.2. Moreover, the highest and lowest pH appeared in the G group and the W group on day 45 (P < 0.05), respectively. The LA content of each group increased significantly on the second day of fermentation (P < 0.001). All the groups were dominated by Firmicutes and Acetobacter, while Proteobacteria and Firmicutes were the top two phyla during the process of ensiling in the three regions. Lactobacillus, Weissella, and Acetobacter were the most dominant genera in all maize silages. Figure 4A shows the abundance of Proteobacteria was the most abundant phylum in the G group, exceeding 95% in raw materials. The dominant bacteria on the surface of maize was Firmicutes, with relative abundances of 90.55 and 71.70%, respectively, and Proteobacteria had relative abundance exceeded 70%. The Lactobacillus abundance decreased while that of Firmicutes increased rapidly and became the dominant phylum with increasing fermentation time. At the genus level, the most dominant bacterial genera were Acetobacter and Weissella, which were the most dominant genera in all samples during the ensiling process. After ensiling, Lactobacillus was the dominant microbial genus, and its relative abundance increased 70%. The Lactobacillus abundance significantly increased with the extension of fermentation time, while those of Weissella and Acinetobacter significantly decreased.

Microbial Diversity of Whole-Plant Maize During Ensiling

The bacterial alpha diversities of the silages were evaluated by the Chao1 and Shannon indexes (Figures 2A, B). The bacterial diversity increased with the extension of fermentation time in the Z group. However, the bacterial diversity of silages in the G group and the W group was richer after 2 days of fermentation. The relative abundances of bacterial communities at the phylum and genus levels were presented in Figures 3, 4. As seen in Figure 3, Proteobacteria and Firmicutes were the top two phyla during the process of ensiling in the three regions. Lactobacillus, Weissella, and Acetobacter were the most dominant genera in all maize silages. Figure 4A shows the abundance of Proteobacteria was the most abundant phylum in the G group, exceeding 95% in raw materials. The dominant bacteria on the surface of maize was Firmicutes, with relative abundances of 90.55 and 71.70%, respectively, and Proteobacteria had relative abundances of 92.2 and 27.04%, respectively. At the genus level, Weissella and Lactobacillus were found in large amounts in the Z and W groups, while no obvious dominant bacteria were found in the G group of raw materials. All the groups were dominated by Firmicutes and Proteobacteria during ensiling at the phylum level. The Proteobacteria abundance decreased while that of Firmicutes increased rapidly and became the dominant phylum with increasing fermentation time. At the genus level, the most dominant bacterial genera were Lactobacillus and Weissella in the samples during the ensiling process. After ensiling, Lactobacillus was the dominant microbial genus, and its relative abundance exceeded 70%. The Lactobacillus abundance significantly increased with the extension of fermentation time, while those of Weissella and Acinetobacter significantly decreased.
or disappeared in the subsequent period. The relative abundance of *Acetobacter* in each group decreased with the process of ensiling. By 45 days, the relative abundance of *Acetobacter* in each group decreased to below 1%. In addition, *Pantoea* and *Stenotrophomonas* existed in the whole corn silage period of all three regions.

### Correlation Analysis Between Bacterial Communities and Environmental Factors

The association analysis between bacterial abundance and environmental factors is shown in Figure 5. Negative correlations were observed between the average temperature and the relative abundance of the *Lactobacillus* (−0.73), *Sphingomonas* (−0.67), *Stenotrophomonas* (−0.74), *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (−0.92), *Chryseobacterium* (−0.75), and *Massilia* (−0.71). Altitude was associated with increased abundance of *Rahnella*, *Sphingomonas*, *Chryseobacterium*, *Herbaspirillum*, *Massilia* (p < 0.05), *Serratia*, and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (p < 0.01). The level of precipitation was associated with decreased abundance of *Rahnella*, *Serratia* (p < 0.01) and *Stenotrophomonas, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Chryseobacterium* and *Massilia* (p < 0.05). Humidity was associated with increased abundance of *Weissella, Lactobacillus*, *Stenotrophomonas, Chryseobacterium, Massilia* (p < 0.05), and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (p < 0.001), while it was associated with decreased abundance of *Escherichia–Shigella* (p < 0.05).

### Correlation Analysis of Bacterial Community With Fermentation Products

The association analysis between bacterial abundance and fermentation indexes is shown in Figure 6. Specifically, the pH value was associated with decreased abundance of *Lactobacillus* (p < 0.01). The LA concentration was associated with increased abundance of *Lactobacillus* (p < 0.05), while it was negatively correlated with that of *Pseudomonas* and *Massilia* (p < 0.05). The contents of AA and PA were associated with increased abundance of *Lactobacillus* (p < 0.001), while AA concentration was negatively correlated with the abundance of *Acetobacter* and *Pseudomonas* (p < 0.01) and *Pantoea* (p < 0.05), PA concentration was associated with decreased abundance of *Acetobacter, Pseudomonas* and *Lysinibacillus* (p < 0.01) and *Weissella, Gluconobacter, and Oceanobacillus* (p < 0.05). Finally, the positive correlations have been observed between AN/TN concentrations and *Lactobacillus* (p < 0.01) and *Herbaspirillum* (p < 0.05), while AN/TN concentrations was associated with decreased abundance of *Acetobacter, Pseudomonas* and *Pantoea* (p < 0.05).

### DISCUSSION

#### Nutritional Characteristics of Whole-Plant Maize During Ensiling

The expected DM content for good silage is 30–35% (Guyader et al., 2018). The DM content in each group decreased with the
extension of fermentation time, which was mainly due to the WSC being consumed by LAB and other microorganisms for fermentation (Hu et al., 2009). The different CP contents of raw materials may be related to cultivation and fertilization (Miao et al., 2006). CP is one of the main nutritional components of silage, and the degradation of protein will affect the nutritional value of pastures. The lost nitrogen can only meet the nutritional needs of livestock by supplementing protein feed in the diet, thus increasing the breeding cost (Bilal, 2009). In this study, the CP content of the Z and G groups increased on the 10th and 20th days, respectively, while that of the W group increased on the 5th and 10th days of fermentation, which may be due to the decrease in DM content (Zhang et al., 2018). In addition, when the pH was low, some bacteria composed of protein could not grow and reproduce in the fermentation process and became a part of the feed, which also increased the content of CP (Bilal, 2009). On the other hand, the loss in CP was relatively small due to good fermentation.

The WSC content decreased significantly throughout the fermentation process, and the loss rate of each group reached 70%. As the substrate of silage fermentation, the WSC content will be decomposed by LAB to produce LA (Moselhy et al., 2015). The content of ADF and NDF affects the chewing time of ruminants and indirectly affects feed digestibility in domestic animals (Jang et al., 2017). In the present study, the contents of NDF and ADF in each group decreased with increasing fermentation time, which indicated that silage fermentation had a certain degradation effect on the fiber components of whole-plant maize, thus improving the digestibility of silage.

**The Fermentation Property of Whole-Plant Maize During Ensiling**

The pH value of silage is an important index to evaluate the success of silage, and well-fermented silage should have a pH of 3.8–4.2 (Kung et al., 2018). The low pH value ensures that harmful bacteria are inhibited and finally contributes to the good fermentation of whole-plant maize (Keshri et al., 2018). The pH was less than 4.0 and reached the lowest point at 20 days in all the silages except for that in the W group, which agreed with the results of a previous study (Sun et al., 2021).
FIGURE 3 | Circos map of bacterial communities at the phylum (A) and genus levels (B) for whole-plant maize silage.

FIGURE 4 | The relative abundance of bacteria community proportions at the phylum (A) and genus (B) level across the treatments (as a percentage of the total sequence).
According to the fermentation mode, LA fermentation can be divided into homofermentative and heterofermentative types (Guo et al., 2018). Homofermentation mainly produces LA, whereas heterofermentation also produces AA, ethanol and CO$_2$ in addition to LA (Pahlow et al., 2003). In this study, LAB grew rapidly and produced enough LA in each group, which inhibited the growth and reproduction of harmful microorganisms. With increasing LA content, homofermentative LAB are inhibited, while heterofermentative LAB begin to dominate due to the stronger tolerance to AA and pH value, and fermentation gradually changes from homofermentative LAB fermentation to heterofermentative LAB fermentation (Shao et al., 2002). The AA content of each group gradually increased with increasing fermentation time. In addition, when the silage is fermented to a certain extent and the pH is low, the fermentation of LAB will also be inhibited. At the same time, some anaerobic microorganisms that may exist in silage begin to decompose LA and produce other organic acids, such as AA and PA, which leads to a decrease in LA content (Shao et al., 2002). In this study, with the extension of fermentation time, the LA content decreased and the AA and PA contents increased gradually in the G group, which indicated that the type of fermentation was heterofermentative. The contents of LA and AA were relatively high, and the ammonia nitrogen content was within a limited range. These indexes showed the excellent fermentation quality of all the silages. BA is a product produced by decomposing protein, glucose and LA by spoilage bacteria and BA bacteria, respectively (Addah et al., 2012). It has been reported that BA content $> 5$ g/kg DM affects the palatability of feed and reduces the feed intake by livestock (Muck, 2010). During the whole fermentation process, BA was not detected in any group.

During the ensiling process, the ammonia nitrogen is mainly produced by the degradation of protein by plant enzymes and the utilization of protein and amino acids by microbial decomposition (Kung et al., 2018). In general, the ammonia-N content is recommended to be less than 5% for maize silage (Zhang et al., 2016). The high ammonia-N content indicates that proteolysis occurred to a deep extent during ensiling (Mu et al., 2021). In the present study, the content of AN/TN in each group was lower than 5%, indicating that harmful bacteria were effectively inhibited.

Microbial Diversity of Whole-Plant Maize During Ensiling

The microbe numbers and species composition are varied with different silage materials or ensiling processes (Khota et al., 2016). Various epiphytic bacterial communities in raw
materials might originate from unique growth environments (McGarvey et al., 2013). Gharechahi et al. (2017) reported a special bacterial community in whole-plant corn from different geographical locations. In this study, the differences at the species level in the epiphytic microorganisms of the Whole-plant fresh samples from the three areas were observed. The sampling locations in three areas belong to a subtropical monsoon climate and are close to each other. The phylum-level bacteria mainly attached to the surface of silage maize in the three regions were Firmicutes and Proteobacteria. Additionally, the main LAB genus in fresh forages was Weissella. This indicated that Weissella might play a major role in the early stage of fermentation (Sun et al., 2021). This genus is a selective anaerobic bacterium, which converts water-soluble sugars into LA and AA in the early stage of silage fermentation (Cai et al., 1998). In the current study, Weissella with low abundance was detected in silages during the ensiling process (Figure 4), which might lead to relatively high AA content in all silages (Table 2).

The nature of silage is due to complex microbial succession (Yang et al., 2019), in which bacteria play a key role (Li P. et al., 2019). In this study, the abundance of Firmicutes increased rapidly and became the dominant phylum during ensiling. This observation might be explained by the fact that the growth of Firmicutes is related to the low pH and anaerobic conditions formed during ensiling (Wang et al., 2019; Zhang et al., 2019). Although Lactobacillus was usually not the dominant bacteria in fresh corn of the Z group, it began to dominate at 2 days of ensiling. This observation suggested that the LAB count in the raw materials is enough to ensure the fermentation quality of the final silage. Lactobacillus, Lactococcus, Weissella, and Enterococcus are common LA-producing bacteria in silage, and their abundance changes are closely related to the quality of silage (Ni et al., 2018). Generally, LA-producing cocci are the dominant LAB. These initiate LA fermentation during the early stages of ensiling, whereby Lactobacillus will grow rapidly into the dominant bacteria (Liu et al., 2019). This result suggested that Lactobacillus was an important genus for silage fermentation during ensiling. Many studies have shown the dominance of Lactobacillus in ensiled corn silages (Li and Nishino, 2011; Ogunade et al., 2017) due to its desirable functions during ensiling (Li et al., 2017; Liu et al., 2019). The study showed that Lactobacillus and Weissella were the only LAB with high abundance in the whole-plant maize silage process in different regions, which was consistent with the research of Ogunade et al. (2017), and the reason for the difference with the research of
Tohno et al. (2013) and Ni et al. (2017b) may be related to the research materials and locations. Gluconobacter and Acetobacter were found in minor abundance during the early stages of ensilage. Acetobacter is a kind of AA-producing and nitrogen-fixing bacteria (Kumiko et al., 2001) and may lead to the decline in pH at the early stage of the ensiling process. Other bacteria, such as Pantoea, Rahnella, and Sphingomonas, were evenly distributed during the ensiling process in lower amounts are consistent with previous studies (Gharechahi et al., 2017; Ni et al., 2017a), and some of them are undesirable in silage (McDonald et al., 1991). Muck (2010) reported that Bacillus, Paenibacillus, Enterobacter, Enterococcus, and Clostridium are the main microorganisms that decompose proteins into ammonia nitrogen and cause protein loss. None of these bacteria were found in the top 10 bacteria regarding relative abundance, which was consistent with the previous results of low AN/TN contents in the present study.

**Correlation Analysis Between Bacterial Communities and Environmental Factors**

There are many factors (moisture, WSC, regional factors) that affect the microbiome and influence the fermentation quality of silage (McEniry et al., 2010; Bernardes et al., 2018). Usually, regional factors include temperature, humidity, precipitation, longitude, latitude, and altitude. Previous reports showed that high temperatures and rainfall had detrimental effects on the fermentation process and silage quality (Kim and Adesogan, 2006). In addition, high temperature affected the transformation of microorganisms in corn silage from a homofermentative to a heterofermentative LAB community, which had been previously reported (Guan et al., 2020). In this study, altitude was positively correlated with the abundance of Stenotrophomonas, Chryseobacterium, and Massilia, while precipitation was negatively correlated with these bacteria. This indicates that precipitation was the factor affecting the epiphytic bacteria of the silage material. The Lactobacillus abundance in fresh maize did not change regularly with increasing altitude, and the reason for the difference may be related to the temperature and precipitation in the three regions. The correlation analysis (Figure 5) showed that the relative abundance of Lactobacillus was negatively correlated with temperature. This also explains why the temperature of the G group was the highest (21.35°C), while the relative abundance of attached Lactobacillus was the lowest (2.75%). The temperature of the W group was the lowest (17.32°C), while the relative abundance of attached Lactobacillus was the highest (63.77%). Altitude and precipitation influence some microorganisms in silage, but they do not relate to the main bacteria in silage, such as Lactobacillus and Weissella. This shows that when silage enters a completely anaerobic environment, the influence of climate factors on the main microorganisms will become smaller. Cai et al. (1999) reported that a certain amount of LAB in forage will have a great impact on silage fermentation. In this study, Lactobacillus and Weissella were correlated with temperature and humidity, and the most dominant bacterial genera were Lactobacillus and Weissella in the samples during the ensiling process. This result indicated that the main factors affecting the microbial diversity of silages were humidity and average temperature. Environmental factors affect the community of epiphytic bacteria on raw materials, which have a greater impact on the initial stage of silage fermentation (Zi et al., 2021b). We speculate that the influence of environmental factors on the microbial community will decrease during the silage fermentation process, thus reducing its influence on silage quality.

**Correlation Analysis of Bacterial Community With Fermentation Products**

Microorganisms affect silage quality through a series of metabolites. For example, Lactobacillus species mainly affect LA production (Guan et al., 2018). In the present study, the Lactobacillus was positively correlated with the LA content, which dominated the bacterial community in the fermentation process and had a negative correlation with pH in all silages. This result was consistent with the report of Sun et al. (2021). Pseudomonas is an undesirable bacterium that can survive in an anaerobic environment, and its production of biogenic amines leads to the decrease in protein content (Roberson and Firestone, 1992; Dunière et al., 2013). The AN/TN content was negatively correlated with the abundance of Pseudomonas, indicating that the existence of Pseudomonas may contribute to the preservation of protein (Ogunade et al., 2018). Other studies have confirmed these findings, indicating that the fermentation characteristics are highly correlated with the microflora of silage and affect the overall fermentation quality (Ren et al., 2019; Yang et al., 2019).

**CONCLUSION**

All of the whole-plant maize silages had satisfactory fermentation quality. The bacterial community of fresh raw material was mainly composed of Weissella and Proteobacteria. Although the bacterial community varied during ensiling, Lactobacillus dominated the ensiling process. Lactobacillus had a negative correlation with pH in all silages and grew well under low pH conditions, produced LA during ensiling, and effectively influenced fermentation quality. Altitude and precipitation influenced some specific microorganisms in silage, but they did not affect the main bacteria in silages. The humidity and average temperature significantly influenced the abundances of Lactobacillus and Weissella of fresh whole-plant maize and had a greater impact on the whole fermentation process.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article-supplementary material, further inquiries can be directed to the corresponding author/s.
AUTHOR CONTRIBUTIONS

JH and CC contributed to conception and design of the study. LL, SD, and CW performed the statistical analysis. YH wrote the manuscript and interpretation of data. All the authors read and contributed to the manuscript.

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