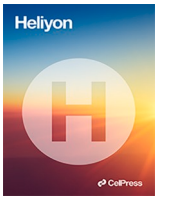
Heliyon 9 (2023) e15949

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Effect of rhizosphere microorganisms on aflatoxin contamination of maize

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ARTICLE INFO

Keywords:
Maize
Aflatoxin contamination
Microbiota
Correlation analysis
Soil physicochemical properties

ABSTRACT

The continued large consumption of maize makes it one of the most important food crops worldwide. However, the yield and quality of maize are greatly affected by global warming, and mycotoxin pollution keeps increasing. The effect of environmental factors, especially rhizosphere microorganisms, on mycotoxin pollution of maize is not completely clear, so we carried out relevant studies. In this study, we found that microbial communities inhabiting the maize rhizosphere, which consists of soil particles firmly attached to roots, as well as the soil, have a significant influence on the aflatoxin pollution of maize. The ecoregion and soil properties also had considerable effects on the microbial structure and diversity. The bacterial communities from the rhizosphere soil were profiled using a high-throughput next-generation sequencing method. The ecoregion and soil properties had considerable effects on the microbial structure and diversity. A comparison of the aflatoxin high concentration group with the low concentration group found that bacteria of the phylum *Gemmatimonadetes* and order *Burkholderiales* were significantly more abundant in the high concentration samples. Furthermore, these bacteria were significantly correlated with aflatoxin contamination and could aggravate its contamination of maize. The results of these analyses showed that seeding location could cause significant shifts in the root microbiota of maize, and the bacteria enriched in high aflatoxin contamination area soils should attract special concern. These findings will support strategies for improving maize yield and aflatoxin contamination control.

1. Introduction

Maize is grown worldwide, and its total output and unit yield rank first among food crops and plays an important role in ensuring world food quantity [1]. According to the 2021 National Statistical Yearbook, the total output and planting area of maize in China were 260 million tons and 43.32 million hectares (Index number: 410A0-0402-202202-0006), respectively, far higher than those of rice and wheat, ranking first among food crops in China. Maize can be divided into maize for grain, fresh maize and silage maize according to its mode of harvest and use, among which the grain is the main maize product in China. In recent years, 10% of the total maize production in China has been used as grain ration, 70% as feed, and more than 20% as raw materials for industrial processing. The maize yield has

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https://doi.org/10.1016/j.heliyon.2023.e15949
Received 6 December 2022; Received in revised form 22 April 2023; Accepted 27 April 2023
Available online 29 April 2023

become an important factor affecting grain supply and demand and the development of animal husbandry in China. At the same time, the quality and safety of maize and its products are related to the sustainable development of China's agriculture and its national health [2].

The quality of maize grains in China is affected by soil conditions, diseases and insect pests, environmental conditions (light, temperature, water, etc.) and harvesting and storage conditions [3]. The quality and safety of maize grains are faced with many problems, among which mycotoxin contamination is the most important [3,4]. Mycotoxins are the primary natural pollutants of agricultural products, with strong pathogenicity, which reduces raw grain yield and quality. It also causes great harm through the ingestion of mycotoxin in contaminated food by animals and humans and has always been a hot spot in global food trade disputes [4].

Mycotoxins are the most important factor affecting the safety and quality of maize and its products [3–6]. According to the FAO, 25% of crops worldwide are contaminated with mycotoxins in varying degrees [4]. Maize and its products in China are seriously affected by mycotoxins due to environmental factors, harvesting and storage conditions [6]. Gao et al. tested for aflatoxin in 279 maize samples from Jilin, Henan, Hubei, Sichuan, Guangdong, and Guangxi, and found a positive rate of 75.6% [7]. With the increase in global climate warming and soil drought stress, the contamination of aflatoxins in maize has shown an increasing trend in China.

Many researchers have carried out researches on the mechanism of mycotoxin contamination from the aspects of varieties and planting patterns, but the correlation of soil microorganisms with the mycotoxin contamination is poorly understood [8,9]. The functions and effects of plant symbiotic microorganisms in different maize planting regions or microbial niches need to be further studied. They affect the detection, early warning and comprehensive prevention and control of maize aflatoxin contamination and subsequently affect the quality and safety of maize products. Therefore, we have carried out relevant research.

In view of the abovementioned facts, the ecological environment and soil conditions are vital to maize production, and the microbiome in maize is critical for aflatoxin production and food safety [10]. Therefore, it is important to explore the microbiome construction of the maize rhizosphere and how it impacts its toxin-producing ability. Its high complexity, unknown factors, and the restrictions of traditional pure culture technology have led to the slow development of understanding of the maize rhizosphere microbiome. In the past, most of its microorganisms could not be cultured separately, which remarkably hindered the investigation of the environmental microbiome [11–13]. Since next-generation sequencing technology has come into widespread use, it is possible to research the microbiome of the maize rhizosphere on a large scale [11,12].

We characterized the distribution of the rhizosphere microorganisms of maize in different planting areas (Fuxin of Liaoning (LF), Tangshan of Hebei (HT), Linyi of Shandong (SL), Hongan of Hubei (HH), Zhangshu of Jiangxi (JZ) and Zhanjiang of Guangdong (ZJ)) in China. We collected rhizosphere soil samples from different planting areas, and high-throughput amplicon sequencing of the 16 S rRNA gene was performed to compare the microbial community structures among different sites. We investigated the main factors affecting the community, and explored the co-occurrence of bacteria and fungi. This will provide a new perspective and approach to mycotoxin contamination of maize and its effective control.

2. Materials and methods

2.1. Soil sample collection and analysis of physical and chemical properties

In 2020, we carried out field trials and sample collection in different regions. The rhizosphere soil samples (n = 90) of maize and bulk soil samples (n = 30) which were more than 10 cm from the rhizosphere were collected from various regions in China. These regions are the main production areas of maize, they are Zhanjiang of Guangdong (ZJ), Zhangshu of Jiangxi (JZ), Hongan of Hubei (HH), Linyi of Shandong (SL), Tangshan of Hebei (HT) and Fuxin of Liaoning (LF). Among the six regions, HT, SL and LF are located in the north and HH, JZ and ZJ are located in the south. The sampling areas of different regions were selected from the sites with high corn yield, obvious regional advantages and significant climatic characteristics. But most importantly, the sampling areas in southern sites including ZJ, JZ, HH were cities with higher aflatoxin pollution, while sampling areas in northern sites including SL, HT and LF with lower aflatoxin pollution according to the aflatoxin pollution monitoring data in China [14].

The concentrations of organic matter, nutrients, and cations were measured for soil samples from themaize rhizospheres. Soil pH was determined by potentiometry [15]; soil organic matter was determined by potassium dichromate volumetric method [15]; soil hydrolyzed nitrogen was determined by the alkali diffusion method [15]; soil available phosphorus was determined by the spectrophotometric method [15]; and the content of microelements in the soil was analyzed by a plasma emission spectrometer [16].

2.2. Analysis of aflatoxin B_1 by chromatography (HPLC)

Aflatoxin B1 was analyzed in maize samples (n = 120) by high performance liquid chromatography (HPLC). The detection of aflatoxin B_1 can be divided into three steps: extraction, purification and detection. Extraction of aflatoxin using methanol: water (6:4) was first conducted. Then, the sample of residue aflatoxin was purified and extracted by immunoaffinity column (B_1). Finally, HPLC was used to test the purified samples extracted from purification. HPLC test condition: flow phase acetonitrile: water for 3:7; flow velocity for 1 mL min^{-1}; chromatographic column for C184.6 mm × 250 mm, for 0.5 μL; column temperature for room temperature; excitation wavelength for 360 nm; test wavelength for 440 nm; injection volume of 20 μL.

2.3. DNA extraction and sequencing

Microbial DNA was extracted from the maize rhizosphere using the E. Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.). A

16 S rRNA library for the Illumina HiSeq 2500 platform was prepared. The primer set 515 (5′-GTGCCAGCMGCCG CGG-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′) with 8 nt barcodes were used for pair-ended sequencing to target the 16 S rRNA V4 region [Ultrahigh-throughput microbial community analysis on the Illumina HiSeq platform]. These primers amplify bacterial 16 S rRNA genes. The PCR amplification program included initial denaturation at 95 °C for 5 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s, and a final extension at 72 °C for 10 min. Three PCRs were conducted for each sample and combined after PCR amplification. The PCR products were subjected to electrophoresis using a 2.0% agarose gel. The band with the correct size was excised and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) and quantified with QuantiFluor: trademark: ST (Promega, U.S.). All samples were pooled together with equal molar amounts from each sample and applied to an Illumina HiSeq 2500 PE 250 for sequencing.

2.4. Analysis of the sequencing data

Sequences were processed using the QIIME (v1.9.0, http://qiime.org/index.html) analysis pipeline. Reads were binned according to the index sequence. Overlapping regions within the paired-end reads were then aligned to generate tags using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/). If more than 10% mismatches were discovered, the paired-end sequences were discarded. All sequences with ambiguous base calls were also discarded. OTUs (operational taxonomic units) were clustered at 97% similarity using Uparse (v7.0.1001, http://drive5.com/uparse/) with the left effective tags. After low-quality and chimeric tags were filtered out, a total of 3,398,272 high-quality tags were obtained. For taxonomy-based analysis, USEARCH combined with the SILVA database (v128, http://www.arb-silva.de/) was used. Rarefaction curves were constructed for all 16 S rRNA gene libraries generated from different maize-cropping sequences. Within-sample diversity was calculated for each sample using the observed species and Shannon indices via the alpha diversity in QIIME from the final OTU table. Rarefaction curves were calculated using alpha rarefaction. Bray Curtis and weighted and unweighted UniFrac distances between samples were calculated from the normalized OTU table. A PCoA plot to visualize the differences among the groups of samples was drawn based on the Bray–Curtis distances. The distribution tree of samples was also calculated based on the Bray−Curtis distances with a mathematic average (UPGMA) dendrogram.

2.5. Statistical analysis

To assess the influence of the different taxa on the rhizosphere microbiomes among the experimental fields, an RDA (redundancy analysis) was performed using the function from the R package vegan. The rhizosphere microbial communities of different regions were further compared using LDA Exact Size (LESe, http://huttenhower.sph.harvard.edu/galaxy) [17], and the LDA score cutoff was set to 3.5. Genera between the low and high aflatoxin contamination regions were picked out using Metastats (http://metastats.cbcb.umd.edu) [18]. The R package vegan was also used to calculate the Spearman correlation between the genera and environmental factors.

3. Results

3.1. Bacterial community of the maize rhizosphere in different planting areas

We collected 120 samples of maize rhizosphere soils and bulk soils (CK) from LF, HT, and SL in the north and HH, JZ, and ZJ in the south (Fig. 1A). Furthermore, we determined the coamplification of nontarget 16 S rRNA (chloroplast, mitochondrial and unassigned sequences) and the number of singletons identified within each sample. We generated a total of 3,316,158 effective tags with an

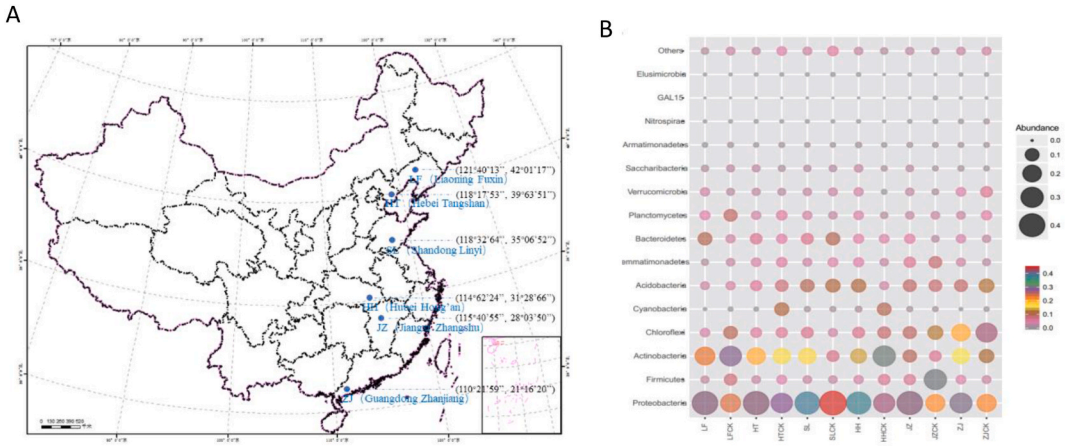

Fig. 1. Geographic distribution of sampling points (A) and the bacterial phylum construction of maize rhizosphere soils and bulk soils from each site. Circle size indicate abundance and similar abundances were color-coded (B).

average length of 256 bp for further analysis (Table 1).

Based on their shared sequence similarity at a 97% threshold, sequences were clustered into 16,197 OTUs. In each sample, there was an average of 3401 bacterial OTUs ranging from 1623 to 4454 OTUs (Table 1). The taxonomy and abundance of all OTUs are shown in Supplementary Table S1. OTUs were further assigned to different taxa and their relative taxonomic abundance was estimated across the different samples. We were able to classify the majority of tags and only a relatively small proportion of reads could not be assigned to phylum taxa (ranging from 0.98% to 8.02%). Assigned tags were classified into 46 bacterial phyla, mainly including *Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria* and *Bacteroidetes.* Fourteen phyla were represented in all soil samples (Fig. 1B). *Proteobacteria* was the most abundant phylum inhabiting the soils, accounting for 35.80% of the total bacterial community. The second most abundant phylum in the community was *Actinobacteria*, and the proportion of these types of bacteria was 16.71%. The two most abundant phyla made up more than half of the bacterial community, which was of vital importance to the structure and composition of the microbial community. Other phyla including *Aminicenantes, Lentisphaerae*, and *Nitrospinae*, were all less abundant and accounted for less than tens of millions of the clones. In total, 658 genera belonging to 335 families were recorded from these samples.

3.2. Bacterial diversity analysis

The community diversity indices were calculated based on the OTUs with 97% sequence similarity. Rarefaction curves were constructed for each individual group showing the number of observed OTUs relative to the number of total identified bacterial rRNA tags (Fig. 2A). Rarefaction curves of most samples tended to be flat, suggesting that a reasonable sequencing depth has been attained, although extra rare bacterial taxa are likely present in the sample. The Shannon–Wiener curve agrees with this claim (Fig. S1). For the observed species, the community richness indices of the HH, ZJ and HT rhizospheres were comparable and were higher than those of the SL, JZ, and LF rhizospheres, implying that additional OTUs were likely present in the HH, ZJ and HT rhizospheres, which saturated more than 3000 OTUs. As expected, almost all bacterial communities in the bulk soils were less diverse than in the rhizospheric communities (Fig. 2A and B). The Shannon diversity indices, which integrate evenness and species richness, suggested that the taxonomic diversity of the bacterial community was also higher in the HT and ZJ rhizospheres than in the other rhizospheres (Fig. 2B). The alpha diversity was fairly consistent from sample to sample in ZJ, the southernmost city. In contrast, there was a considerable difference in bacterial composition between samples from LF, the northernmost city.

We evaluated beta diversity at the OTU level (OTUs defined at a 97% similarity cutoff). To compare the composition of the identified community members within different soil samples, a weighted UniFrac dissimilarity matrix was calculated. The density changes along the inner-group weighted UniFrac distances computed from the OTU profile showed the beta diversity within each rhizosphere soil sample group (Fig. 2C). The weighted UniFrac distance values between samples in ZJ were mainly smaller than 0.30. However, a double peak distribution was observed in LF, with values of 0.20 and 0.35, respectively. Other locations where inner-group distances of samples had more than two peaks also showed a larger composition difference between samples.

3.3. Bacterial community variation across locations

Significant variation in the tag number of bacterial OTUs per sample based on location was observed. Multiresponse permutation procedures (MRPPs) showed that all intergroup distances were greater than the intergroup distances ($A>0$), indicating that the differences in the bacterial community of the maize rhizosphere between locations were much larger than those between individuals. MRPP also revealed that the microorganisms from different sites were significantly different from each other (Table 2).

We also evaluated the influence of geographical locations and microhabitat on the maize rhizosphere microbiota structure using the Bray–Curtis distance (OTUs defined at $\geq$97% identity). Overall, similarities in the bacterial community structures among the samples were displayed using principal component analysis (PCoA). The PCoA showed that the samples from the rhizosphere soil in HH had similar reactivities to ZJ and JZ, and samples from HT had similar structures to SL and LF, since the samples from these rhizospheres clustered together. PCoA analyses revealed strong clustering of rhizosphere bacterial communities according to the

Table 1
Data summary.

Total of reads after QC	3,398,272
Singletons	48,907
Unassigned/mitochondria/chloroplast tags	33,207
Total of assigned tags after QC	3,316,158
Average assigned tag length after QC	256 ± 0.00
Average of assigned tags after QC	36,846 ± 512
Total of OTUs	16,197
Average of OTUs	3401 ± 68

A singleton is a read with a sequence that is present exactly once, i.e. is unique among the reads. Reads that were singletons afterquality filtering and global trimming were therefore discarded. Tags assigned to mitochondria and chloroplast were also abandoned, and tags assigned to Bacteria kingdom were used as input for OTU clustering. The average tag length and quantity were calculated based on soil samples across all planting sites.

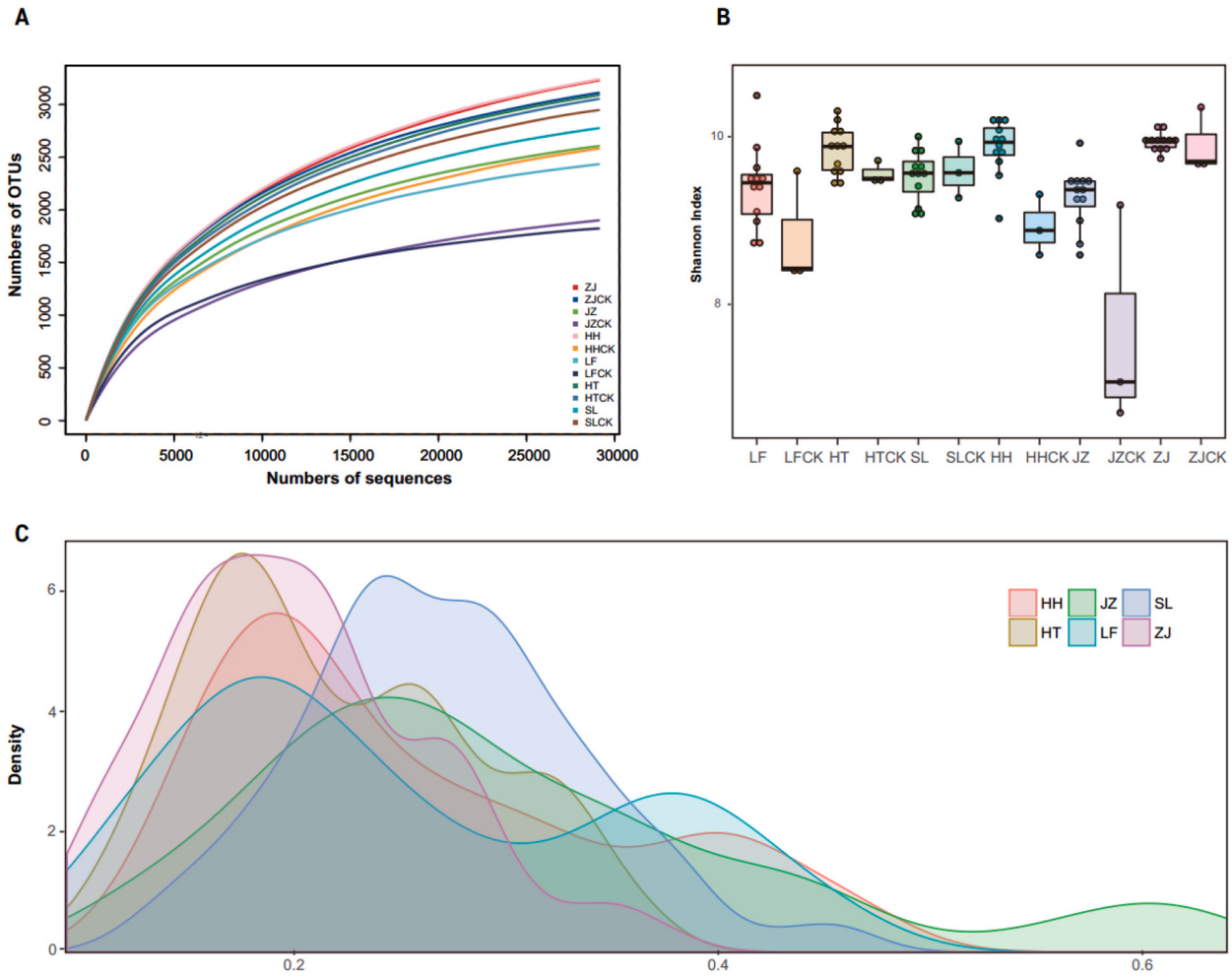

Fig. 2. Diversity from microbiomes of rhizospheries and bulk soil (CK). Rarefaction curve analysis for the soil microbiomes with operational taxonomic units (OTUs) with 97% nucleotide sequence similarity (A) and boxplots for Shannon index were displayed (B). Distribution of the weighted UniFrac pairwise distances between all samples from each site based on OTU-97% communities (C).

different sites. The rhizosphere microbiota separated mainly along the first principal coordinate, basically following a geographical distribution (Fig. 3A).

Moreover, hierarchical clustering of Bray-Curtis-based distance displayed a clustering structure, with communities grouped by

Table 2
Multi-response Permutation Procedures (MRPP) at the OTU level between rhizosphere soil groups from different sites. Significance levels: *P ≤ 0.01.

Group	A:	Observed-delta	Expected-delta	Significance
HH-LF	0.23	0.44	0.57	*
LF-ZJ	0.28	0.42	0.58	*
HH-ZJ	0.18	0.41	0.50	*
LF-SL	0.18	0.47	0.58	*
HH-SL	0.13	0.47	0.53	*
SL-ZJ	0.19	0.44	0.55	*
JZ-LF	0.18	0.49	0.60	*
HH-JZ	0.15	0.48	0.57	*
JZ-ZJ	0.21	0.46	0.58	*
JZ-SL	0.16	0.51	0.61	*
HT-LF	0.11	0.44	0.49	*
HH-HT	0.19	0.43	0.53	*
HT-ZJ	0.25	0.41	0.55	*
HT-SL	0.14	0.46	0.54	*
HT-JZ	0.17	0.48	0.58	*

sites. All samples from different geographic locations were well separated except LF18. T, where T was merged into the HT group. We could see that the HT group was closest to the LF group in the cluster. Accordingly, we found that samples from both regions were from northern China, and HT was neighboring LF on the map (Fig. 3B). In another group (SL) from northern China, the rhizosphere microbiota from this group was pretty consistent with HT and LF at the genus level. These results demonstrated that geographic locations contributed the most to the microbiota variations in the maize rhizosphere.

Finally, we took a closer look at the individual bacterial components at separate levels, which differentiated the bacterial communities in the rhizosphere soil sites. LEfSe analysis using the factorial Kruskal–Wallis test showed that the many phylogenetic groups could be significantly distinguished among the six soil sites. LEfSe identified 1, 13, 6, 5 and 7 bacterial clades in HH, JZ, LF, SL and ZJ, respectively, which consistently explained the significant differences among the six microbial communities. The most differentially abundant bacterial taxa at the six sites belonged to *Proteobacteria*, which concretely expressed *Rhizobiales* and *Sphingomonadaceae* (*Alphaproteobacteria*) enriched in LF, *Burkholderiales* (*Betaproteobacteria*) in JZ and *Xanthomonadales* (*Gammaproteobacteria*) in SL (Fig. 3C). The overrepresented clades of LF also included *Sphingobacteriales* (*Sphingobacteriia*) and *Actinobacteria* (*Actinobacteria*), which is different from those of ZJ (SBR2076, *Ktedonobacteria* and *Anaerolineae* of *Chioroflexi*) and JZ (*Gemmatimonadaceae* of *Gemmatimonadetes*, *Bacilli* of *Firmicutes* of *Chioroflexi*), indicating the beta-diversity of these communities.

3.4. Environmental factors affect the rhizosphere bacteria

To statistically assess the effects of pH and various chemical elements on the rhizosphere bacterial composition, we performed RDA (redundancy analysis) on the OTU profile. The first two constrained RDA components could explain 49.21% of the total variation (Fig. S2). In the RDA model, pH, organic matter and other inorganic elements were not found to produce significantly different effects in explaining the environmental variables in the distribution of bacterial groups among rhizospheres. Nevertheless, combining the top 50 abundant genera in rhizosphere soils from six sites and the environmental factors, we found that these factors were closely associated with the sites. The genera in the heatmap were clustered into three main groups; they were more abundant in HH and ZJ; more abundant in JZ; and more abundant in SL, HT and LF, which could be roughly divided into two groups, south (HH, ZJ, JZ) and north

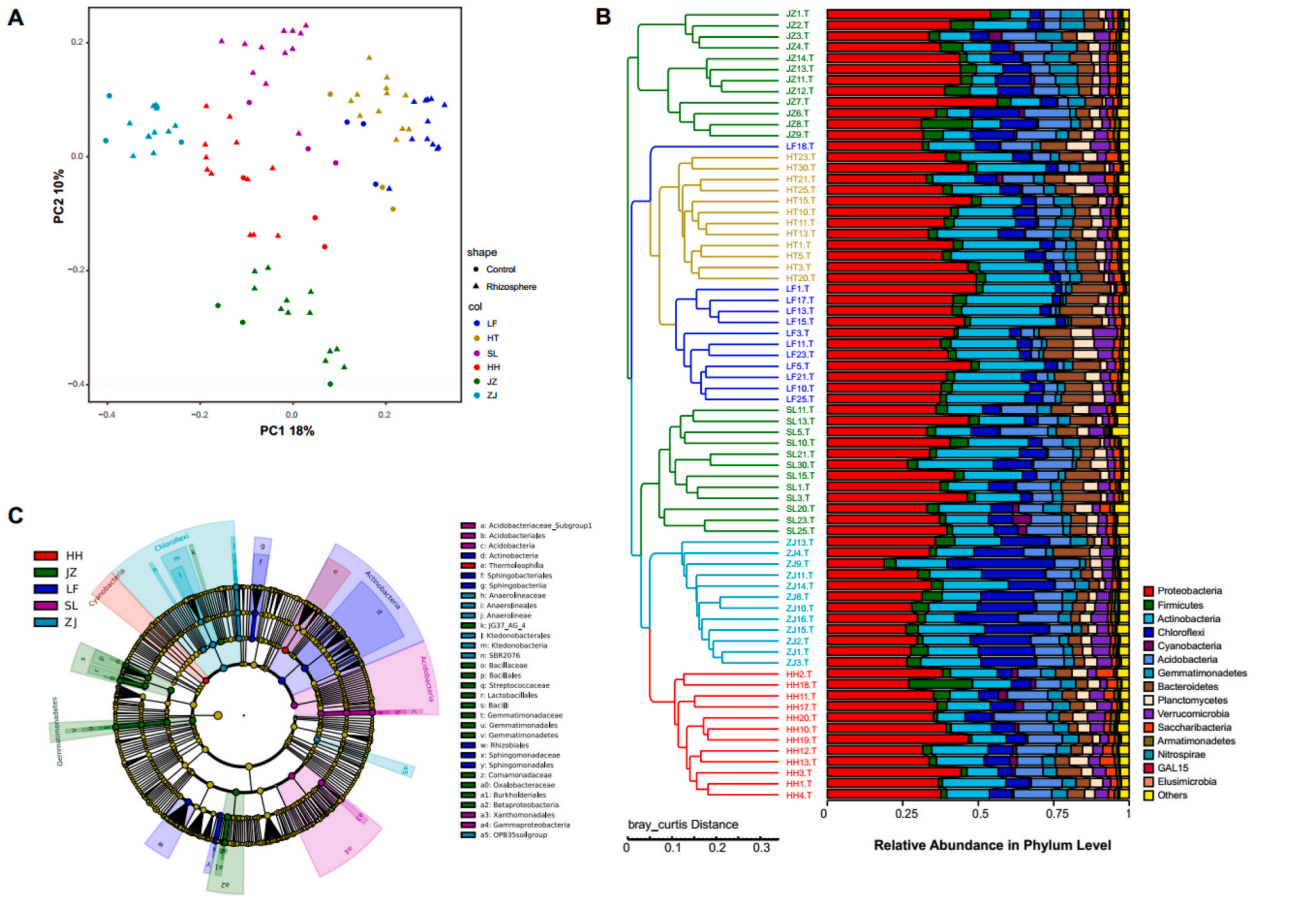

Fig. 3. Principal Coordinate Analysis (PCoA) of distribution of bacteria in six sites (A). Overall distribution of bacteria at phylum level in maizes' rhizosphere soils of bray curtis distance with mathematic average (UPGMA) dendrogram (B). And LEfSe results on maize rhizosphere microbiomes (C).

(SL, HT, LF). Spearman's correlations (Fig. 4) showed that the more abundant genera in the south were significantly ($p < 0.05$) negatively related to pH but positively correlated with phosphorus, manganese, zinc, iron and organic matter. In contrast, the more abundant genera in the north were significantly ($p < 0.05$) positively related to pH and were negatively correlated with phosphorus, manganese, zinc, iron and organic matter. The soil phosphorus, manganese, zinc, iron and organic matter seemed to be in line with aflatoxin levels when correlated with the screened genera, and all of their changes showed an opposite correlation direction to the pH.

To investigate the effects of aflatoxin on the maize rhizosphere microbiome, we measured the concentration of aflatoxin in each sample. Samples from the southern region (JZ, ZJ and HH) produced many more aflatoxins than samples from the northern region (SL, LF and HT) (Table S2). We compared the aflatoxin high concentration (southern) with the low concentration group (northern). Ten genera had significantly different abundances between the southern and northern samples (Table 3). At the genus level, *Bacillus* ($q = 0.005$) of the phylum *Firmicutes*, *Gemmatimonas* ($q = 0.003$) of the phylum *Gemmatimonadetes*, and *Ramlibacter* ($q = 0.003$) and *Haliangium* ($q = 0.003$) of the phylum *Proteobacteria*, were enriched in the southern samples while *Mycobacterium* ($q = 0.003$) of the phylum *Actinobacteria*, *Mucilaginibacter* ($q = 0.003$) of the phylum *Bacteroidetes* and *Bradyrhizobium* ($q = 0.003$), *Rhizomicrobium* ($q = 0.007$), *Sphingomonas* ($q = 0.009$), *Burkholderia-Paraburkholderia* ($q = 0.003$) of the phylum *Proteobacteria* were more strongly associated with the northern samples. To verify whether these differences were affected only by site, we also compared the bulk soil samples from southern and northern China. The results showed that all of genera selected above had no significant differences in the bulk samples, which further illustrated that the differences between the southern and northern maize rhizosphere microbial communities resulted from not only planting sites but also the integration of planted maize and its rhizosphere bacteria.

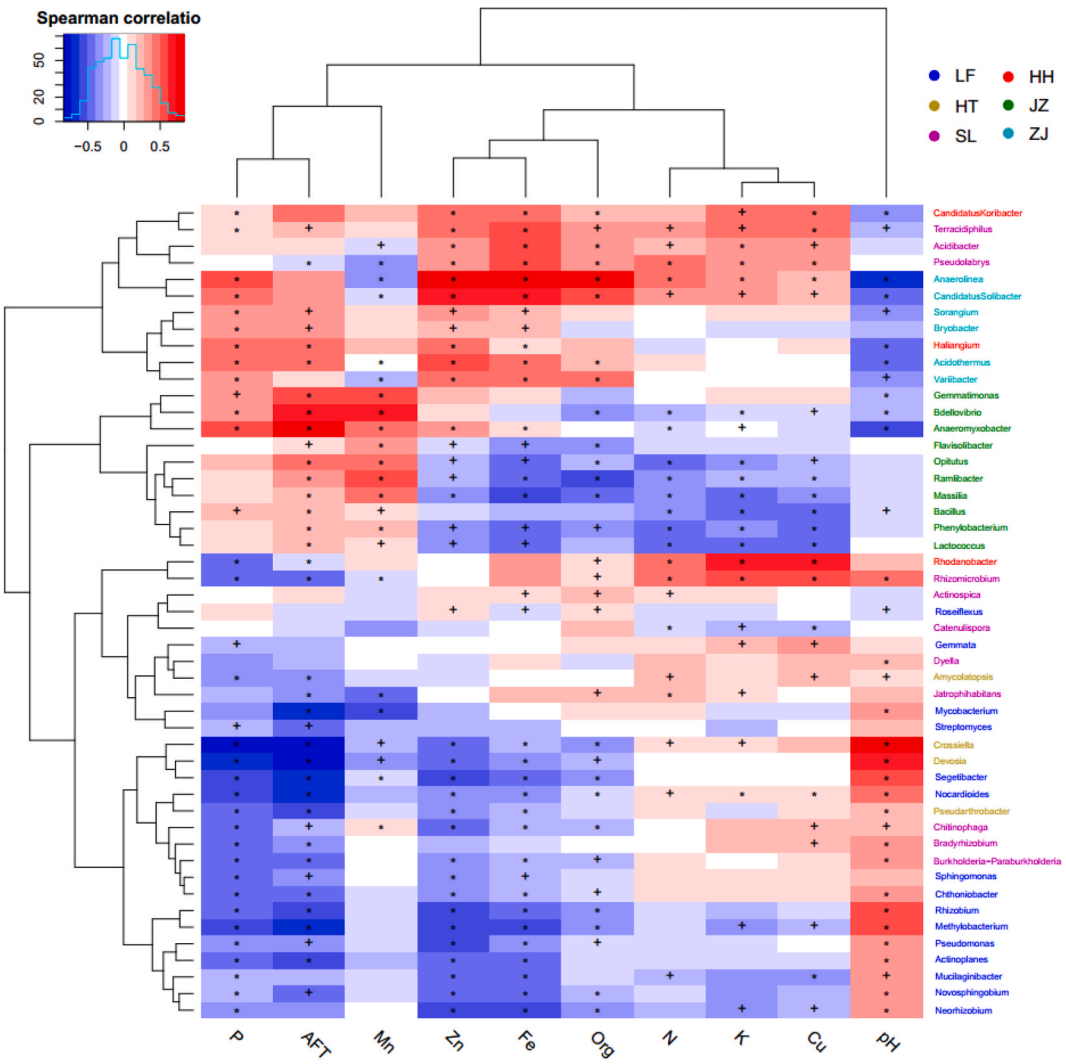

Fig. 4. Heatmap and hierarchical clustering of the mean of top 50 genera' relative abundance of rhizosphere samples against environmental factors, including aflatoxin content. The genera were colored according to the most abundant groups. OTUs negatively and positively correlated with the yield were marked with different colors; negative: blue; positive: red. +: $p < 0.05$; *: $p < 0.01$

Table 3
Metastats analysis of bacteria community composition of southern and northern rhizosphere soil samples at genus level. Genus mean abundance more than 0.5% were showed in the table.

Genus	Enriched group	Relative abundance (%)		FDR
		Southern	Northern	
Bacillus	Southern	0.023	0.012	0.005
Gemmatimonas	Southern	0.026	0.015	0.003
Ramlibacter	Southern	0.017	0.007	0.003
Haliangium	Southern	0.017	0.010	0.003
Mycobacterium	northern	0.006	0.010	0.003
Mucilaginibacter	northern	0.005	0.011	0.003
Bradyrhizobium	northern	0.010	0.014	0.003
Rhizomicrobium	northern	0.008	0.013	0.007
Sphingomonas	northern	0.029	0.040	0.009
Burkholderia-Paraburkholderia	northern	0.012	0.027	0.003

4. Discussion

In recent years, maize has witnessed the fastest growth in area and yield among major grain crops in China, and maize has become the main force in increasing its grain output [19]. Maize plays an important role in China's agricultural production, and it is of strategic significance to develop maize production to ensure national food security [19]. However, with the destruction of the soil structure caused by global warming and continuous planting, maize diseases and insect pests tend to increase, especially mycotoxin pollution of maize, which seriously limits the improvement of maize yield and quality [19]. Little is known about the changes in maize microbial populations caused by climate change and the interaction between microorganisms and mycotoxins, making it necessary to study the relationship between them. In this study, we characterized the rhizosphere microbial community composition of maize from different experimental fields in China. Our fields were located in distinct climatic regions, with three fields in northern China and three in southern China. Our design allowed us to test the influence of planting locations on the maize rhizosphere microbial community across the fields. We also quantified the relative contributions of factors explaining the variation in maize rhizosphere-associated bacterial consortia. It also helped us to assess the degree to which these microbe-aflatoxin interactions depend upon the fields in which they are measured. We found that the bacterial composition significantly influences the concentration of aflatoxin across field environments.

We described the bacterial community structure of maize rhizosphere soils at the phylum level. Moreover, the alpha diversity results showed that the soil microbiomes were sufficiently sampled in this study since rarefaction curves were asymptotic in these samples. We observed remarkably dissimilar shapes of the OTU rarefaction curves when comparing rhizosphere soil and bulk samples. Bacterial alpha diversity decreased from the maize rhizosphere to the bulk microbiota. These findings were consistent with reports in other studies [20,21], indicating that maize root bacteria follow the general rule of microbiota establishment. Alpha and beta diversity analyses revealed that root microbiomes were variable between sampled sites. In addition, the beta community diversity of the LF, HH and JZ fractions was more variable between sampling locations than for the other fractions. The reason for the variation in these microbiomes is unknown, and we speculated that it might be related to uneven humidity. It has been proven that plant microbial diversity increases with high moisture treatment [22].

We found that the microbiota of the six fields clustered grossly by geographic proximity: the three northern soils harbored more similar microbiota compared with the three southern fields. However, the microbiota of the three northern fields were not most similar to each other, as the SL soil microbiota showed greater similarity to the HH microbiota than the HT and LF microbiota. It is noteworthy that SL is the southernmost field in the northern fields, and HH is the northernmost field in the northern field; they are geographically close to each other, suggesting that the regional and climatic differences among soils from different fields are sufficient to explain the biogeographic patterns observed herein. Previous studies concluded that the composition of the soil bacterial community was unrelated to temperature and latitude; in contrast, it was structured according to pH rather than geographical distance [23,24]. In a subsequent study, another analysis pointed out that geographical distance was significantly influenced by the bacterial community structure [23]. In our study, we also demonstrated that geographical distance played a major role in shaping the composition of bacterial communities. On the other hand, the microbial diversity and richness showed no sign of any strong associations with latitude and geographical distance, which agrees with a previous study [25].

Throughout the rhizosphere microbiota, *Proteobacteria* are adapted to the crop rhizosphere generally and across diverse sites. This finding is not uncommon, as *Proteobacteria* are well known to respond to labile carbon sources and are weedy fast-growing microbiota whose populations fluctuate opportunistically [25]. When examining the site variation, we observed that the soil samples showed strong statistically significant taxonomic differences in microbial community composition. The differential abundance analysis in the soil showed that many *Chloroflexi* bacteria were enriched in ZJ. The phylum *Chloroflexi* has been estimated to dominate the microbial community of some seafloor sediments and can make up more than 10% of the community in the B horizon of temperate grasslands and alpine meadows [26,27]. *Chloroflexi* contains several isolated bacteria that have been found to respire a diverse array of halogenated anthropogenic chemicals. The phylum *Gemmatimonadetes*, another frequent and persistent bacteria highly abundant in soils, was significantly enriched in JZ soil. Jennifer et al. [28]reported that *Gemmatimonadetes* seemed to be more abundant in arid soils, suggesting an adaptation to low-moisture environments, which is similar to our result. In contrast, HH was also far from the seaside, but no evidence showed that *Gemmatimonadetes* was enriched in the soil of this place, which suggested that factors other than moisture affect

Gemmatimonadetes bacteria. The phyla Actinobacteria and Bacteroidetes, in particular, have been shown to be enriched in the LF rhizosphere and were the next most significant source of variation in the microbiota composition. The different identities could structure the root-associated communities. In addition, our results support the hypothesis that maize planted in different locations harbors dissimilar rhizosphere microbial communities.

The high nutritive value of maize makes it a perfect substrate for fungal growth and potential mycotoxin contamination. Aflatoxins are a highly carcinogenic and mutagenic group of mycotoxins produced by *Aspergillus* species, mainly *A. flavus* and *A. parasiticus* [29]. In this study, the immunoaffinity column (B_1) was used to purify aflatoxin B_1. This method has been used widely in recent years, as a reference method, due to their accuracy and sensitivity. Limitations of this purifying method are, however, the high cost of immunoaffinity column, and costly and time-consuming protocols. However, we still preferred this because it was typically characterized by high specificity. The bacterial abundance and community composition, to a certain degree, were also affected by the concentration of aflatoxin. RDA and Spearman's rank correlations were used to investigate the relationship between the relative abundance of the microbial genera and the soil characteristics in this study. The soils under the different fertilization regimes showed significantly ($p < 0.05$) different fertility characteristics, which also played an important role in driving the composition and distribution of the microbial communities, since 48 genera showed significantly ($p < 0.05$) positive or negative correlations with one or more of the soil nutrient parameters.

We also explored the role of the maize rhizosphere bacterial community in aflatoxin contamination. The results demonstrated that maize planted in southern China had the highest level of aflatoxin, while northern samples had the lowest level. A previous study indicated that hot climates, prevalent in the tropics, promote the growth of fungi that infect maize in the field [30]. Cities in southern China are very close to the Equator, and the temperature was generally higher at the three southern sites, indicating that temperature was enough to explain the difference in aflatoxin at different sites. Similar to the fact that aflatoxin status can influence the microbiome, the soil microbiome can also significantly influence the concentration of aflatoxin. It is remarkable that bacteria of the genera *Bacillus, Gemmatimonas, Ramlibacter and Haliangium* were most abundant in the southern samples and were strongly associated with high concentrations of aflatoxin. *Mycobacterium, Mucilaginibacter, Bradyrhizobium, Rhizomicrobium, Sphingomonas* and *Burkholderia-Paraburkholderia* were enriched in northern samples, which showed a lower concentration of aflatoxin. All of the above results were in accordance with the Pearson's correlation conclusions. The genera noted above may have a special association with aflatoxin; for instance, Bacillus subtilis strain UTBSP1 isolated from pistachio nuts could degrade AFB_1 with its extracellular constitutively produced enzyme [31]. Perhaps that is why Bacillus are abundant in the low aflatoxin group; they break down aflatoxin. However, *Mycobacterium* was enriched in the low aflatoxin group in our study, which leads to the question of what the real relationship between bacteria and aflatoxin is. All we are certain of is that these genera were closely related to aflatoxin, and further research need to be carried out to determine if this was a cause or a result of the aflatoxin infection. The results above open a new perspective to further explore the connections between the bacterial community and fungal contamination. It is necessary for us to study the mechanism of these interactions in depth.

5. Conclusions

Maize production and quality are influenced by rhizospheric microorganisms, which change significantly in response to different environmental factors. Here, we analyzed the microbiota from 6 major maize-planting areas in China and illustrated the structural characteristics of the microbiota associated with different environmental conditions. Our work shows that the structure and diversity of microbiomes inhabiting the rhizosphere of maize are distinctive under different biogeographies. We also found that microbiota occupying different niches of the maize rhizosphere are closely related to A. flavus abundance and aflatoxin production. Aflatoxins are produced predominantly by *A. flavus* and *A. parasiticus*. These fungi vary in physiological, morphological and genetic traits, while *A. flavus* strains are most common and more frequently isolated from soil in China, and the strains of *A. parasiticus* are vary rare in China [4]. Therefore, our research indicates that microbial-microbial and environment-microbial interactions contribute to the microbiota, structure, and diversity in different niches, which have significant effects on aflatoxin contamination. This knowledge can be translated into a better understanding of disease control and could be used for the production of resilient, healthy maize. However, additional studies are necessary to focus on mechanisms of the interactions including microbial-microbial, environment-microbial and environment-microbial-aflatoxin, which will help to clarify the main reasons for aflatoxin pollution in maize, help to clarify the structure and function of rhizospheric microorganism and its regulatory mechanism on aflatoxin pollution, and enrich its ecological theory. These developed parts will help to provide theoretical basis for the development of effective control technology on aflatoxin pollution.

Author contribution statement

Suyan Gao: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yanpo Yao: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Feng Ju: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zhaolin Du: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Peisheng Yan: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Bei Niu: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was funded by State Key Laboratory of North China Crop Improvement and Regulation (NCCIR2021KF-14).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15949.

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