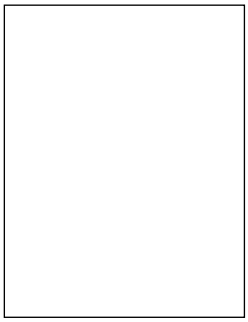

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Soil Characteristics Overwhelm Cultivar Effects on the Structure and Assembly of Root-Associated Microbiomes of Modern Maize

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ABSTRACT

Modern breeding primarily targets the traits of crop yield and is likely to influence the root-associated microbiomes which play significant roles in plant growth and health. The relative importance of soil and cultivar factors in shaping the root-associated microbiomes of modern maize remains uncertain. We conducted a pot experiment in a controlled environment using three soils (Mollisol, Inceptisol and Ultisol) and four contrasting cultivars which are widely planted in China (Denghai 605, Nonghua 816, Qiaoyu 8 and Zhengdan 958). We applied 16S rRNA gene amplicon sequencing to characterize the bacterial communities in the bulk soil, rhizosphere and endosphere. Our results showed that the four cultivars had different shoot biomass and root exudation levels. The microbiomes in the bulk soil, rhizosphere and endosphere were different. We observed the apparent community divergence between soils rather than cultivars, thereinto, edaphic factors substantially contributed to microbiome variation. Moreover, permutational multivariate analysis of variance corroborated significant contributions of soil type but not cultivar on the root-associated microbiome structure. Differential abundance analysis confirmed that each soil presented a distinct root microbiome, while network analysis indicated different co-occurrence patterns of the root microbiome among the three soils. The core root microbiome members are implicated in plant growth promotion and nutrient acquisition in the roots. In conclusion, the root-associated microbiomes of modern maize are much more controlled by soil characteristics than by cultivar root exudation. Our study is anticipated to help contribute to improved breeding strategies through integrative interactions of soils, cultivars and their associated microbiomes.

Key Words: amplicon sequencing, edaphic properties, endosphere, indigenous microbes, rhizosphere, root exudates

INTRODUCTION

Focus at the root-soil interface reveals a highly active and coordinated microbiome which can be pivotal in plant growth promotion, nutrient acquisition, disease suppression, and a range of other functions that benefit agriculture and biotechnology (Rodriguez *et al.*, 2008; Weyens *et al.*, 2009; Berendsen *et al.*, 2012; Bulgarelli *et al.*, 2013; Panke-Buisse *et al.*, 2015; van der Heijden *et al.*, 2016). The zone of soil found between 0 and 2 mm distance from the root surface, referred to as the rhizosphere, is profoundly influenced by plant through the exudation of nutrient sources and phytoalexins. This makes the rhizosphere a 'hotspot' microhabitat where increased microbial abundance, interactions and genetic exchange are found (Bulgarelli *et al.*, 2013; Turner *et al.*, 2013). This results in a differentiation between the rhizosphere microbiome and that of the bulk soil (Broeckling *et al*., 2008; Peiffer *et al.*, 2013; Schreiter *et al.*, 2014; Nuccio *et al.*, 2016). In contrast to the rhizosphere and bulk soil, the endosphere (root interior) features a highly specific microbiome, in which diversity is much lower than that estimated for microbiomes outside the roots (Peiffer *et al.*, 2013; Quiza *et al.*, 2015). Recently, the structure and functions of the endosphere microbiomes of various plants have been reported (Lundberg *et al.*, 2012; Schlaeppi *et al.*, 2014; Edwards *et al.*, 2015; Chen *et al.*, 2016; Wagner *et al.*, 2016), and overall, the endosphere microbiome is preferentially colonized by the phyla *Proteobacteria*, *Actinobacteria, Bacteroidetes* and *Firmicutes* (Schlaeppi *et al.*, 2014; Hardoim *et al.*, 2015; Vandenkoornhuyse *et al.*, 2015).

The structure and assembly of the root-associated microbiomes are affected by soil characteristics (including indigenous soil microbial communities and physico-chemical edaphic parameters), regional climatic characteristics (e.g., moisture and temperature), plant species (or genotype) and anthropogenic activity (Götz *et al.*, 2006; Edwards *et al.*, 2015; Tkacz *et al.*, 2015; Chen *et al.*, 2016; Nuccio *et al.*, 2016). Recent studies have shown that plant roots assemble their associated microbiomes by a two-step selection model: 1) the recruitment step: rhizodeposits (mainly root exudates) attract and facilitate growth of certain microbes, causing a general recruitment into the rhizosphere from the bulk soil biome; 2) the selection step: the microbes thriving in proximity to the roots are selected to permit entry inside the roots (Bulgarelli *et al.*, 2013; Edwards *et al.*, 2015; van der Heijden and Schlaeppi, 2015). The two-step selection model depends on soil type and plant host. Soil type can be the dominant factor structuring rhizosphere bacterial communities (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Peiffer *et al.*, 2013). Yet, in other cases, plant host can be the determinant of rhizosphere bacterial assemblages (Wieland *et al.*, 2001), and plant-specific composition of fungal communities in the rhizosphere can also be detected (Costa *et al.*, 2006).

High-throughput sequencing has been used to provide new insights into the root-associated microbiomes of various crops, such as maize, rice, barley and potato (Peiffer *et al.*, 2013; Marques *et al.*, 2014; Bulgarelli *et al.*, 2015; Edwards *et al.*, 2015). However, our current understanding of the root-associated microbiomes of different cultivars within a single crop species is still limited. Better understanding of the extent of a crop's influence over its associated microbiomes could help improve crop breeding strategies to increase resource use efficiency and crop productivity through interactions of plant host with beneficial microbial consortia. Several studies have suggested that the genetic variation in crop cultivars can influence the root microbiome establishment through microbe-microbe and host-microbe interactions (Bouffaud *et al.*, 2014; Bulgarelli *et al.*, 2015). Peiffer *et al.* (2013) found that the rhizosphere of maize cultivars exhibited a small but significant proportion of variation in the bacterial microbiome structure across fields (each with unique soils and management conditions), and substantially more variation between replicates within the same field. Earlier, Aira *et al.* (2010) reported that maize genotype strongly modified the structure and growth of the rhizosphere

microbiome of maize in an experimental field. However, Johnston-Monje *et al.* (2016) found that the rhizosphere bacterial microbiome of juvenile maize primarily originated from the seed and soil transmitted communities, with genotype only affecting the rhizosphere microbiome of juvenile maize grown in sterile sand. Cotta *et al.* (2014) reported that the abundance but not structure of the rhizosphere microbiome was responsive to the changes in maize genotypes under field conditions. Since the study of field grown crops for their influence on the microbiome is hindered by environmental complexity and heterogeneity, thus far, the relative importance of soil and cultivar factors in shaping maize root-associated microbiomes has not been determined.

In order to deepen our understanding of the root-associated microbiomes of modern maize, we conducted a greenhouse pot experiment involving four representative maize cultivars planted in three different soils under controlled environmental conditions. We used high-resolution amplicon sequencing of the bacterial 16S rRNA gene to unravel the structure, variation, and assembly of rhizosphere and endosphere bacterial microbiomes of maize cultivars among different soils. Since the establishment of the root-associated microbiomes is a result of interactions between soil, plant roots and environmental conditions, we controlled environmental variables (e.g., temperature, light, humidity, irrigation and fertilization) and only took soil and cultivar effects into consideration. Specifically, we examined: (i) what differences in root exudation occurred among cultivars, (ii) how much variation in the root-associated bacterial microbiomes resulted from soil and cultivar factors, (iii) which members comprised the core root microbiome across soils and cultivars, and (iv) whether the root microbial co-occurrence was different in the three soils. We hypothesized that the difference in root exudate profiles among cultivars would not be sufficient to bring about significant changes in the root-associated microbiomes (because modern maize breeding has selected primarily for the traits of crop yield over ecosystem interactions), with the microbiome instead being more strongly determined by soil characteristics.

MATERIALS AND METHODS

Description of soil types and maize cultivars

Three soils typical of latitude zones (subtropical, warm temperature, and cold temperature zones) were collected from three Agro-Ecological Experimental Stations of Chinese Academy of Sciences located at Yingtan (28°15′ N, 116°55′ E), Fengqiu (35°00′ N, 114°24′ E) and Hailun (47°26′ N, 126°38′ E), China. The soil in the Yingtan is an acid loamy clay derived from Quaternary red clay (an Ultisol in the USDA soil taxonomy (USST)). The soil in the Fengqiu is a fluvo-aquic soil which has developed from alluvial sediments of the Yellow River (an Inceptisol in the USST). The soil in the Hailun is a black soil derived from loam loess (a Mollisol in the USST). Cropping systems for each soil were as follows: Ultisol - continuous maize, Inceptisol - maize-wheat rotation, and Mollisol - maize-soybean rotation.

The three soils were collected from top 20 cm soil layer in July 2015. Visible rocks and plant residues were removed, and the soils were air-dried and sieved < 2 mm. Soil pH, soil organic C (SOC), total N (TN), total P (TP), available P (AP), available K (AK) and cation exchange capacity (CEC) were determined by methods of Lu (2000) (Table I). We selected four modern maize cultivars with large genetic differences (Denghai 605, Nonghua 816, Qiaoyu 8 and Zhengdan 958). The four cultivars are widely planted across China owing to their high yields across the range of latitudes represented (Fig. 1). Denghai 605 was designated as ‘DH’, Nonghua 816 as ‘NH’, Qiaoyu 8 as ‘QY’, and Zhengdan 958 as ‘ZD’.

TABLE I
The general soil physicochemical properties

	pH (H_2O)	C/N	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	CEC (cmol kg^{-1})
Mollisol	6.12	14.68	26.86	1.83	1.12	28.75	171.90	7.88
Inceptisol	8.31	14.50	10.44	0.72	1.19	14.05	154.71	7.84
Ultisol	5.53	25.66	10.52	0.41	0.79	9.85	326.61	8.31

Fig. 1 Main planting areas of maize cultivars Denghai 605, Nonghua 816, Qiaoyu 8 and Zhengdan 958 in China.

Greenhouse pot experiment

10 kg soil (oven-dry basis) was uniformly given 3.6 g urea, 1.7 g superphosphate and 1.1 g potassium sulfate (equivalent to 1 mg each N, P and K for 6 g soil), and then added to the pot with a size of 25 cm in diameter and 26 cm in height. Soils were watered at the weight ratio of 140 mg H_2O g^{-1}.

Maize seeds were surface sterilized by washing in 30% H_2O_2 for 30 min, and rinsing several times with sterile deionized water. Seeds were transferred to petri plates containing nutrient agar, and germinated under sterile conditions at 25 °C for 4 days. Only those seedlings that showed no microbial colonization were used. Similar sized maize seedlings (approximately 10 cm) were transplanted to the pots (two seedlings per pot). Plants were grown in a greenhouse with a 14 h light (day at 28 °C) and 10 h dark (night at 18 °C) at 70% relative humidity. During plant growth, all plants were equally watered using sterile deionized water when needed. All weeds were manually removed as they occurred. Twelve treatments (3 soils × 4 cultivars) were established with three replicates of each.

Bulk soil, rhizosphere and endosphere sampling

At the late jointing stage, the plants along with soil were removed from each pot. Plants were gently shaken by hand, and the surrounding detached soil was collected as the bulk soil. The roots with firmly adhering soil (approximately 0–2 mm from root surface) were placed in a 50 mL Falcon tube, capped and stored on liquid nitrogen, and taken to the lab. 30 mL of phosphate buffered saline (PBS) was added to the tube, stirred vigorously for 30 s (vortex intensity 6.0), and the roots were removed. Soil suspension was centrifuged for 2 min at 10,000 *g*. The supernatant was discarded, and the soil was freeze-dried and stored as the rhizosphere sample at –80 °C. The roots were transferred to a new Falcon tube with 30 mL PBS, and sonicated for 30 s at 60 Hz (output frequency 42 kHz, 90 W). The microbes that inhabit the root surface were thus separated from the roots. Two more sonication procedures using clean PBS were performed to clean root surfaces. The PBS was discarded and sonicated roots were stored at –80 °C until DNA extraction of endophytes. All materials used (e.g. tubes, forceps, bibulous papers, and PBS) had been autoclave-sterilized before use.

Root exudate collection and analysis

To investigate the profiles of root exudates from the maize cultivars, we first collected root exudates from the harvested plants in the Inceptisol (in which all four cultivars are widely cultivated). The plant roots were

cleaned by a minimum of 5 rinses with sterile deionized water till the root surface showed no soil residues. Each plant with clean roots was placed in a high glass beaker with 100 mL of sterile 0.5% $CaCl_2$ solution (preventing root cell disruption), and all roots were submerged in the solution. The beakers were wrapped with black plastic film to exclude light. Plants were grown in a growth chamber with a 14 h/28 °C light (light intensity 3500 lux) and 10 h/18 °C dark at 70% relative humidity for one day. The solution containing root exudates was filtered through a 0.45 μm filter membrane. 50 mL solution was concentrated to a volume of 2 mL and frozen at –20 °C until analysis. Plant shoots and roots were divided, oven-dried at 60 °C and weighed.

Low molecular weight organic acids in root exudates were analyzed using an Ultra Fast Liquid Chromatograph (Shimadzu, Japan) fitted with an Agilent XDB-C18 column. The mobile phase consisted of 98% phosphate buffer and 2% pure methanol with isocratic elution of 0.8 mL min^{-1} for 30 min. Organic acid standards (malic acid, oxalic acid, citric acid and formic acid) and root exudates were injected (20 μL) into the chromatographic system sequentially and consistently. Organic acids in root exudates were identified and quantified by elution time and peak areas relative to standards. In addition, total organic C (TOC) content in root exudates was determined using a Multi C/N 3100 TOC analyzer (Analytik Jena AG, Jena, Germany).

DNA extraction from roots and soil

The root tissues were pre-homogenized before the DNA extraction by bead beating for 1 min (Mini Beadbeater, BioSpec, USA) (Edwards *et al.*, 2015), and then the endosphere DNA was extracted using a FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol. The extracted DNA was dissolved in 30 μL of TE buffer, and purified using an UltraClean DNA Purification Kit (MoBio, Carlsbad, CA, USA). Microbial DNA in the bulk soil and rhizosphere soil were also extracted using the FastDNA Spin Kit (MP Biomedicals). The DNA was dissolved in 50 μL of TE buffer. DNA quality and concentrations were assessed based on absorbance at 230, 260 and 280 nm (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, DE, USA). The 108 DNA samples were stored at –80 °C for amplicon library preparation.

16S rRNA gene V4 amplification and MiSeq sequencing

The 16S rRNA gene V4 amplification was carried out using primers designed to amplify fragments belonging to the variable region from 515 to 806 of the 16S rRNA gene (Caporaso *et al.*, 2011). For parallel sequencing of a sample set, the forward primer was extended at the 5′ end with a sample-specific barcode sequence. PCR components in final concentrations included 1 U TransStart FastPfu DNA polymerase (TransGen, Beijing, China), 1× FastPfu buffer, 0.3% BSA, 2 mM of $MgCl_2$, 250 μM of dNTPs and 200 nM of each primer. Twenty-seven thermal cycles (30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C) were conducted with a final extension at 72 °C for 10 min. The quality of reaction products was verified in a 1% agarose gel. The reaction products were cleaned using a QIAquick PCR Purification Kit (Qiagen, Shenzhen, China), and quantified using a NanoDrop spectrophotometer (NanoDrop Technologies). The amplicons were pooled in equimolar concentrations and loaded on a MiSeq Reagent Kit V2, and dual index sequencing of paired-end 250 bp was run on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA). The sequence data have been submitted to NCBI Sequence Read Archive under BioProject PRJNA358644 and BioSample accessions SAMN06176978–SAMN06177085.

Community bioinformatics and statistics

The raw sequence data were processed using the UPARSE pipeline (Edgar, 2013), based on the following workflow: i) quality filtering sequences using a "maxee" value of 1 and trimmed to a consistent length; ii) dereplicating identical sequences and removing singleton reads; iii) building a de novo dataset of > 97% similar sequence clusters and simultaneously removing chimeras on this non-redundant dataset, using self dataset and RDP Gold sequence as references (Cole *et al.*, 2014); iv) generating an OTU abundance table by mapping the total reads to representative sequences. Taxonomic annotation was assigned to each OTU representative sequence by UCLUST (Edgar, 2010) in QIIME (version 1.9.0) (Caporaso *et al.*, 2010) against the Greengenes 13_8 database. All sequences unsigned and signed to archaea and chloroplast were removed in downstream analyses. To avoid potential bias caused by sequencing depth, all sequence data were rarefied to 13,148 sequences per sample for the bacterial diversity analyses. Taxonomy assignment, rarefaction and alpha diversity calculations were conducted in QIIME.

The effects of soil type, compartment, cultivar, and their interactions on alpha diversity were analyzed using multi-way ANOVA. A cluster dendrogram based on the Bray-Curtis dissimilarity was generated using the package Vegan (Dixon, 2003) in R (R Development Core Team, 2010). Principal coordinate analyses of the unweighted and weighted UniFrac distances (Lozupone and Knight, 2005) were calculated to determine the separation of beta diversity using the R package Ape (Paradis *et al.*, 2004). We performed a canonical analysis of principal coordinates (CAP) constrained by edaphic factors pH, C/N, SOC, TN, TP, AP, AK and CEC. Effect significance of these factors was calculated by running the Vegan's permutest function over the CAP model using a maximum of 500 permutations. Permutational multivariate analysis of variance (PERMANOVA) was performed to measure effect size and significance of soil, compartment, cultivar, and their interactions on beta diversity. Differences in plant biomass, root exudates, and relative abundances of major phyla/classes were detected by one-way ANOVA based on Tukey's HSD test.

Differential abundances of OTUs were tested using the R package DESeq2 (Love *et al.*, 2014). We filtered out those OTUs for which the normalized counts across samples were 0. We adjusted *P*-values for multiple testing using the procedure of Benjamini and Hochberg (Benjamini and Hochberg, 1995), selecting a false discovery rate (FDR) of 10% to denote statistical significance (Love *et al.*, 2014). Enriched and depleted OTUs were defined as OTUs with differential abundance greater than 1.0 and adjusted *P*-value of < 0.1.

Network analysis

The most abundant OTUs with relative abundances of ≥ 0.1% in the endosphere were used for network analysis. Pairwise correlation comparisons were conducted based on the maximal information coefficient (MIC) in MINE software (Reshef *et al.*, 2011). The MIC is a highly useful score that reveals the strength of linear and non-linear associations among variables (Reshef *et al.*, 2011). The top 1000 interactions were selected according to their MIC strength. Relationships between OTUs were significant at a FDR of 10%. To reduce network complexity, the resulting OTUs with significantly strong ($r > 0.6$) positive linear relationship, strong ($r < –0.6$) negative linear relationship and strong (MIC-$\rho^2 > 0.6$) nonlinear relationships (Banerjee *et al.*, 2016) were used for network construction. Network topological features were calculated using NetworkAnalyzer tool in Cytoscape (version 3.2.1) (Shannon *et al.*, 2003). Modular structure and clusters of highly interconnected nodes were analyzed using the MCODE application with default parameters (Banerjee *et al.*, 2016). OTUs with maximum betweenness centrality scores were considered to be keystone species (Vick-Majors *et al.*, 2014; Banerjee *et al.*, 2016).

RESULTS

Plant biomass and root exudation among cultivars

The cultivars Denghai 605 (DH) and Qiaoyu 8 (QY) had significantly larger shoot biomass than the cultivars Nonghua 816 (NH) and Zhengdan 958 (ZD), and the four cultivars had no significant differences in root biomass (Fig. 2a). The quantities of root exudates indicated by TOC, oxalic acid, formic acid and citric acid concentrations were normalized to root weight. There was a significantly higher content of TOC in root exudates from the cultivar ZD compared to the three other cultivars, significantly higher content of oxalic acid from the cultivars DH and ZD compared to the cultivars NH and QY, and significantly different content of citric acid among all the four cultivars (Fig. 2b).

Fig. 2 Plant biomass (a) and root exudation patterns (b) of maize cultivars Denghai 605 (DH), Nonghua 816 (NH), Qiaoyu 8 (QY) and Zhengdan 958 (ZD). The concentration of total organic C (TOC) was expressed as mg C g^{-1} root, and organic acids as mg acid g^{-1} root. Different letters indicate significant differences at the 0.05 level.

The root-associated bacterial microbiome composition

A total of 3,425,939 high-quality bacterial sequences clustered into 8,631 OTUs across all 108 samples. After normalized rarefaction, 8,626 OTUs (1,696 ± 674 OTUs) were generated (Supplementary Table I). The main phyla in the rhizosphere were *Proteobacteria* (*Alpha-*, *Beta- and Gammaproteobacteria*), *Acidobacteria*, *Actinobacteria* and *Chloroflexi*. Compared to the Ultisol, the Mollisol and Inceptisol rhizospheres showed significantly increased abundance of *Alphaproteobacteria* and decreased *Gammaproteobacteria* (Supplementary Fig. 1a). In all soils, the endosphere community was dominated by *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria*. In the Mollisol endosphere, the cultivar DH showed significantly more abundant *Gammaproteobacteria* than three other cultivars. *Actinobacteria* was significantly more abundant in the Ultisol endosphere than in the Mollisol and Inceptisol endospheres (Supplementary Fig. 1b). *Betaproteobacteria* was significantly more abundant in the Mollisol and Ultisol than in the Inceptisol (Supplementary Fig. 1c). A heat map was constructed using the genera with relative abundances of ≥ 0.1% in at least one group. The endosphere samples in the Mollisol and Inceptisol diverged much from those in the Ultisol soil (Supplementary Fig. 2). The genera *Streptomyces* and *Massilia* dominated the endosphere community in the Mollisol, *Pseudomonas* and *Cellvibrio* in the Inceptisol, and *Streptomyces* and *Rhodanobacter* in the Ultisol (Supplementary Fig. 2).

Root-associated microbiome structure and variation

Phylotype richness, phylogenetic and Shannon diversity from a subset of 13,148 sequences were used to estimate bacterial alpha-diversity. Regardless of soil type and maize cultivar, alpha-diversity in the rhizosphere and bulk soil was considerably higher than that in the endosphere (Supplementary Table II). Multi-way ANOVA showed that soil, compartment, and their interaction all affected alpha-diversity (except for Shannon), whereas cultivar had no significant effect on alpha-diversity (Supplementary Table II).

The hierarchical clustering of Bray-Curtis dissimilarities revealed that the bacterial communities in the

rhizosphere and bulk soil were distinct from the endosphere, in which the bacterial community was also different between soils, observed from the second and third hierarchical clusters (Supplementary Fig. 3). No apparent divergences between cultivars were observed in any soil (Supplementary Fig. 3). Principal coordinate analyses of unweighted UniFrac (UUF) and weighted UniFrac (WUF) distances were used to investigate separation patterns of community structure (beta-diversity). The WUF metric takes abundance of taxa into consideration (whereas the UUF does not) and is thus more sensitive to rare taxa. The community separation between soils was observed along the first coordinate of UUF (Fig. 3a), and the second coordinate of WUF (Fig. 3b). Similarly, the bacterial community in the endosphere was separated from the rhizosphere and bulk soil along the second coordinate of UUF (Fig. 3c), and the first coordinate of WUF (Fig. 3d). No clear separation of bacterial community between cultivars occurred in each soil (Fig. 3e, f). We quantified the contribution of edaphic factors (i.e. pH, C/N, SOC, TN, TP, AP, AK and CEC) on bacterial community variation by using canonical analysis of principal coordinates (CAP). These factors contributed significantly to bacterial community variation (35.69% of variation, $P = 0.002$, UUF; 16.35% of variation, $P = 0.002$, WUF) (Supplementary Fig. 4). Permutational multivariate analysis of variance (PERMANOVA) corroborated the significant ($P = 0.001$) effects of soil type on the root-associated bacterial microbiome structure. Soil type largely explained the variation in the rhizosphere microbiome (62.72% explained, UUF; 75.98% explained, WUF) and endosphere microbiome (41.09% explained, UUF; 37.94% explained, WUF), whereas maize cultivar had little influence on the root-associated microbiome variation (Table II).

Fig. 3 Principal coordinate analysis plots of the OTU-based unweighted (a, c and e) and weighted (b, d and f) UniFrac distances showing the variation in the bacterial communities between samples. Panels a and b indicate variation source from soils (Mollisol, Inceptisol and Ultisol), c and d from compartments (bulk soil, rhizosphere and endosphere), and e and f from cultivars (Denghai 605, Nonghua 816, Qiaoyu 8 and Zhengdan 958).

TABLE II

Permutational MANOVA based on unweighted and weighted UniFrac distance metrics revealing the relative contributions of soil type, compartment and cultivar on bacterial microbiome variations. The significance was examined by F-test based on sequential sum of square from 999 permutations of the OTU data

		Unweighted UniFrac				Weighted UniFrac		
		Sum Sq.	% explained	Sig.		Sum Sq.	% explained	Sig.
Whole data	Soil	8.24	35.69	0.001	Soil	6.26	17.42	0.001
	Compartment	3.25	14.09	0.001	Compartment	18.99	52.89	0.001
	Cultivar	0.31	1.35	0.269	Cultivar	0.45	1.25	0.041
	Soil × Compartment	2.40	10.41	0.001	Soil × Compartment	2.76	7.68	0.001
	Soil × Cultivar	0.56	2.42	0.437	Soil × Cultivar	0.55	1.53	0.228
	Compartment × Cultivar	0.60	2.60	0.303	Compartment × Cultivar	0.42	1.16	0.534
	Soil × Compartment × Cultivar	1.09	4.73	0.479	Soil × Compartment × Cultivar	1.09	3.02	0.197
	Residuals	6.63	28.71		Residuals	5.40	15.05	
	Total	23.08			Total	35.91		
Rhizosphere	Soil	3.94	62.72	0.001	Soil	3.70	75.98	0.001
	Cultivar	0.21	3.33	0.466	Cultivar	0.19	3.87	0.066
	Soil × Cultivar	0.42	6.68	0.486	Soil × Cultivar	0.17	3.59	0.595

	Residuals	1.71	27.28		Residuals	0.81	16.56	
	Total	6.28			Total	4.87		
Endosphere	Soil	3.07	41.09	0.001	Soil	2.02	37.94	0.001
	Cultivar	0.45	6.01	0.271	Cultivar	0.32	6.06	0.351
	Soil × Cultivar	0.79	10.62	0.467	Soil × Cultivar	0.69	12.99	0.262
	Residuals	3.16	42.28		Residuals	2.29	43.01	
	Total	7.48			Total	5.33		
Bulk soil	Soil	3.63	59.77	0.001	Soil	3.29	49.01	0.001
	Cultivar	0.25	4.18	0.283	Cultivar	0.35	5.25	0.245
	Soil × Cultivar	0.44	7.19	0.452	Soil × Cultivar	0.77	11.41	0.118
	Residuals	1.75	28.85		Residuals	2.31	34.33	
	Total	6.07			Total	6.71		

Enriched and depleted OTUs in the rhizocompartments

To identify OTUs that are correlated with community separation between compartments, we conducted differential abundance analysis by fitting a generalized linear model with a negative binomial distribution to normalized values for each of the 8,626 OTUs and testing for differential abundance using a Wald test. Using OTU counts from corresponding bulk soil as a control and adjusted *P* values of < 0.1, there were distinctly different numbers of OTUs that were significantly enriched and depleted in the rhizocompartments between soils (Fig. 4a–f). The endosphere was enriched in fewer OTUs and depleted in more than 2,400 OTUs compared to the rhizosphere (Fig. 4a–f). There were 61, 91 and 26 OTUs that were exclusively enriched in the endospheres of the Mollisol, Inceptisol and Ultisol, respectively (Fig. 4g–i). Among these root unique OTUs, the dominant OTUs belonged to *Alphaproteobacteria*, *Bacteroidetes* and *Actinobacteria* for the Mollisol, Inceptisol and Ultisol, respectively (Fig. 4j–l). We identified the core root microbiome that was consistently detected across soils and cultivars. The OTUs that showed differential abundance and were present in at least 50% of the endosphere communities of four cultivars in each soil were selected. 19 OTUs were shared by the endosphere microbiomes in the three soils, and therefore comprised the core root microbiome (Fig. 5a). The core root microbiome had 7 *Actinobacteria* members (3 within *Micrococcales*, 2 within *Propionibacteriales*), 6 *Alphaproteobacteria* members (4 within *Rhizobiales*, 2 within *Rickettsiales*), 3 *Betaproteobacteria* members (2 within *Burkholderiales*), and 3 members (within *Xanthomonadales*, *Sphingobacteriales* and *Planctomycetales*) of *Gammaproteobacteria*, *Bacteroidetes* and *Planctomycetes* (Fig. 5b).

Fig. 4 Rhizocompartments are enriched and depleted for certain bacterial OTUs. OTUs enrichment and depletion were observed for the rhizosphere compared with corresponding bulk soil in the Mollisol (a), Inceptisol (b) and Ultisol (c), and for the endosphere in the Mollisol (d), Inceptisol (e) and Ultisol (f). Venn diagrams showed the number of differentially enriched OTUs between rhizosphere and endosphere in the Mollisol (g), Inceptisol (h) and Ultisol (i). Pie charts illustrated the proportion and number of unique OTUs enriched in the endosphere in the Mollisol (j), Inceptisol (k) and Ultisol (l).

Fig. 5 Venn diagram showing the core root microbiome of maize. The OTUs that showed differential abundance and were present in at least 50% of the endosphere communities of maize cultivars Denghai 605,

Nonghua 816, Qiaoyu 8 and Zhengdan 958 in each soil were selected. (a) the number of unique OTUs and OTUs shared between root microbiome in the Mollisol, Inceptisol and Ultisol, (b) summarized taxonomic composition of the core root microbiome.

Co-occurrence and modularity within the root microbiome

Network co-occurrence and modularity of the most abundant OTUs in the endosphere varied by soil type (Fig. 6). The networks for the three soils comprised different number of OTUs (nodes) and significant associations (edges). Structural attributes of the overall network such as clustering coefficient, network centralization and mean shortest paths were different among the three soils (Supplementary Table III). The edges for the three soils were predominantly composed of significant positive associations and nonlinear associations (Fig. 6). MCODE analysis revealed different network modular structure among the three soils. The subnetworks showed 3 rank clusters with network scores of 7.14 and 3.00 for the Mollisol (Fig. 6a), 5 rank clusters with network scores ranging from 6.00 to 3.00 for the Inceptisol (Fig. 6b), and 4 rank clusters with network scores ranging from 3.60 to 2.50 for the Ultisol (Fig. 6c). Betweenness centrality score discerns the nodes that are most important in maintaining connectivity in an ecological network, and is used for identification of keystone taxa. The keystone taxa identified were *Chitinophaga*, *Devosia* and one member within *Xanthomonadaceae* in the roots in the Mollisol, two members within *Methylophilaceae* and *Sphingomonadaceae* in the Inceptisol, and *Burkholderia* and one member within *Alcaligenaceae* in the Ultisol (Supplementary Table III).

Fig. 6 Network analysis exhibiting co-occurrence patterns and modular clusters of the most abundant OTUs in the endosphere in the Mollisol (a), Inceptisol (b) and Ultisol (c). Gray line, black line and blue line represent significantly strong ($r > 0.6$) positive linear relationship, strong ($r < –0.6$) negative linear relationship and strong (MIC-$\rho^2 > 0.6$) nonlinear relationships, respectively. Colored nodes signify corresponding OTUs assigned to major phyla and classes of *Proteobacteria*. The widths of edges are proportional to correlation values.

DISCUSSION

The main purpose of this study was to disentangle the relative importance of soil and cultivar factors in shaping the root-associated microbiomes of modern maize under controlled environmental conditions. By deep 16S rRNA gene amplicon sequencing, we found that soil type strongly affected the structure of the rhizosphere and endosphere bacterial microbiomes while maize cultivar had little influence on these microbiome structures. Each soil presented a distinct root microbiome, and co-occurrence patterns of the root microbiome were different among soils. Taken together, these findings suggest that soil characteristics overwhelm cultivar effects on the structure and assembly of root-associated microbiomes of modern maize.

We found that soil characteristics affected the rhizosphere microbiome establishment more than cultivar. This includes compound effects from indigenous microbial communities and soil properties. Bakker *et al.* (2015) distinguished the effects of the resident soil microbial communities from soil properties. They used chemical amendments to alter the microbial communities in given soil types, and found that the amendment-altered microbial communities can substantially influence selection of the rhizosphere microbiome by maize plants. The present study confirms the soil-dependent establishment of the rhizosphere microbiome of maize in natural (non-amended) soils. We constrained the effects of the measured soil

properties including pH, C/N, SOC, TN, TP, AP, AK and CEC on the bacterial communities in the bulk soil, rhizosphere and endosphere, and found that these properties substantially (P = 0.002) contributed to the variation in these community structures. Castellanos *et al.* (2009) found that the maize rhizosphere microbiome structure and diversity are also driven by soil salinity, calcium and SOC content. In other studies, site- and management-specific soil properties were implied to shape the structure of the rhizosphere bacterial microbiome of maize (Aira *et al.*, 2010; Peiffer *et al.*, 2013; Li *et al.*, 2014). The present study confirms this and also corroborates studies on other crop-species showing the deterministic roles of soil characteristics in structuring the rhizosphere microbiome (Schreiter *et al.*, 2014; Edwards *et al.*, 2015; Chen *et al.*, 2016). Previous investigations of the root microbiome of *Arabidopsis thaliana* grown under controlled environments revealed soil type as major source of variation in root microbiome membership and provided evidence for only limited genotype-dependent variation (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Schlaeppi *et al.*, 2014). Recent deep profiling of plant microbiomes of a wild perennial mustard also confirmed that host genetic control of the microbiome was not evident in the roots and varied substantially among site-specific soils (Wagner *et al.*, 2016). In the present study, the same pattern was observed.

We did not find significant effects of maize cultivar or its interaction with soil type on the rhizosphere microbiome structure, despite different shoot biomass and contents of TOC, oxalic acid and citric acid in root exudates found among the cultivars studied. While shifts in the soil bacterial community structure have indeed been linked with various inputs of low molecular weight root exudates to soils (Eilers *et al.*, 2010), the structure of microbial communities was found to be more determined by the composition and/or quality rather than the quantity of root exudates (Aira *et al.*, 2010). Aira *et al.* (2010) found differences in the maize rhizosphere microbial communities between two cultivars, which had different capacity to store sugars and starch and accordingly resulted in different composition of root exudates. In previous work, we also found that the root-associated microbiomes of perennial ryegrass were little affected by quantitative changes in root exudates caused by elevated atmospheric CO_2 (Chen *et al.*, 2016). Modern maize breeding, primarily targeting the traits of crop yield through successive parent hybridization, has presumably led to some convergence of the composition and/or quality of root exudates among cultivars. The present study detected similar types of root exudate compounds among cultivars (Supplementary Fig. 5). Similar types of root exudate compounds among cultivars could partly explain little impact of maize cultivar on the rhizosphere microbiome structure, because the types of C compounds added to soil have been found to be the main factor determining the shifts in the bacterial community structure (Eilers *et al.*, 2010; Shi *et al.*, 2011; Pascault *et al.*, 2013). It is likely that different root exudation levels among cultivars tested here would have some impact on the rhizosphere microbiome structure, but that their effects are overshadowed by the overwhelming effects from three distinct soils.

In addition to root exudates, some other plant traits such as differences in developmental timing (Chaparro *et al.*, 2014; Li *et al.*, 2014), root morphology (Szoboszlay *et al.*, 2015), plant defense signaling (Doornbos *et al.*, 2012) and seed microbial communities (Johnston-Monje *et al.*, 2016) also influence the maize root-associated microbiome assemblage. We harvested maize plants at the late jointing stage when plants still have a great ability of root exudation. The abundance of certain bacterial groups in the rhizosphere responds to changed root exudation at distinct plant developmental stages (Li *et al.*, 2014), and the rhizosphere microbiome structure often changes dramatically in the early stage of plant growth but later remains more stable (Chiarini *et al.*, 1998; Chaparro *et al.*, 2014). The endosphere microbiome structure approaches steady state within two weeks (Edwards *et al.*, 2015). Most probably, the seed-borne microbes did not have any influence, since the seeds were surface sterilized and only those seedlings that showed no microbial colonization were used in the experiment.

We identified the core root microbiome across soils and cultivars. This data can be used to strengthen our understanding of the maize root microbiome functions. The core microbiome was comprised of 19 bacterial members within the orders *Micrococcales* (mainly *Arthrobacter*), *Rhizobiales*, *Burkholderiales*, *Xanthomonadales*, *Sphingobacteriales* and *Rickettsiales*. Also, *Devosia*, *Burkholderia* and an unclassified *Xanthomonadaceae* within *Rhizobiales*, *Burkholderiales* and *Xanthomonadales* were identified as the keystone taxa in the root microbiomes. *Arthrobacter* within *Micrococcales* can produce unique siderophore to facilitate solubilization and uptake of diverse elements (Brantley *et al.*, 2001) and increase maize productivity by promoting chlorophyll synthesis (Sharma *et al.*, 2016). *Arthrobacter*'s metabolic versatility can not only alleviate nutrient stress but also combat toxic pollutants (Mongodin *et al.*, 2006). A recent study of rhizobia and arbuscular mycorrhizal fungi revealed that they complemented each other to promote plant N and P acquisition and seedling establishment (van der Heijden *et al.*, 2016). *Burkholderiales* is capable of promoting plant growth through degradation of pollutants (Siciliano *et al.*, 2001), pathogen suppression (Santos *et al.*, 2004; Rosenblueth and Martinez-Romero, 2006), fixing N (Estrada-De los Santos *et al.*, 2001; Perin *et al.*, 2006; Caballero-Mellado *et al.*, 2007), lowering plant ethylene levels (Onofre-Lemus *et al.*, 2009) and synthesizing phytohormones (Suarez-Moreno *et al.*, 2012). *Xanthomonadales* members are known hydrocarbon decomposers, but have also been shown to obtain C from other microorganisms co-occurring in the same space (Lueders *et al.*, 2006). *Sphingobacteriales* members have ability to attach and compete for nutrients and space on the roots (Haichar *et al.*, 2008). Some other bacteria (e.g., the *Rickettsiales* members) are obligate intracellular bacteria. They colonize root tissues together with their hosts (a range of root herbivores) with whom they have either parasitic or symbiotic relationships. Importantly, the members of *Rickettsiales* investigated were not found to suppress maize plant defenses against these root herbivores (Robert *et al.*, 2013). Collectively, within the core root microbiome, most of the bacterial members identified are implicated in plant growth promotion and preferential colonization in the roots for nutrient acquisition.

CONCLUSION

Soil type strongly affected the maize root-associated microbiome structure, whereas maize cultivar had no effect despite different shoot biomass and content of root exudates among maize cultivars Denghai 605, Nonghua 816, Qiaoyu 8 and Zhengdan 958. The root unique OTUs in the Mollisol, Inceptisol and Ultisol mainly belonged to *Alphaproteobacteria*, *Bacteroidetes* and *Actinobacteria*, respectively. The three soils presented different co-occurrence patterns of the root bacterial microbiomes. Thus, our study suggests that the root-associated microbiomes of modern maize are much more controlled by soil characteristics than by cultivar root exudation.

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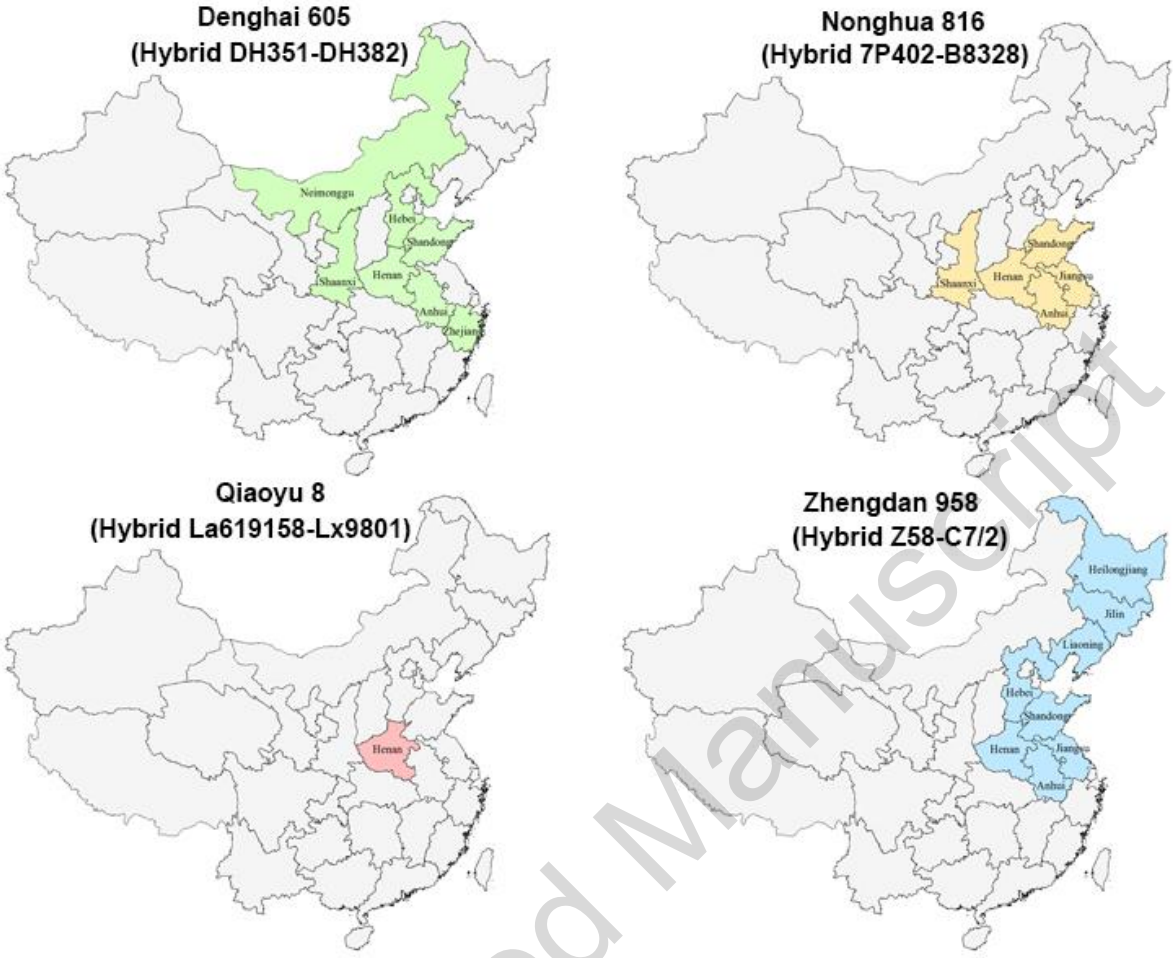


Fig. 1

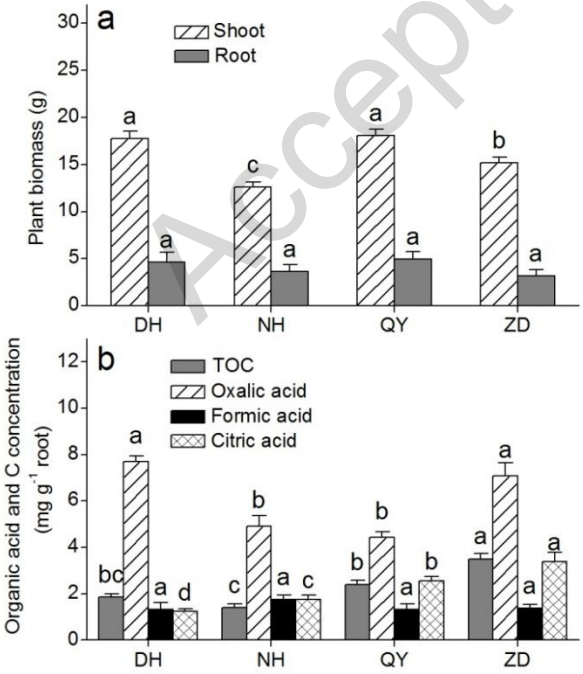


Fig. 2

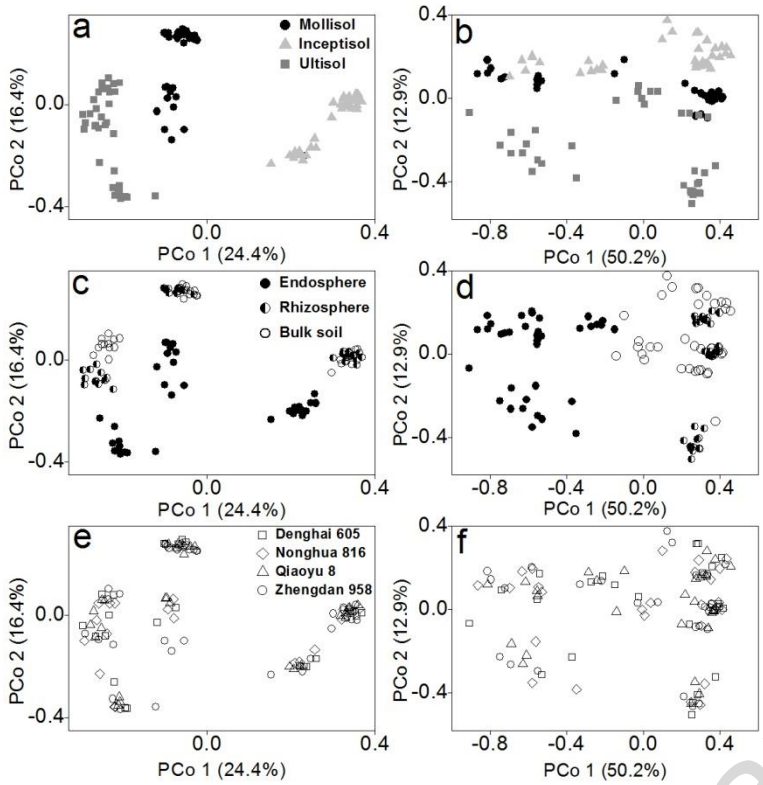


Fig. 3

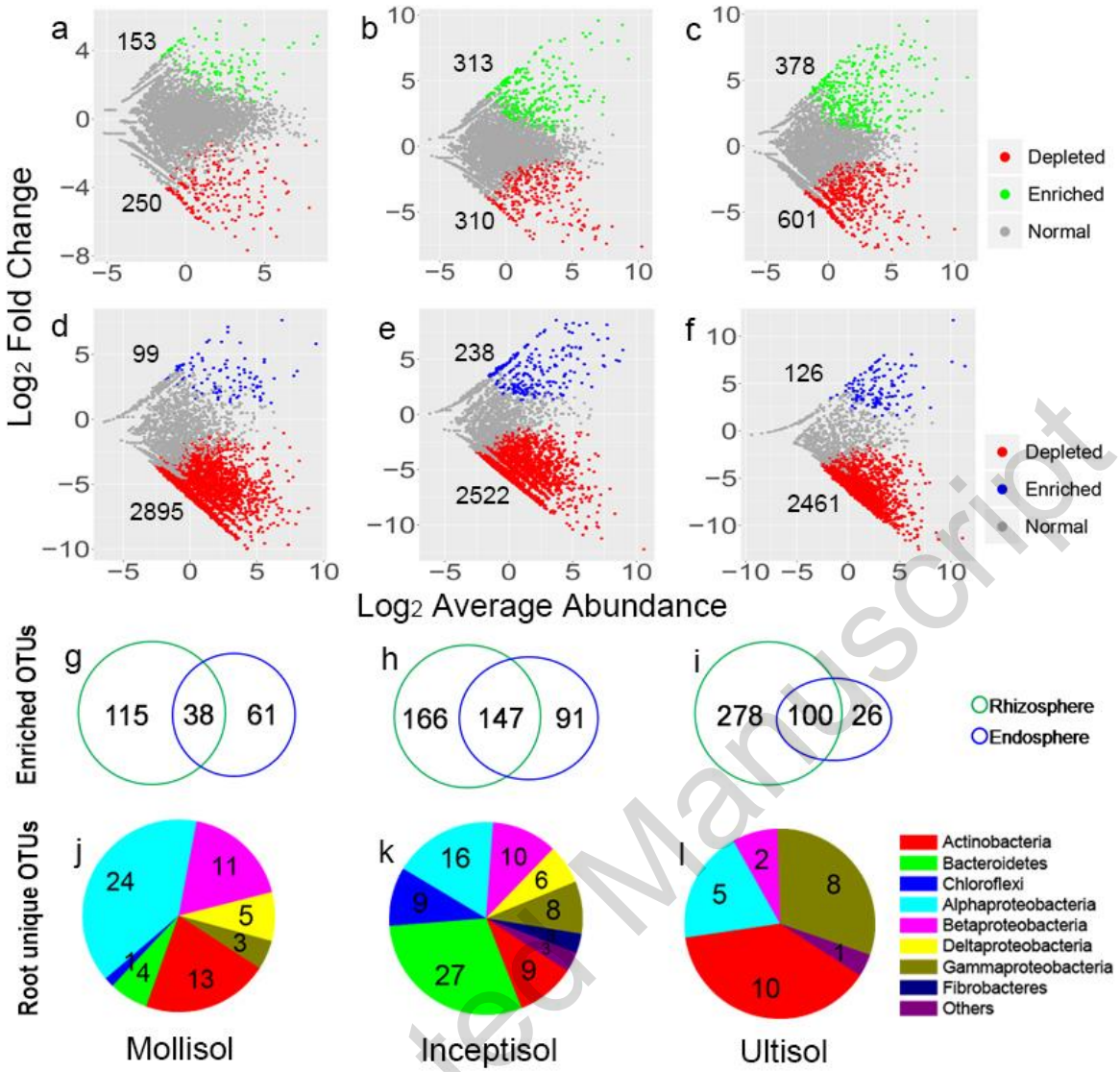
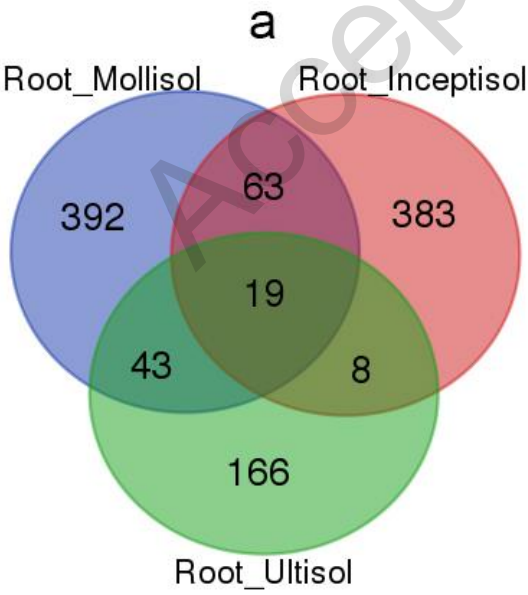


Fig. 4



b

Core root microbiome		
	Order	Genus
Actinobacteria (7 OTUs)	*Corynebacteriales*	*Mycobacterium*
	Micrococcales	*Tetrasphaera*
	Micrococcales	*Arthrobacter*
	Micrococcales	*Arthrobacter*
	Propionibacteriales	*Nocardioidaceae* member
	Propionibacteriales	*Marmoricola*
	Streptosporangiales	*Nonomuraea*
Alphaproteobacteria (6 OTUs)	*Rhizobiales*	*Bradyrhizobiaceae* member
	Rhizobiales	*Devosia*
	Rhizobiales	*Mesorhizobium*
	Rhizobiales	*Rhizobium*
	Rickettsiales	*Rickettsiales* member
	Rickettsiales	*Rickettsiales* member
Betaproteobacteria (3 OTUs)	*Burkholderiales*	*Comamonadaceae* member
	Burkholderiales	*Comamonadaceae* member
	SC-I-84	SC-I-84 member
Gammaproteobacteria	*Xanthomonadales*	*Thermomonas*
Bacteroidetes	*Sphingobacteriales*	*Flavisolibacter*
Planctomycetes	*Planctomycetales*	*Pirellula*

Fig. 5

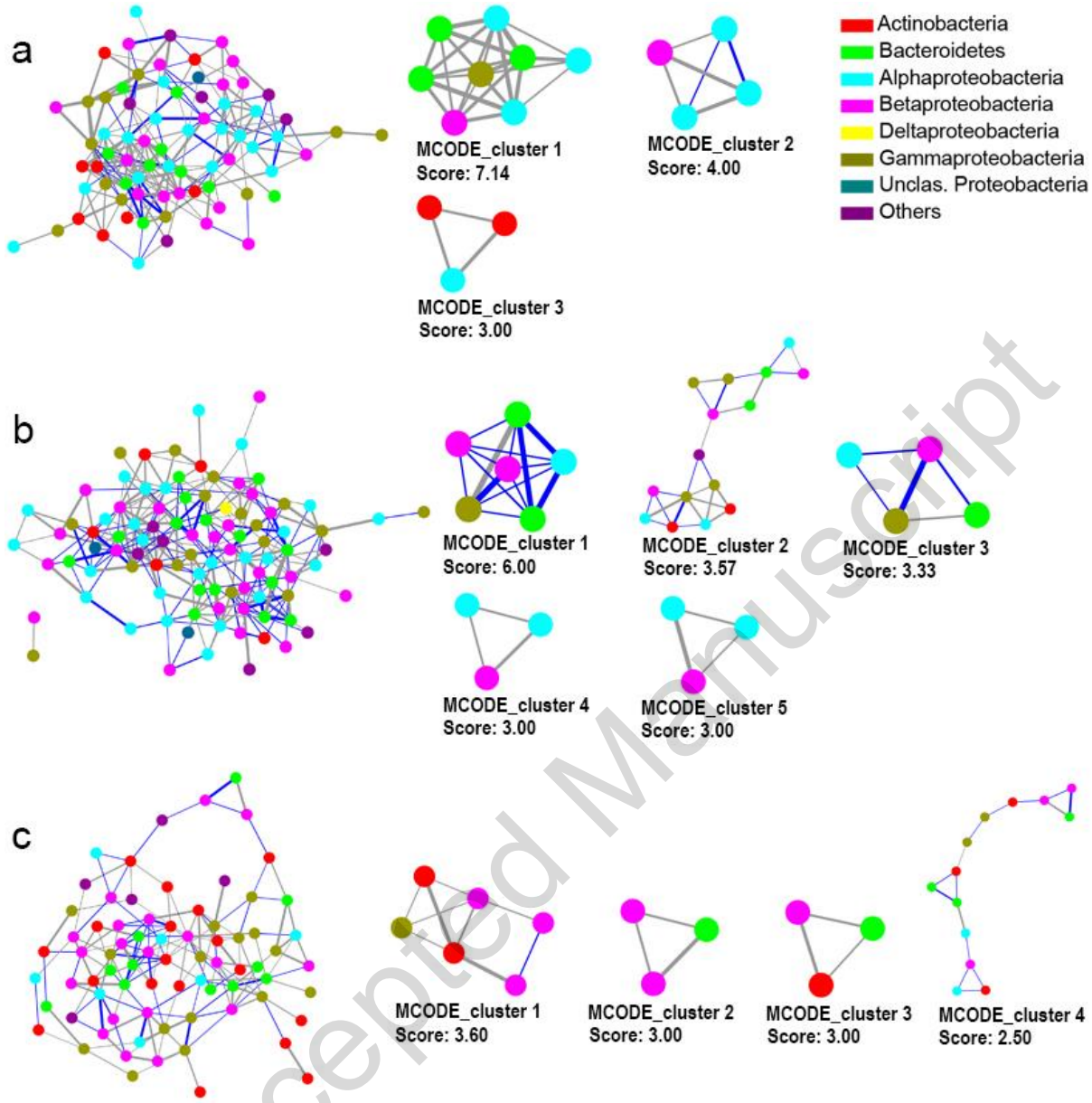


Fig. 6

Supplementary Material

Supplementary Table I

Detailed information of bacterial sequences and OTUs for all 108 samples

Soil type	Compartment	Maize cultivar	BioSample	Sequences	OTUs	Rarefied OTUs	Good's coverage
Mollisol	Rhizosphere	Denghai 605	SAMN06176978	28428	2684	2563	0.966
Mollisol	Rhizosphere	Denghai 605	SAMN06176979	32849	2679	2475	0.968
Mollisol	Rhizosphere	Denghai 605	SAMN06176980	39552	2806	2452	0.968
Mollisol	Rhizosphere	Nonghua 816	SAMN06176981	26393	2509	2459	0.967
Mollisol	Rhizosphere	Nonghua 816	SAMN06176982	31299	2524	2327	0.968
Mollisol	Rhizosphere	Nonghua 816	SAMN06176983	31977	2496	2310	0.969
Mollisol	Rhizosphere	Qiaoyu 8	SAMN06176984	35808	2537	2276	0.971

Mollisol	Rhizosphere	Qiaoyu 8	SAMN06176985	25468	2642	2619	0.964
Mollisol	Rhizosphere	Qiaoyu 8	SAMN06176986	29004	2811	2667	0.964
Mollisol	Rhizosphere	Zhengdan 958	SAMN06176987	26019	2425	2385	0.968
Mollisol	Rhizosphere	Zhengdan 958	SAMN06176988	26015	2355	2300	0.968
Mollisol	Rhizosphere	Zhengdan 958	SAMN06176989	24452	2515	2515	0.965
Mollisol	Endosphere	Denghai 605	SAMN06176990	25386	1487	1069	0.979
Mollisol	Endosphere	Denghai 605	SAMN06176991	51943	1343	929	0.983
Mollisol	Endosphere	Denghai 605	SAMN06176992	27560	1411	1118	0.978
Mollisol	Endosphere	Nonghua 816	SAMN06176993	22443	1456	1194	0.978
Mollisol	Endosphere	Nonghua 816	SAMN06176994	16996	1344	1008	0.978
Mollisol	Endosphere	Nonghua 816	SAMN06176995	23724	1566	1305	0.976
Mollisol	Endosphere	Qiaoyu 8	SAMN06176996	29438	1780	1365	0.975
Mollisol	Endosphere	Qiaoyu 8	SAMN06176997	24594	1519	1268	0.977
Mollisol	Endosphere	Qiaoyu 8	SAMN06176998	13148	1438	1301	0.975
Mollisol	Endosphere	Zhengdan 958	SAMN06176999	15948	1027	846	0.983
Mollisol	Endosphere	Zhengdan 958	SAMN06177000	13735	1003	891	0.982
Mollisol	Endosphere	Zhengdan 958	SAMN06177001	23214	750	591	0.988
Mollisol	Bulk soil	Denghai 605	SAMN06177002	34822	2625	2364	0.969
Mollisol	Bulk soil	Denghai 605	SAMN06177003	49357	2873	2352	0.969
Mollisol	Bulk soil	Denghai 605	SAMN06177004	38035	3141	2778	0.964
Mollisol	Bulk soil	Nonghua 816	SAMN06177005	39915	2716	2337	0.968
Mollisol	Bulk soil	Nonghua 816	SAMN06177006	26986	2470	2358	0.968
Mollisol	Bulk soil	Nonghua 816	SAMN06177007	38236	2657	2331	0.969
Mollisol	Bulk soil	Qiaoyu 8	SAMN06177008	20807	2212	2090	0.968
Mollisol	Bulk soil	Qiaoyu 8	SAMN06177009	27395	2445	2306	0.967
Mollisol	Bulk soil	Qiaoyu 8	SAMN06177010	31487	2643	2413	0.967
Mollisol	Bulk soil	Zhengdan 958	SAMN06177011	40933	2500	2139	0.969
Mollisol	Bulk soil	Zhengdan 958	SAMN06177012	35278	2502	2209	0.969
Mollisol	Bulk soil	Zhengdan 958	SAMN06177013	35548	2450	2139	0.969
Inceptisol	Rhizosphere	Denghai 605	SAMN06177014	29558	2246	2097	0.97
Inceptisol	Rhizosphere	Denghai 605	SAMN06177015	28737	2674	2532	0.963
Inceptisol	Rhizosphere	Denghai 605	SAMN06177016	29218	2516	2384	0.967
Inceptisol	Rhizosphere	Nonghua 816	SAMN06177017	33980	2761	2469	0.964
Inceptisol	Rhizosphere	Nonghua 816	SAMN06177018	41777	2619	2236	0.969
Inceptisol	Rhizosphere	Nonghua 816	SAMN06177019	31128	2639	2468	0.968
Inceptisol	Rhizosphere	Qiaoyu 8	SAMN06177020	34975	2636	2340	0.968
Inceptisol	Rhizosphere	Qiaoyu 8	SAMN06177021	39533	2347	2020	0.97
Inceptisol	Rhizosphere	Qiaoyu 8	SAMN06177022	33476	2459	2191	0.966
Inceptisol	Rhizosphere	Zhengdan 958	SAMN06177023	28796	2420	2287	0.966
Inceptisol	Rhizosphere	Zhengdan 958	SAMN06177024	27315	2093	2027	0.971
Inceptisol	Rhizosphere	Zhengdan 958	SAMN06177025	34062	2186	1961	0.97
Inceptisol	Endosphere	Denghai 605	SAMN06177026	35990	1226	967	0.983
Inceptisol	Endosphere	Denghai 605	SAMN06177027	22152	1110	932	0.982
Inceptisol	Endosphere	Denghai 605	SAMN06177028	40385	1496	1114	0.98

Inceptisol	Endosphere	Nonghua 816	SAMN06177029	20826	1675	1403	0.974
Inceptisol	Endosphere	Nonghua 816	SAMN06177030	21884	1174	906	0.982
Inceptisol	Endosphere	Nonghua 816	SAMN06177031	24339	1085	849	0.985
Inceptisol	Endosphere	Qiaoyu 8	SAMN06177032	32523	1315	1043	0.981
Inceptisol	Endosphere	Qiaoyu 8	SAMN06177033	46434	1213	834	0.984
Inceptisol	Endosphere	Qiaoyu 8	SAMN06177034	28236	1320	947	0.981
Inceptisol	Endosphere	Zhengdan 958	SAMN06177035	24755	951	786	0.984
Inceptisol	Endosphere	Zhengdan 958	SAMN06177036	28079	1225	1045	0.982
Inceptisol	Endosphere	Zhengdan 958	SAMN06177037	45417	1172	847	0.985
Inceptisol	Bulk soil	Denghai 605	SAMN06177038	35814	2499	2212	0.971
Inceptisol	Bulk soil	Denghai 605	SAMN06177039	39690	2660	2303	0.968
Inceptisol	Bulk soil	Denghai 605	SAMN06177040	31987	2450	2224	0.968
Inceptisol	Bulk soil	Nonghua 816	SAMN06177041	31898	2462	2272	0.971
Inceptisol	Bulk soil	Nonghua 816	SAMN06177042	29641	2365	2200	0.966
Inceptisol	Bulk soil	Nonghua 816	SAMN06177043	31483	2217	1995	0.969
Inceptisol	Bulk soil	Qiaoyu 8	SAMN06177044	33694	2397	2195	0.971
Inceptisol	Bulk soil	Qiaoyu 8	SAMN06177045	33420	2698	2467	0.97
Inceptisol	Bulk soil	Qiaoyu 8	SAMN06177046	34738	2199	1964	0.974
Inceptisol	Bulk soil	Zhengdan 958	SAMN06177047	37887	1879	1572	0.973
Inceptisol	Bulk soil	Zhengdan 958	SAMN06177048	32948	2290	1977	0.969
Inceptisol	Bulk soil	Zhengdan 958	SAMN06177049	37220	2783	2513	0.97
Ultisol	Rhizosphere	Denghai 605	SAMN06177050	34475	1648	1461	0.976
Ultisol	Rhizosphere	Denghai 605	SAMN06177051	37214	1758	1557	0.977
Ultisol	Rhizosphere	Denghai 605	SAMN06177052	34745	1804	1584	0.973
Ultisol	Rhizosphere	Nonghua 816	SAMN06177053	31940	1788	1662	0.978
Ultisol	Rhizosphere	Nonghua 816	SAMN06177054	36136	1688	1442	0.974
Ultisol	Rhizosphere	Nonghua 816	SAMN06177055	39706	1968	1636	0.973
Ultisol	Rhizosphere	Qiaoyu 8	SAMN06177056	30191	1974	1824	0.972
Ultisol	Rhizosphere	Qiaoyu 8	SAMN06177057	58802	2083	1558	0.975
Ultisol	Rhizosphere	Qiaoyu 8	SAMN06177058	28609	1959	1869	0.974
Ultisol	Rhizosphere	Zhengdan 958	SAMN06177059	33170	1566	1408	0.976
Ultisol	Rhizosphere	Zhengdan 958	SAMN06177060	32082	1744	1619	0.976
Ultisol	Rhizosphere	Zhengdan 958	SAMN06177061	34949	1519	1320	0.976
Ultisol	Endosphere	Denghai 605	SAMN06177062	14615	461	380	0.992
Ultisol	Endosphere	Denghai 605	SAMN06177063	23580	551	468	0.991
Ultisol	Endosphere	Denghai 605	SAMN06177064	29137	865	711	0.987
Ultisol	Endosphere	Nonghua 816	SAMN06177065	31726	706	495	0.989
Ultisol	Endosphere	Nonghua 816	SAMN06177066	23092	608	579	0.99
Ultisol	Endosphere	Nonghua 816	SAMN06177067	25202	994	806	0.983
Ultisol	Endosphere	Qiaoyu 8	SAMN06177068	25923	642	494	0.99
Ultisol	Endosphere	Qiaoyu 8	SAMN06177069	28572	740	549	0.989
Ultisol	Endosphere	Qiaoyu 8	SAMN06177070	44718	916	563	0.989
Ultisol	Endosphere	Zhengdan 958	SAMN06177071	29923	675	454	0.991
Ultisol	Endosphere	Zhengdan 958	SAMN06177072	18971	448	378	0.992

Ultisol	Endosphere	Zhengdan 958	SAMN06177073	30068	755	578	0.988
Ultisol	Bulk soil	Denghai 605	SAMN06177074	36183	2010	1781	0.977
Ultisol	Bulk soil	Denghai 605	SAMN06177075	38759	2197	1906	0.973
Ultisol	Bulk soil	Denghai 605	SAMN06177076	42321	2426	2003	0.968
Ultisol	Bulk soil	Nonghua 816	SAMN06177077	36888	2208	1873	0.972
Ultisol	Bulk soil	Nonghua 816	SAMN06177078	29404	1849	1722	0.973
Ultisol	Bulk soil	Nonghua 816	SAMN06177079	33030	2215	2004	0.971
Ultisol	Bulk soil	Qiaoyu 8	SAMN06177080	34113	2019	1788	0.973
Ultisol	Bulk soil	Qiaoyu 8	SAMN06177081	26862	2055	1912	0.974
Ultisol	Bulk soil	Qiaoyu 8	SAMN06177082	36544	2367	2096	0.973
Ultisol	Bulk soil	Zhengdan 958	SAMN06177083	34744	2391	2134	0.97
Ultisol	Bulk soil	Zhengdan 958	SAMN06177084	39385	2207	1867	0.972
Ultisol	Bulk soil	Zhengdan 958	SAMN06177085	41973	2463	2032	0.97

Supplementary Table II

The bacterial alpha-diversity in the bulk soil, rhizosphere and endosphere of maize cultivars Denghai 605 (DH), Nonghua 816 (NH), Qiaoyu 8 (QY) and Zhengdan 958 (ZD) planted in the Mollisol, Inceptisol and Ultisol

		Phylotype richness [a]			Phylogenetic diversity [a]			Shannon diversity [a]		
		Rhizosphere	Endosphere	Bulk soil	Rhizosphere	Endosphere	Bulk soil	Rhizosphere	Endosphere	Bulk soil
Mollisol	Cultivar DH	2497 ± 59 [b]	1039 ± 98	2498 ± 243	232.1 ± 4.2	125.1 ± 8.5	238.7 ± 5.8	9.4 ± 0.1	5.1 ± 0.7	9.6 ± 0.2
	Cultivar NH	2365 ± 82	1169 ± 150	2342 ± 14	232.5 ± 2.6	136.8 ± 11.1	227.2 ± 4.1	9.3 ± 0.2	4.7 ± 1.0	9.2 ± 0.1
	Cultivar QY	2521 ± 213	1311 ± 49	2270 ± 165	232.3 ± 5.5	141.5 ± 4.5	222.1 ± 13.4	9.4 ± 0.2	5.0 ± 0.8	8.6 ± 0.8
	Cultivar ZD	2400 ± 108	776 ± 162	2162 ± 40	226.5 ± 6.5	100.6 ± 12.4	219.3 ± 3.5	9.1 ± 0.1	3.8 ± 0.1	8.6 ± 0.3
Inceptisol	Cultivar DH	2338 ± 221	1004 ± 97	2246 ± 49	233.5 ± 14.5	118.8 ± 9.2	227.2 ± 4.0	9.0 ± 0.3	5.8 ± 0.4	9.0 ± 0.3
	Cultivar NH	2391 ± 134	1053 ± 305	2156 ± 144	241.8 ± 4.8	129.2 ± 18.6	216.0 ± 9.3	9.1 ± 0.3	4.6 ± 0.5	8.6 ± 0.4
	Cultivar QY	2184 ± 160	941 ± 105	2209 ± 252	219.2 ± 12.0	115.9 ± 7.8	233.1 ± 13.3	8.3 ± 0.6	5.5 ± 0.6	9.2 ± 0.3
	Cultivar ZD	2092 ± 172	893 ± 135	2021 ± 472	224.4 ± 13.9	113.7 ± 13.9	208.4 ± 30.1	8.4 ± 0.3	5.3 ± 0.7	8.1 ± 1.3
Ultisol	Cultivar DH	1534 ± 65	520 ± 171	1897 ± 111	167.7 ± 2.3	74.4 ± 18.0	203.8 ± 3.4	6.9 ± 0.1	4.1 ± 1.0	8.4 ± 0.3
	Cultivar NH	1580 ± 120	627 ± 161	1866 ± 141	170.2 ± 12.1	82.1 ± 13.9	196.4 ± 7.7	7.3 ± 0.8	4.5 ± 0.4	8.2 ± 0.1
	Cultivar QY	1750 ± 168	535 ± 36	1932 ± 155	176.7 ± 9.1	71.6 ± 4.4	201.5 ± 11.1	8.0 ± 0.4	4.4 ± 0.2	8.6 ± 0.8
	Cultivar ZD	1449 ± 154	470 ± 101	2011 ± 135	156.7 ± 5.4	66.6 ± 8.5	214.7 ± 7.1	6.6 ± 0.5	3.8 ± 0.7	8.7 ± 0.5
Multi-way ANOVA [c]										
		Sum Sq.	% explained	Sig.	Sum Sq.	% explained	Sig.	Sum Sq.	% explained	Sig.
Soil		6316698	11.5	0.014	46400.8	13.3	0.026	23.5	5.3	NS
Cultivar		496882	0.9	NS	2154.6	0.6	NS	4.8	1.1	NS
Compartment		43733404	79.8	< 0.001	276217.3	78.9	< 0.001	360.1	81.1	0.004
Soil × Cultivar		243862	0.4	NS	719.1	0.2	NS	2.6	0.6	NS
Soil × Compartment		1157061	2.1	0.007	7655.5	2.2	0.004	13.8	3.1	0.010
Cultivar × Compartment		138112	0.3	NS	1540.3	0.4	NS	1.0	0.2	NS
Soil × Cultivar × Compartment		536707	1.0	NS	3288.2	0.9	NS	7.8	1.8	NS
Residuals		2199423	4.0		12144.1	3.5		30.3	6.8	
Total		54822149			350119.9			443.9		

[a] Phylotype richness, phylogenetic and Shannon diversity were calculated based on rarefaction to 13,148 sequences per sample.

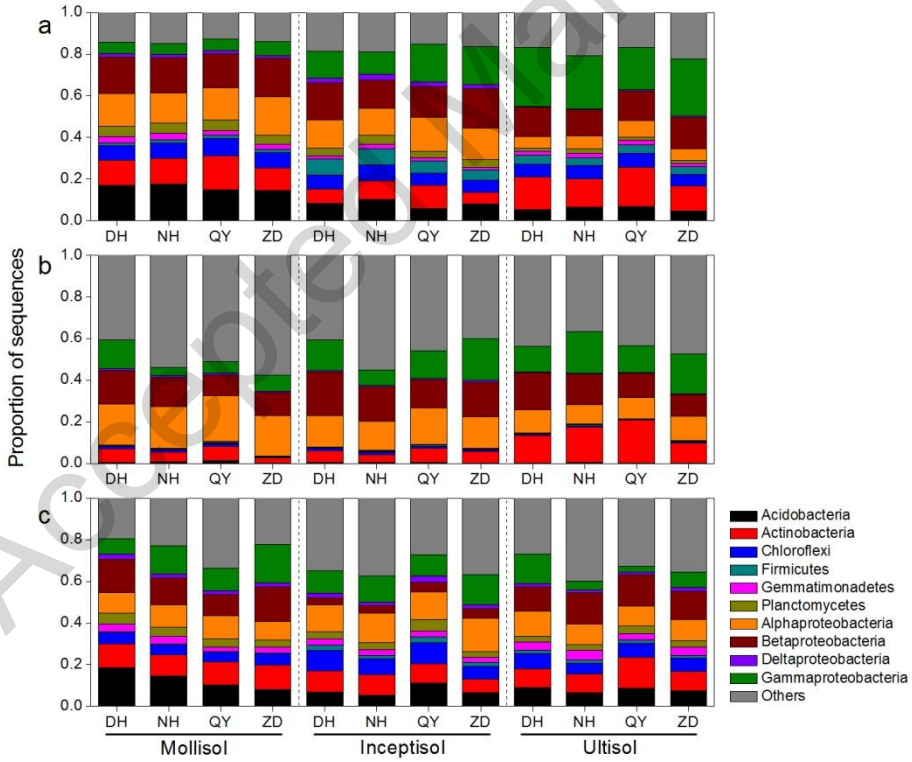
[b] Means ± standard deviations (n=3).

[c] The effect significances of soil type, maize cultivar, compartment, and their interactions were analyzed using a multi-way ANOVA. NS means no significance.

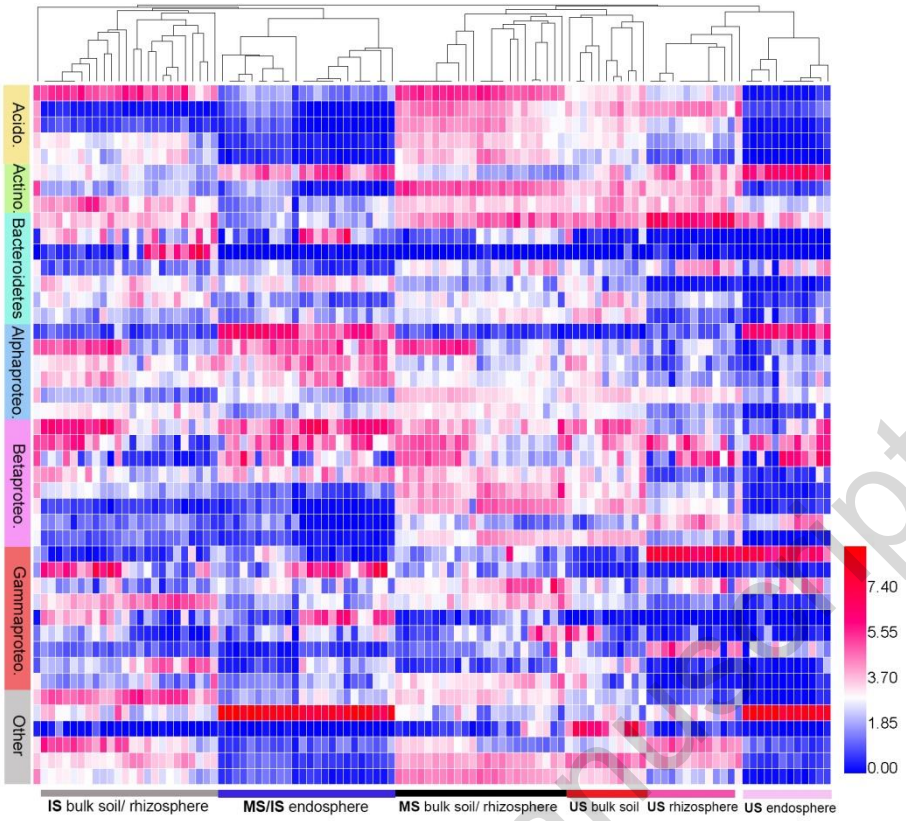
Supplementary Table III

Network topological parameters calculated by NetworkAnalyzer tool and MCODE app in Cytoscape v.3.2.1

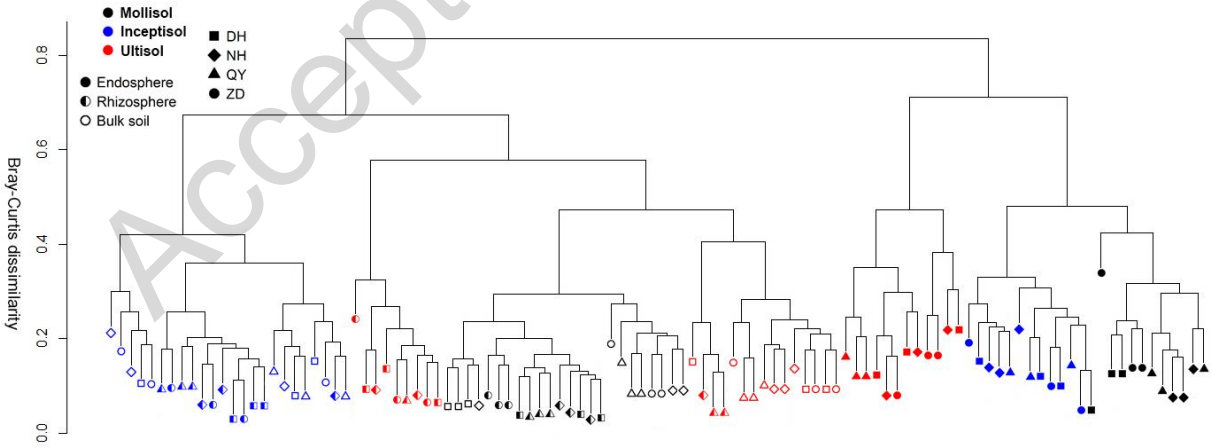
	Mollisol	Inceptisol	Ultisol
Number of nodes	73	96	75
Number of edges	219	261	167
Network density	0.08	0.06	0.06
Network heterogeneity	0.59	0.56	0.52
Clustering coefficient	0.32	0.28	0.24
Connected components	1	2	1
Network diameter	7	8	8
Network radius	4	1	5
Network centralization	0.16	0.08	0.11
Shortest paths	5256	8744	5550
Characteristic path length	3.01	3.24	3.54
Avg. number of neighbors	6.00	5.44	4.43
Keystone taxa	*Chitinophaga*	Unclassified *Methylophilaceae*	*Burkholderia*
	Devosia	Unclassified *Sphingomonadaceae*	Unclassified *Alcaligenaceae*
	Unclassified *Xanthomonadaceae*		



Supplementary Fig. 1 Proportion of sequences of the main bacterial phyla and classes of Proteobacteria in the rhizosphere (a), endosphere (b) and bulk soil (c) of the cultivars Denghai 605 (DH), Nonghua 816 (NH), Qiaoyu 8 (QY) and Zhengdan 958 (ZD) planted in the Mollisol, Inceptisol and Ultisol. The differences in the relative abundances of major phyla/classes were detected by one-way ANOVA.

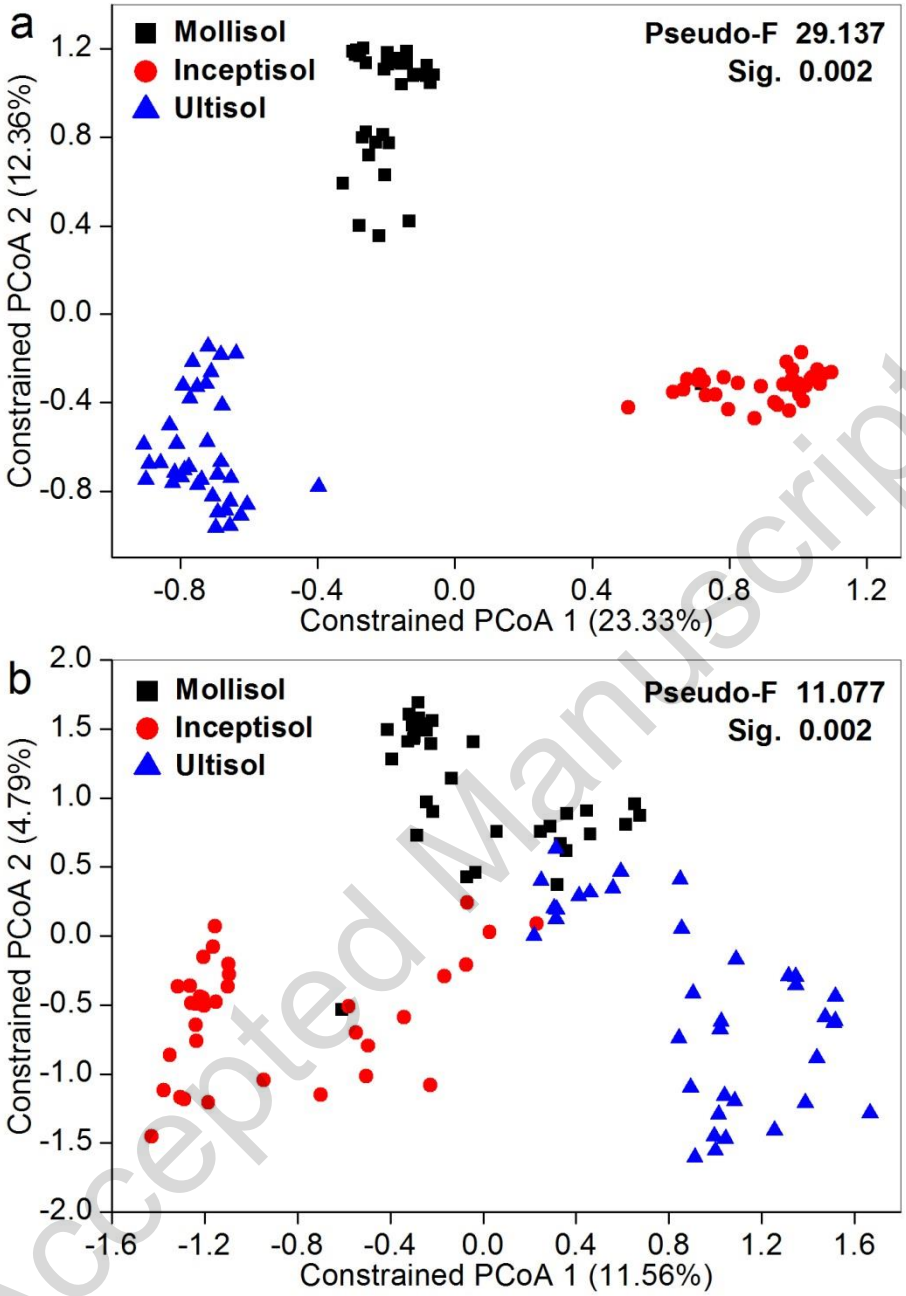


Supplementary Fig. 2 Heat map showing the distributions of the main bacterial population in all samples. The top 44 most abundant genera with relative abundances of ≥ 0.1% in at least one group were selected, and values were transformed following the formula $\log_2 (1000x + 1)$, where x is the proportion of sequences for individual taxon. Hierarchical clustering was based on Bray-Curtis dissimilarity with group-average linkage. MS, Mollisol; IS, Inceptisol; US, Ultisol.

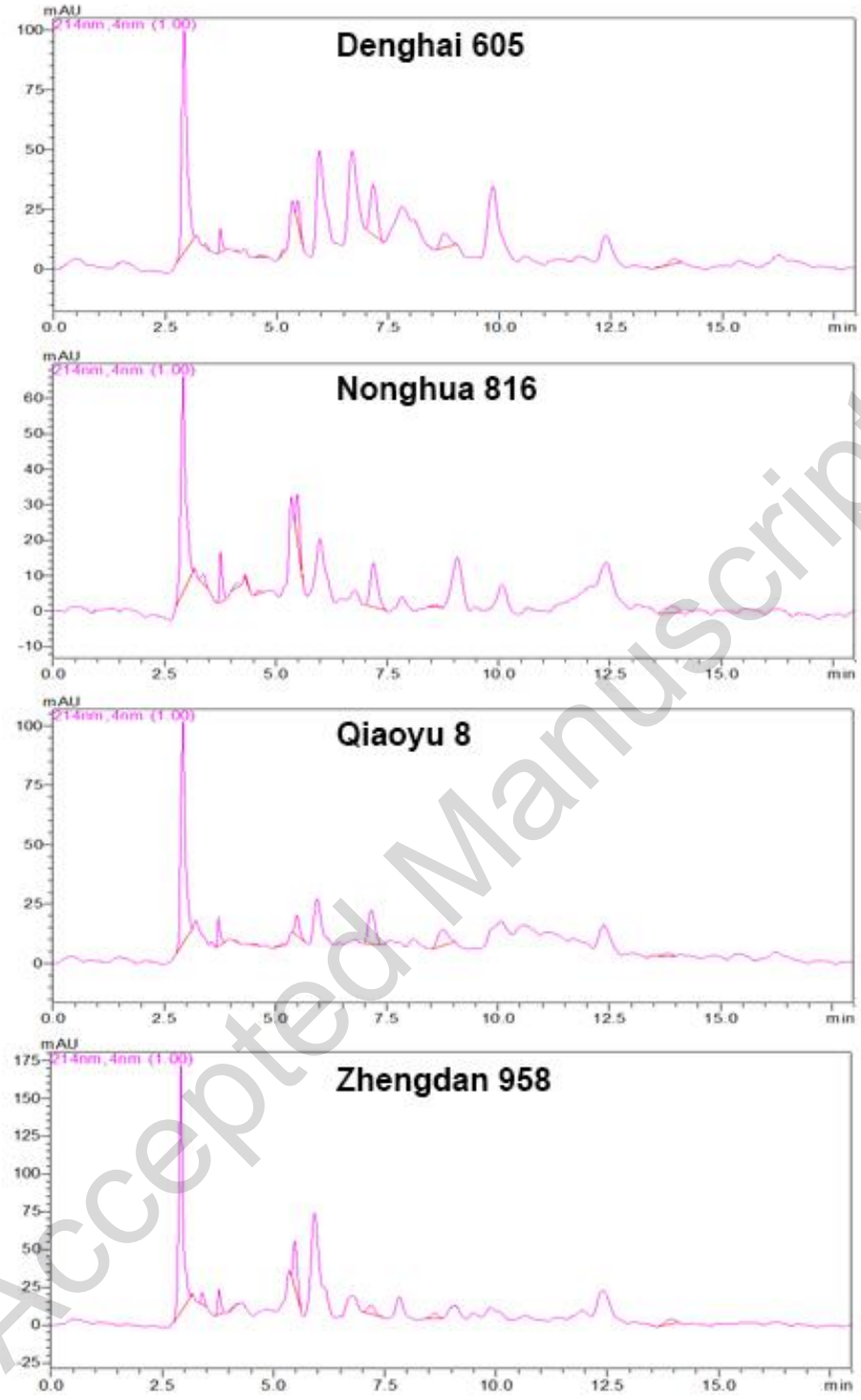


Supplementary Fig. 3 The cluster dendrograms illustrating Bray-Curtis dissimilarity between samples for the bacterial community using unweighted pair-group method with arithmetic means (UPGMA). Log_2-transformed OTU abundance was used to calculate a Bray-Curtis dissimilarity matrix. The Mollisol, Inceptisol and Ultisol were labeled by black, blue and red colors, respectively. The solid, half-solid and hollow shapes represented the endosphere, rhizosphere and bulk soil, respectively. The square, diamond, triangle and circle designated the cultivars Denghai 605 (DH), Nonghua 816

(NH), Qiaoyu 8 (QY) and Zhengdan 958 (ZD), respectively.



Supplementary Fig. 4 Canonical analysis of principal coordinates (CAP) of unweighted (**A**) and weighted (**B**) UniFrac distances quantifying the impacts of soil properties on bacterial community composition. CAP was constrained to the factors pH, C/N, SOC, TN, TP, AP, AK and CEC. The effect significance was calculated by permutation test for all constrained factors. The percent variation explained by the PCs is indicated on the axes and refers to the fraction of the total variance.



Supplementary Fig. 5 Liquid chromatograms showing similar root exudate components among maize cultivars Denghai 605, Nonghua 816, Qiaoyu 8 and Zhengdan 958. The chromatogram was randomly selected from three replicates of each cultivar.