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Biogeography and ecological processes affecting root-associated bacterial communities in soybean fields across China

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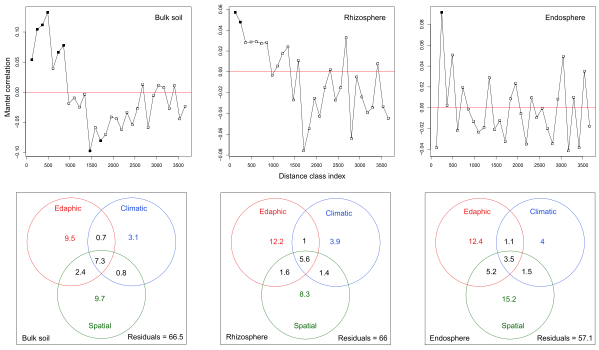
HIGHLIGHTS

- Bacterial communities in bulk soil, soybean rhizosphere and endosphere were compared.
- High–throughput sequencing was performed on an Illumina HiSeq 2500 platform.
- Environmental rather than spatial factors governed bacterial community turnover.
- Edaphic factors were more important than climatic factors for community turnover.
- Bacteria in the three compartments displayed different biogeographic patterns.

GRAPHICAL ABSTRACT



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ABSTRACT

Root-associated bacteria have profound effects on plant health and productivity, but their biogeographic patterns across large spatial scales remain poorly understood. Here, we used high–throughput sequencing to compare the bacterial distributions in the bulk soil, rhizosphere, and endosphere across 51 soybean fields in China. Environmental variables were more important than spatial variables, and edaphic variables were more important than climatic variables, for governing bacterial community turnover in each soil-root compartment. Both bacterial richness and community turnover were significantly correlated with different environmental and spatial variables among the three compartments. Their different spatial autocorrelation ranges for bacteria suggested distinct bacterial biogeographic patterns were present. The distributions of nearest taxon index (NTI) showed that deterministic processes dominated local bacterial communities, while its importance decreased from the bulk soil to the endosphere. These results provide new insights into the assembly of root–associated bacterial communities at a continental scale.

1. Introduction

Plant roots provide habitats for the diverse microorganisms in the rhizosphere and endosphere, where complex plant-microbe interactions play an essential role in plant nutrient uptake, disease suppression, and resistance to abiotic stress (Bulgarelli et al., 2013; Mendes et al., 2011; Reinhold-Hurek et al., 2015). Using next-generation sequencing technologies, numerous studies have investigated the composition and structure of root–associated bacterial communities and the main factors affecting them, such as soil types, plant species, and host developmental stage (Bulgarelli et al., 2012; Edwards et al., 2015;

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Lundberg et al., 2012; Xiao et al., 2017). However, only a few studies have explored the biogeographic patterns of root–associated bacterial communities (Fan et al., 2017; Nuccio et al., 2016).

Biogeography is the study of organism distribution patterns over space and time (Hanson et al., 2012). Gaining knowledge about the biogeographic patterns of microorganisms is particularly important because it can provide key insights into the mechanisms that generate and maintain microbial diversity (Martiny et al., 2006), which would help to better predict ecosystem-level responses to environmental change. Microbial biogeographic patterns are driven by local environmental factors (i.e., deterministic processes) and regional processes, such as dispersal limitations, mass effects, and historical factors (Hanson et al., 2012; Vellend, 2010; Wang et al., 2013). The bacterial biogeographic patterns in bulk soil have been extensively investigated in multiple ecosystems (i.e., both natural and agricultural) and at various spatial scales (i.e., local, regional, and global). Studies have revealed the importance of spatial factors (Caruso et al., 2011; Dumbrell et al., 2010) and environmental factors, such as soil pH (Fierer and Jackson, 2006; Griffiths et al., 2011), carbon content (Chu et al., 2016), C/N ratio (Högberg et al., 2007), precipitation (Angel et al., 2010), and temperature (Zhou et al., 2016), in shaping bacterial spatial distribution patterns. Nonetheless, current knowledge of bacterial biogeographic patterns in the rhizosphere and endosphere is relatively limited, such that bacterial biogeographic patterns have not been compared among the bulk soil, rhizosphere, and endosphere compartments.

More importantly, microbial biogeographic patterns and their causal ecological processes are scale-dependent (Levin, 1992; Talbot et al., 2014). For example, Martiny et al. (2011) demonstrated that geographic distance determines ammonia-oxidizing bacterial community composition at the local but not regional scale. However, most studies investigating root–associated bacterial communities have collected their samples from a small number of sites or from greenhouse systems. A notable exception is the study by Nuccio et al. (2016) spanning a > 500-km north–south gradient in California and that by Fan et al. (2017) across c. 1000 km of northern China. Nuccio et al. (2016) found that the wild oat rhizosphere communities were most influenced by climatic factors, while the bulk soil communities responded more to edaphic factors. Fan et al. (2017) showed that the bacterial communities in wheat rhizosphere were controlled more by geographic distance than by environmental factors. As both studies were performed at the regional scale, the root–associated bacterial distribution patterns at larger (i.e., continental) spatial scales remain largely undetermined.

Soybean (*Glycine* max) is a major economic crop and is widely distributed across China (Li et al., 2008). This enables sampling across a broad range of geographic locations and environmental gradients. Here, we used high–throughput sequencing to compare the bacterial communities of soybean in three distinct soil-root compartments (bulk soil, rhizosphere, and endosphere) in 51 fields across China. The objectives of this study were to determine and compare bacterial biogeographic patterns among the three soil–root compartments at a continental scale. We hypothesized that (i) bacteria in the three compartments show different biogeographic patterns; and (ii) the ecological factors and their relative importance in governing bacterial community turnover are different among the three compartments.

2. Materials and methods

2.1. Sample collection

Samples were collected from 51 soybean fields in China, these fields were located between 19°–50° N and 81°–130° E (Fig. 1). The distances between locations ranged from 63 to 3700 km. All fields were cultivated under conventional (i.e., pesticides and chemical fertilizers use permitted) but not organic (i.e., organic, manure, or compost fertilizers used) practices, and were planted with modern domesticated and locally adapted soybean cultivars. Sampling sites and relevant climate information are detailed in Supplementary Table S1. The sampling was conducted between May and August of 2015, which corresponds to the flowering period of the plant in all fields. In each field, a ~100 m^2 plot was chosen, and the bulk soil samples were taken at random from the topsoil layer (0–20 cm) in five cores (5 cm diameter) and pooled. Along with the surrounding soil, 15–20 randomly picked healthy plants were carefully removed from each field using a spade. All plants were taken from the central region of each field to avoid edge effects. Roots were carefully shaken to remove loosely attached soil, grouped into one sample per field and placed in plastic bags. All samples were transported to the laboratory on ice and stored at −80 °C until DNA extraction.

2.2. Acquisition of environmental data

A subset of the soil was air-dried and analyzed for its texture, organic C, pH, total/available N, and macronutrient contents (i.e., available P, K, Mg, and Ca) by using the standard soil testing procedures described in Bao (2000). Monthly weather data were extracted from the China Meteorological Database (http://data.cma.cn/). Climate data were collected from 51 weather stations near the sampling sites. Mean annual temperature (MAT) and mean annual precipitation (MAP) were computed based on the average monthly values. We also calculated one, two, and three month sum precipitation (SMP1–3) and mean temperature (MMT1–3) ranges.

2.3. Sample preparation, DNA extraction and sequencing

The rhizosphere and endosphere compartments were separately sampled by sequential washing and sonication treatments following a previously described method (Lundberg et al., 2012; Xiao et al., 2017). A detailed description of how the different compartments were separated in this study is provided in the Supplementary Information. Nodules were removed from the endosphere compartment and a 0.5 g portion of soil (bulk or rhizosphere) was used for DNA extraction. For endosphere samples, ~1 g of roots were lysed by grinding under liquid nitrogen using a mortar and pestle. The DNA for each sample was extracted using the Fast DNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol. DNA concentration and purity was confirmed on 1% (w/v) agarose gels. The V4 region of the bacterial 16S rRNA gene was amplified using the 515F (5′-GTGCCAGC MGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Evans et al., 2014) with unique barcodes. All PCR reactions (30 μL volume) contained: 15 μL Phusion Master Mix (New England Biolabs), 0.2 μM forward and reverse primers, and 10 ng template DNA. The DNA from each sample was individually amplified by PCR in triplicated reactions consisting of: initial denaturation at 98 °C for 1 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, and a final elongation at 72 °C for 5 min. Triplicate PCR products were pooled and purified with the Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing libraries were generated by using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations with index codes. Sequencing (250 bp paired end) was run on an Illumina HiSeq 2500 at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

2.4. Sequence analysis

Paired-end reads were merged using FLASH (Magoc and Salzberg, 2011) and assigned to each sample according to the unique barcodes using QIIME (Caporaso et al., 2010). Sequences <200 bp in length, with average quality <25, or containing ambiguous bases were discarded. Chimeras were detected and removed using UCHIME (Edgar et al., 2011). High-quality sequences with ≥97% similarities were then assigned to the same operational taxonomic unit (OTU)

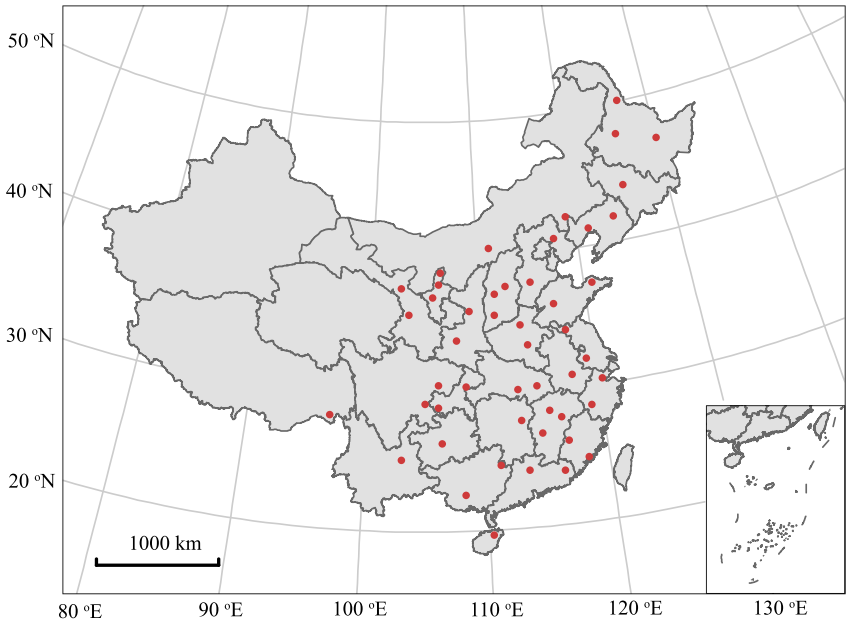

Fig. 1. Geographic distribution of the sampling sites across 51 soybean fields in China.

using the UPARSE pipeline (Edgar, 2010). Taxonomy was assigned by comparison with the Ribosomal Database Project Classifier using a threshold of 80% (Wang et al., 2007). All sequences assigned to chloroplast and mitochondria were removed from the dataset. To account for differences in library size across the samples, the OTU table was rarefied to 5209 sequences per sample in QIIME. OTU richness and Bray–Curtis dissimilarity matrices were calculated by using the rarefied OTU tables. The raw sequence data used in this study are available as an NCBI Small Read Archive (SRA) dataset, under BioProject PRJNA395393, with the accession number SRP113347 and run numbers of SRR5859787–SRR5860093.

2.5. Statistical analysis

All statistical analyses were carried out in R version 3.2.3 (R Core Team, 2013) and PRIMER 7 (Clarke and Gorley, 2015). Permutational multivariate analysis of variance (PERMANOVA) was performed with 999 permutations to test for differences in the bacterial community composition among the compartments. Significant differences in bacterial richness and beta diversity among the three compartments were determined by the pairwise Wilcoxon signed-rank test and the *P*-values were adjusted according to the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). Differences in bacterial communities between samples were visualized by non-metric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarity matrix. Tukey's range test, a single-step multiple comparison procedure, was applied to identify those taxa that significantly differed among the three compartments. Spearman's rank correlations were used to determine the relationships between bacterial richness and environmental factors.

The Mantel test and Mantel correlogram were used to determine the effect of geographic distance on the bacterial communities. The relative influences of edaphic, climatic, and spatial variables on the bacterial communities were determined by using a variance partitioning method (Peres-Neto et al., 2006). Spatial variables—accounting for unmeasured processes, such as dispersal, spatially structured environmental variables, and historical contingencies (Peres-Neto and Legendre, 2010)—were generated by using the principal coordinates of neighbor matrices (PCNM) analysis (Borcard et al., 2011). Environmental variables (except for pH) were log(x + 1)-transformed to improve normality and reduce nonlinearity. Distance-based linear modeling (distLM) analysis was used to select the significant edaphic, climatic, and spatial variables (Legendre and Anderson, 1999), via the forward selection procedure and adjusted-R^2 selection criterion in PRIMER 7. Variation partitioning was performed by the 'varpart' function in the 'vegan' package, with the Bray–Curtis dissimilarity matrix as the response variable, and the significant edaphic, climatic and spatial variables set as the explanatory variables. The significant variables were also used to build distance-based redundancy ordinations (db-RDA).

A maximum likelihood tree was constructed from the representative OTU sequences in Fast Tree using the Jukes-Cantor model (Price et al., 2010), and then converted to a phylogenetic distance matrix using the function 'cophenetic' in the 'picante' package (Kembel et al., 2014). To determine the ecological processes that governed a local community, the nearest taxon index (NTI) was calculated using community data tables and phylogenetic distance matrix, with the function "ses.mntd" in the same package. The "local community", as used here, refers to the community in one sample, relative to the spatial turnover in community composition between samples, and NTI is the negative of the output of 'ses.mntd'. For a single community, NTI value greater than +2 or less than −2 indicates coexisting taxa that are more closely (phylogenetic clustering) or distantly (phylogenetic over-dispersion) related than expected by chance (Stegen et al., 2012). A mean (or median) NTI value taken across all the communities that is above or below zero indicates these communities are phylogenetically clustered or over-dispersed on average, while a larger absolute NTI value indicates greater effects of deterministic processes (Stegen et al., 2012; Wang et al., 2013). Pairwise Wilcoxon signed-rank tests were then used to test whether the NTI distributions differed significantly among the three compartments, and the *P*-values were adjusted following the Benjamini-Hochberg method.

3. Results

3.1. Bacterial diversity and community composition

A total of 22,827 non-singleton OTUs were retrieved from 7,505,187 quality-filtered sequences and 153 samples (51 samples per compartment). Proteobacteria, Actinobacteria, and Acidobacteria largely

dominated all three soil-root compartments (Fig. S1). Tukey's range test showed the relative abundance of Proteobacteria significantly increased with root proximity, and 38.1%, 59.6%, and 74.1% of the reads were assigned to this phylum in the bulk soil, rhizosphere, and endosphere communities, respectively. By contrast, Acidobacteria, Chloroflexi, Gemmatimonadetes, and Planctomycetes all significantly decreased with proximity to roots; for example, Actinobacteria were significantly more abundant in the endosphere (18.5%) compared with the bulk soil (12.6%) and rhizosphere (13.7%) (Fig. S2). Taken together, these results revealed shifts in the bacterial community composition going from the bulk soil to endosphere.

Bacterial richness decreased from the bulk soil to the rhizosphere, and then to the endosphere (Wilcoxon test; Fig. 2A and Table S2), a trend validated by the OTU rarefaction curves (Fig. S3). Measures of between-sample diversity (beta diversity), based on Bray-Curtis dissimilarity index, revealed that the bacterial communities in the rhizosphere had the highest beta diversity (Fig. 2B and Table S2). PERMANOVA based on the Bray–Curtis distance revealed significant differences in bacterial community composition among the compartments ($R^2 = 0.341$, $P = 0.001$). Consistently, NMDS ordination plots revealed separate clustering of bacterial communities by compartment. Additionally, rhizosphere samples had the highest between-sample variation, which corresponded with the highest beta diversity (Fig. 2C).

3.2. Effect of environmental factors on bacterial richness

Spearman's correlations suggested that bacterial richness was related to different environmental factors in the three soil-root compartments (Fig. S4). Bacterial richness in bulk soil was negatively correlated with organic carbon, Ca, and Mg contents, but positively correlated with MAP and MAT. Bacterial richness in the rhizosphere was negatively correlated with both MMT2-3 and SMP2-3, whereas in the endosphere it was only negatively correlated with available nitrogen. Soil pH was not correlated with bacterial richness in any compartment. Additionally, the bacterial richness in the three soil-root compartments showed different relationships with latitude (Fig. 3, Fig. S4). With increasing latitude, a decrease in bacterial richness was observed in bulk soils ($r^2 = 0.155$, $P = 0.003$), while an increase was observed in the rhizosphere ($r^2 = 0.21$, $P < 0.001$). The bacterial richness of endosphere samples was not correlated with latitude.

3.3. Ecological factors governing community spatial turnover

The significant edaphic, climatic, and spatial variables were selected based on the distLM analysis with the forward selection procedure, and visualized by db-RDA ordination (Fig. 4). Four edaphic variables (soil pH, Ca, Mg, and K), two climatic variables (MAP and MAT), and five spatial factors (PCNM 1, 2, 3, 8, and 18) were significantly related to the spatial turnover of the bulk soil bacterial community, ($P < 0.05$; Fig. 4A). Five edaphic variables (soil pH, Mg, available N, moisture, and clay), two climatic variables (MAP and MMT3), and four spatial factors (PCNM 1, 2, 3, and 5) were significantly related to the rhizosphere bacterial community turnover ($P < 0.05$; Fig. 4B). Five edaphic variables (soil pH, Mg, Ca, available N, and clay), two climatic variables (MAP and MMT3), and seven spatial factors (PCNM 1, 2, 3, 6, 8, 25, and 28) were significantly related to the endosphere bacterial community turnover ($P < 0.05$; Fig. 4C).

The variation partitioning analyses revealed that the climatic, edaphic, and spatial variables explained a total of 33.5%, 34% and 42.9% of the variance in the bulk soil, rhizosphere, and endosphere bacterial communities, respectively (Fig. 4). Pure environmental effects—edaphic, climatic and their joint effect, except their intersection with the spatial effect—explained a larger proportion of the bacterial community turnover occurring in bulk soil (13.3%), rhizosphere (17.1%) and endosphere (17.5%) than did the pure spatial effect. Among the environmental factors, edaphic variables were more important than climatic factors for driving bacterial community turnover of bulk soil (9.5%), rhizosphere (12.2%), and endosphere (12.4%). A pure climatic effect explained the smallest proportion of variation in the bacterial community of bulk soil (3.1%), rhizosphere (3.9%), and endosphere (4%) when compared with other two sets of factors.

The Mantel test revealed that geographic distance was negatively correlated with bulk soil bacterial community similarities ($r = 0.279$, $P < 0.001$); however, this relationship was not observed with the bacterial communities of the rhizosphere ($r = 0.124$, $P = 0.055$) or endosphere ($r = 0.060$, $P = 0.201$). The Mantel correlogram showed significant spatial autocorrelation in the bacterial communities but its range differed among the three compartments (Fig. 5). In the bulk soil and rhizosphere, spatial autocorrelation was significant within the first seven (0–794 km) and two (0–185 km) distance classes, respectively. For the endosphere, significant spatial autocorrelation only occurred in the second distance classes (63–185 km). Together, these results clearly revealed different spatial distributions of the bacterial communities in the three compartments.

3.4. Assembly processes driving local community composition

The relative influences of deterministic and stochastic processes for governing local community composition were assessed using

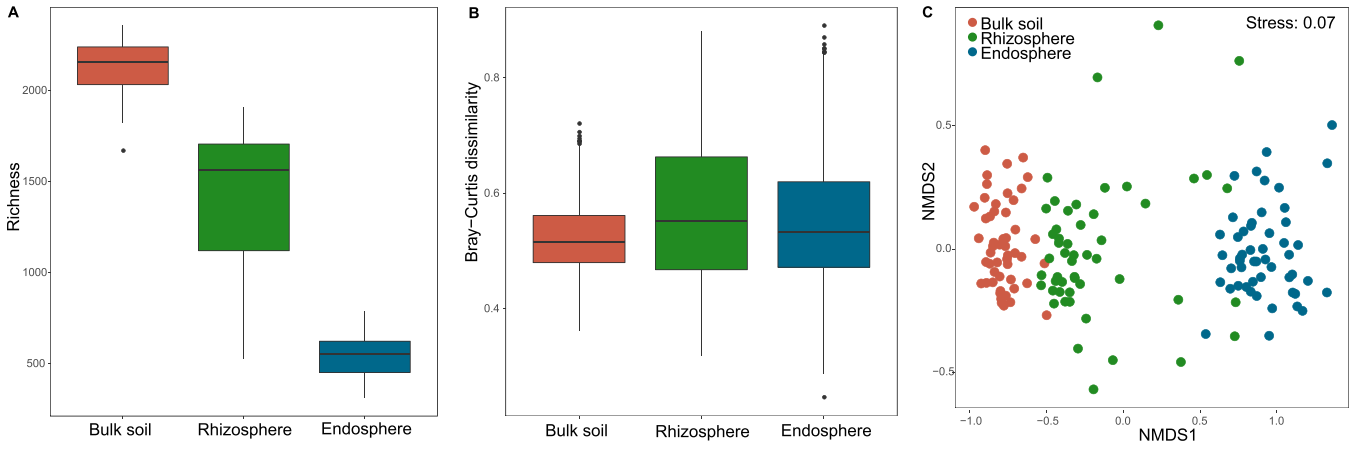

Fig. 2. Bacterial diversity measurements of three soil-root compartments in the soybean fields: (A) a rarefied richness box plot, (B) a Bray-Curtis dissimilarity box plot, (C) a non-metric multidimensional scaling (NMDS) ordination plot of bacterial communities based on Bray-Curtis dissimilarities.

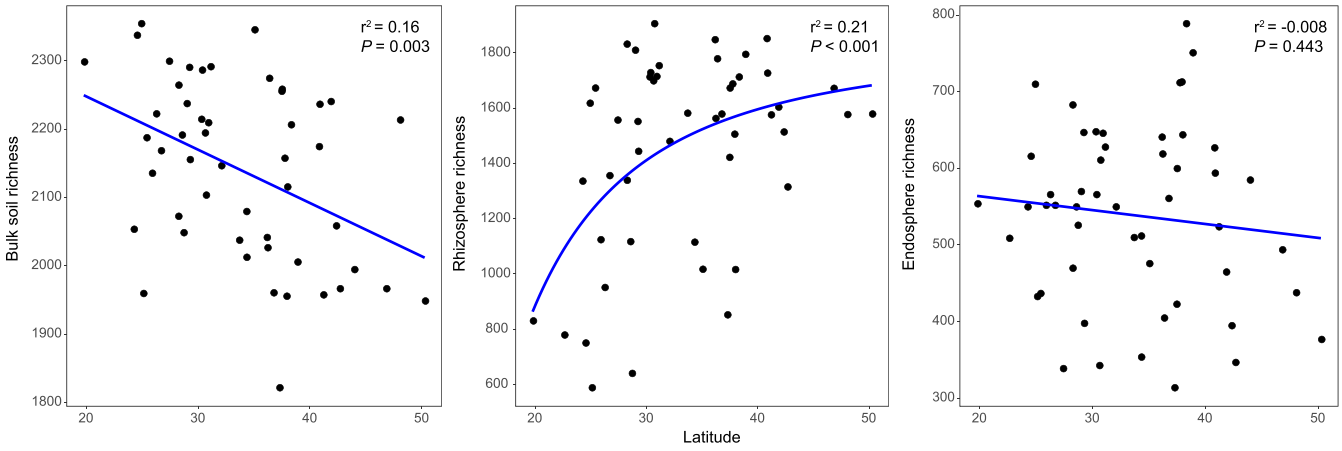

Fig. 3. Relationships between the operational taxonomic unit richness of bacteria in soil-root compartments and latitude in the soybean fields. Lines denote the linear or polynomial regression model of interaction between bacterial richness and latitude.

the NTI (Fig. 6). NTI values across 51 samples showed non-normal distributions in the three root-soil compartments, and their median values were all significantly larger than zero (Wilcoxon signed-rank test, $P < 0.0001$ for all). Additionally, 100%, 96%, and 92% of the 51 bacterial communities were phylogenetically clustered (NTI > 2) in the three compartments. Wilcoxon signed-rank tests showed that the median NTIs significantly decreased with root proximity, 13.17 for bulk soils, 6.27 for rhizosphere samples, and 3.04 for endosphere samples (Table S3).

4. Discussion

In the present study, we compared the biogeography patterns of bacteria communities in the bulk soil, soybean rhizosphere, and endosphere across China. Our results showed that environmental variables were more important than spatial factors in driving bacterial community turnover in all three soil-root compartments. Of the environmental variables measured, edaphic variables were more important than climatic factors. Moreover, the bacterial richness and

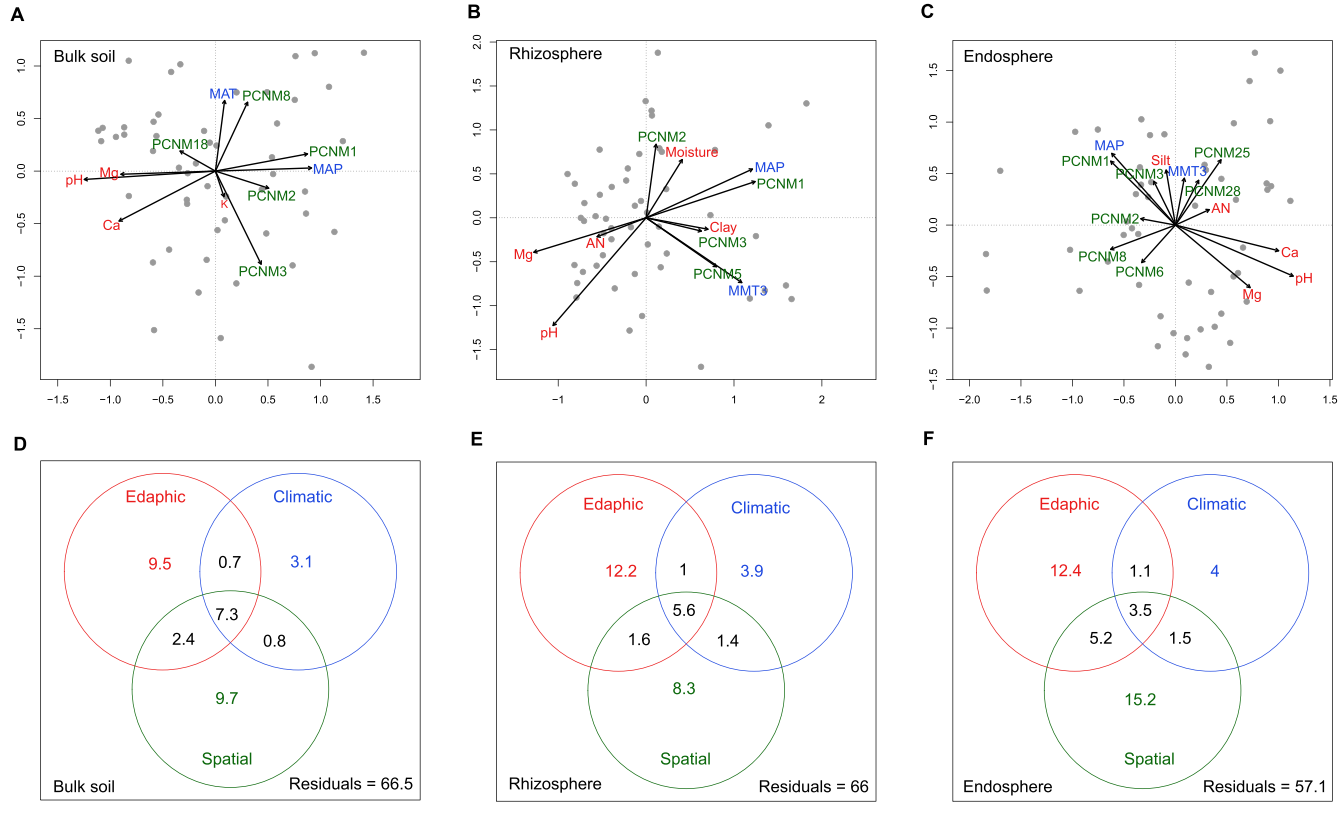

Fig. 4. Effect of ecological factors on bacterial community turnover of three soil-root compartments in the soybean fields. (A–C) Distance-based redundancy ordination showing significant variables that influence bacterial community turnover in three soil-root compartments. (D–F) Variation partitioning (% variation explained) of bacterial community in relation to combined climatic, edaphic, and spatial variables. The numbers inside the sections indicate the percentage of the variation explained. AN, soil available nitrogen; K, Ca and Mg, soil potassium, calcium and magnesium content, respectively; MMT3, three month mean temperature ranges; MAT, mean annual temperature; MAP, mean annual precipitation; PCNM, principal coordinates of neighbor matrices.

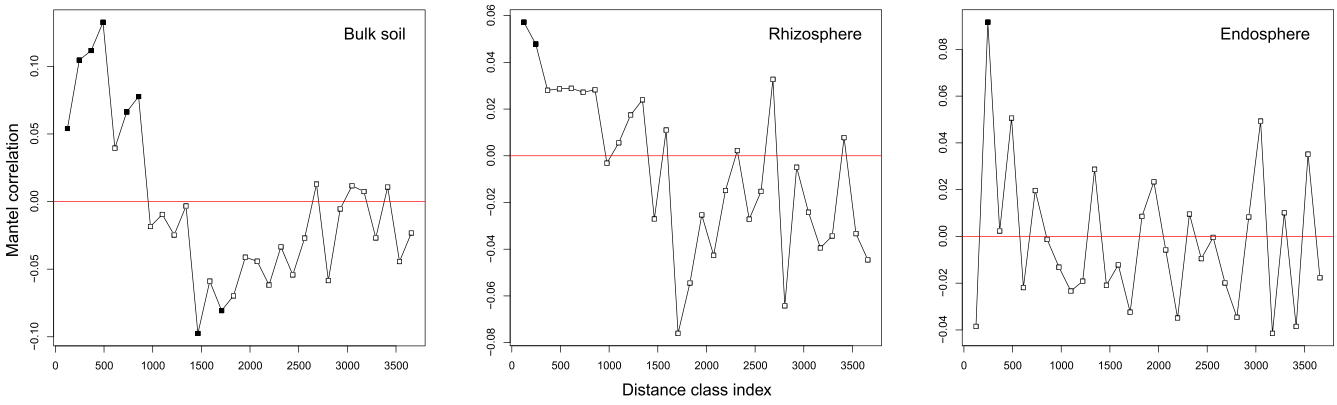

Fig. 5. Mantel correlograms showing significant autocorrelation of bacteria in three soil-root compartments across various distance classes in the soybean fields. Solid symbols denote significant ($P < 0.05$) correlations for each class.

community turnover in the three compartments were significantly related to different environmental and spatial factors, suggesting that bacterial biogeographic patterns of soybean rhizosphere and endosphere are unalike and distinguishable from that of bulk soil.

4.1. Distinct bacterial diversity and community composition in the three compartments

Bacterial richness decreased from the bulk soil to the endosphere in our soybean fields, thus indicating a significant root filtration effect (Xiao et al., 2017). Comparing the bacterial communities in the bulk soil, rhizosphere, and endosphere also revealed dramatically distinct community compositions in the present study. Proteobacteria were gradually enriched going from the bulk soil to endosphere, which is in line with the studies of wheat (Ofeklalzar et al., 2014), poplar (Gottel et al., 2011), and rice (Sessitsch et al., 2012). The relative abundance of Acidobacteria, Chloroflexi, Gemmatimonadetes, and Planctomycetes significantly decreased with root proximity. These results collectively demonstrate how gradual changes in bacterial community composition can occur when transitioning from bulk soil to the endosphere (Reinhold-Hurek et al., 2015).

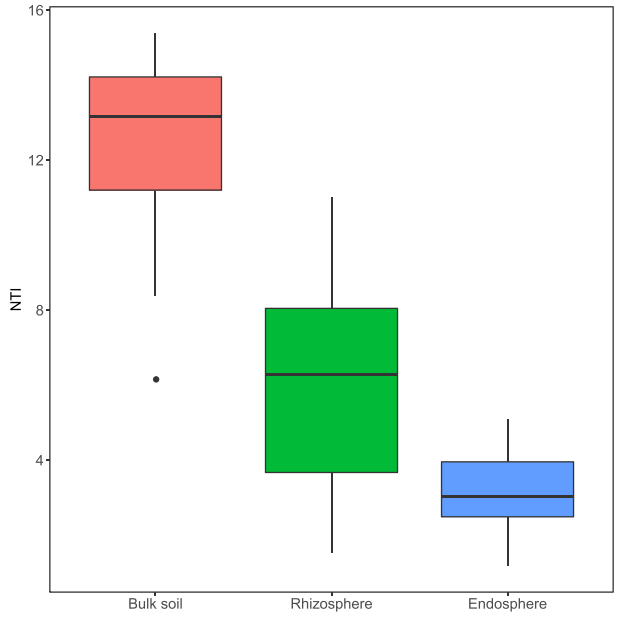

Fig. 6. Nearest taxon index (NTI) of bacterial communities in different soil-root compartments in the soybean fields.

4.2. Distinct biogeographic patterns for bacterial communities in the three compartments

In a manner consistent with biogeographic patterns of plants, animals, and fungi (Hillebrand, 2004; Tedersoo et al., 2014), the overall bacterial richness in the bulk soil decreased when latitude increased. In contrast, the bacterial richness in the rhizosphere, but not in the endosphere, was significantly and positively correlated with latitude. This suggests that environmental filtering is specific for root–associated bacterial communities in soybean fields. Furthermore, various climate factors were negatively correlated with latitude in our study (Fig. S5). Thus, the correlations between climate factors and bacterial richness may offer valuable insights into the mechanisms underlying the latitudinal gradient. Our results show that bacterial richness in the bulk soil is similarly correlated with both latitude and temperature, supporting the hypothesis that diversity increases with increasing environmental temperature due to metabolic kinetics (Brown et al., 2004). In contrast, the bacterial richness in the rhizosphere was negatively correlated with temperature. In addition, bulk soils and the rhizosphere exhibited opposite trends in bacterial richness and precipitation. Water deficiencies may influence bacterial richness in the rhizosphere by stimulating the proliferation of plant growth-promoting rhizobacteria that help plants tolerate drought stress (Yang et al., 2009).

The relative contribution to bacterial community turnover from local environmental—edaphic and climatic—factors exceeded that of spatial factors in all three soil-root compartments (as revealed by the variation partitioning analysis). Edaphic and climatic factors would affect root-associated bacteria indirectly by modulating the bulk soil bacterial communities, from which most of the rhizosphere and endosphere colonizers are recruited (Reinhold-Hurek et al., 2015). These factors may also modulate plant physiology and the plant–root microbiota interactions (Lareen et al., 2016), which could thus affect the bacterial communities associated with roots. Furthermore, edaphic factors were more important than climatic ones for governing bacterial community turnover in all three soil–root compartments. In contrast, Nuccio et al. (2016) found that the rhizosphere communities of wild oats were more influenced by climatic than by edaphic factors. We presume that long–term anthropogenic activity (i.e., irrigation) in the soybean crop system has diminished the relative importance of climatic factors in the present study. A large part of the variation in our data could not be explained by the variables included in our analyses; this unexplained variation may come from unmeasured environmental variables. It should be noted that crop management practices—i.e., crop rotation, and fertilizer and pesticide treatments—and the particular soybean cultivars grown could also influence the below-ground bacterial communities. Although the 51 soybean fields we studied were all cultivated under conventional practices, the pesticide and chemical fertilizer

types in use were not taken into consideration in our analyses. A study of maize found little variation in the rhizosphere community composition arising from host plant genotypes (Peiffer et al., 2013). However, the relative influence of host genotype may be stronger in the endosphere (Reinhold-Hurek et al., 2015). Therefore, additional studies that consider crop management and cultivars should provide more comprehensive insight into the root-associated bacterial biogeography of agricultural ecosystems.

Among the three compartments, a distance–decay relationship was only observed in the bulk soil. However, an effect of geographic distance on bacterial community composition emerged when the community turnover was examined across distance classes, and spatial autocorrelation range was found not the same among compartments. This provides further evidence that bacteria displayed different biogeographic patterns in the bulk soil, rhizosphere, and endosphere in soybean fields. This distance effect could be due to spatially autocorrelated environmental factors or dispersal limitation (Hanson et al., 2012; Liu et al., 2015). Therefore, such distinguished distance effects may support the aforementioned distinct environment–distribution relationship, or instead point to the differentiation of bacterial dispersal rates among the compartments.

4.3. Processes governing local community composition

In addition to community turnover, we also determined the relative influence of ecological processes that may govern local community composition. The median NTIs of three soil-root compartments were all significantly greater than zero in this study, suggesting that deterministic assembly processes dominated bacterial community composition at local scales (Stegen et al., 2012). A previous study has also indicated that the microbial community composition in soybean rhizosphere is driven by deterministic processes and is selected based on the functional traits beneficial to plant health (Mendes et al., 2014). Moreover, the effect of deterministic processes tended to weaken with root proximity, as indicated by the significantly decreased NTI value from bulk soil to endosphere (larger NTI value reflects greater effects (Wang et al., 2013)). Fan et al. (2017) also reported a lower NTI value in the wheat rhizosphere when compared with that of bulk soil. The differences in NTI distributions between the three compartments could be due to differences in resource availability, since deterministic processes are of greater importance in low-resource environments (Chase, 2010; Van der Plas et al., 2012; Zhang et al., 2016). Compared with the rhizosphere, bulk soils represent a niche with relatively low C availability (Philippot et al., 2013). During plant growth, various rhizodeposits are released into the rhizosphere; however, these do not provide sufficient nutrients for all rhizosphere microbes and nutrients from the soil are also required (Tkacz et al., 2015). In contrast, root habitats are rich in organic compounds (Beattie, 2015) and it has been proposed the saprophytic bacteria account for a considerable proportion of root-enriched communities in all plants (Bulgarelli et al., 2012). These imply that nutrient abundance gradually increases from the bulk soil to the endosphere. This conclusion is supported by the increase in the relative abundance of Proteobacteria from the bulk soil to the endosphere observed in our study. Proteobacteria are generally categorized as fast-growing r-strategists that flourish when resources are abundant (Fierer et al., 2007).

5. Conclusions

Our study presents a continental-scale comparison of bacterial biogeographic patterns for three different soil-root compartments: bulk soils, the rhizosphere, and the endosphere, in soybean fields. The relative influence of environmental variables was greater than that of spatial factors for governing bacterial community turnover in all three soil-root compartments, and edaphic variables were more important than climatic variables. The bacterial richness and community spatial turnover in the three compartments were significantly correlated with different environmental and spatial variables, and the spatial autocorrelation range for bacteria differed among the compartments. Collectively, these results imply distinct bacterial biogeographic patterns existing in the three compartments. Although deterministic process governed local bacterial community in all compartments, its importance decreased from the bulk soil to the endosphere. This study broadens our understanding of biogeography patterns in the root-associated bacterial communities of agricultural ecosystems.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.230.

Conflict of interest

The authors declare no conflicts of interest.

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