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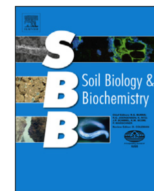

High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China

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ABSTRACT

Black soils (Mollisols) are one of the most important soil resources for maintaining food security of China and are mainly distributed in northeast China. To understand which environmental factors influence the microbial communities and how the communities are distributed in the black soils, we collected 26 soil samples with different soil carbon contents across the black soil zone in northeast China, and the soil bacterial community compositions were estimated using high resolution bar-coded pyrosequencing. A total of 355,813 bacterial 16S rDNA sequences were obtained, which were classified into at least 35 bacterial groups. The dominant groups across all samples (>5% of all sequences) were *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes* and *Planctomycetes*. The composition and diversity of the soil bacterial community were dominantly affected by both soil pH and soil total carbon content, and the effect of soil pH was stronger than that of soil carbon content. Variance partitioning analysis showed that geographic distance contributed 14.75% of the bacterial community variation, and soil environmental factors explained approximately 37.52% of the variation. Pairwise analysis showed that a relatively higher diversity of the bacterial community was observed at lower latitudes, suggesting that a latitudinal diversity gradient of the bacterial community might be present in the black soil zone. In general, our results indicated that contemporary factors, such as soil pH and soil carbon content, were more important than the historical factor of geographic distance in shaping the bacterial community in the black soil zone in northeast China.

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1. Introduction

The biogeographical distributions of macroorganisms, such as plants and animals, across large scales have been studied for centuries (Levin, 1992). In contrast, similar studies of microorganisms are still limited, although the several efforts to discover the microbial biogeography have been conducted recently (Fierer and Jackson, 2006; Ge et al., 2008; Lauber et al., 2009; Chu et al., 2010). It is known that soil microbial community diversity and composition vary distinctly between different soil ecosystems, and this variation is thought to be related to the changes in a number of soil biotic and abiotic factors (Garbeva et al., 2004; Ramette and Tiedje, 2007; Green et al., 2008). Among these factors, soil pH is often observed as an overarching factor in the dominating overall soil bacterial community (Fierer and Jackson, 2006; Baker et al., 2009; Lauber et al., 2009) and the composition of individual bacterial groups (Nicol et al., 2008; Davis et al., 2009; Jenkins et al., 2009; Jones et al., 2009). However, as stated by Rousk et al. (2010), one limitation of these studies is that we do not know whether pH itself is the factor directly shaping bacterial communities or if it is indirectly integrated with many environmental factors, such as soil nutrition, soil available organic carbon, and vegetation type, in changing the soil bacterial community. Therefore, due to the paucity of the detailed and comprehensive studies of soil bacterial biogeography, particularly across larger spatial scales, our understanding of soil microbial biogeography remains incomplete (Lauber et al., 2009).

Black soils, classified as Dark Chernozemes, also named as Mollisols, are one of the most important soil resources for crop production in China and play a crucial role in food security (Liu et al., 2012). The black soils are primarily distributed in a long and narrow area called the black soil zone, which is approximately 900 km from north to south and 300 km from east to west, in three

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provinces of Heilongjiang, Jilin and Liaoning in northeast China. In the black soil zone, the annual average temperature decreases gradually from south to north, while soil total C, N and P, available N and P (Zhang et al., 2007), soil enzyme activities and microbial biomass (Liu et al., 2008) increase from south to north. Because soil microbes play important roles in nutrient cycling (Ingham et al., 1985), we hypothesized that the soil microbial communities in the black soil zone would also be geographically distributed.

The formation of the microbial biogeographical distribution patterns is determined by two general factors, environmental heterogeneity (contemporary factor) and dispersal limitation (historical factor) (Fierer, 2008). Environmental heterogeneity drives biogeographic patterns by the commonly known hypothesis "everything is everywhere, but the environment selects" (Baas Becking, 1934; De Wit and Bouvier, 2006), suggesting the microbial species are widely distributed and the microbial community compositions are governed by ecological Drift and Selection (Stegan et al., 2013). In contrast, when dispersal limitation is the dominating factor driving the biogeographical patterns, the geographic distance should be the best predictor of genetic divergence between the communities (Fierer, 2008). In a previous study using the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method, we preliminary discovered that soil microbial communities in the black soil zone were more similar to their neighboring locations than those at greater distances (Mi et al., 2012). However, the four soil sampling sites and the limited resolution of DGGE fingerprinting that was used in that study were not able to assess the microbial biogeographical distributions and identify which environmental factors exerted the strongest influence on these distributions. Therefore, more sampling sites and a higher resolution technique are essential to study the biogeographical distribution of microbial community in the black soils of northeast China.

Recently, bar-coded pyrosequencing was prevalently used in the analysis of microbial community compositions in broad and fine scales (Acosta-Martínez et al., 2008; Lauber et al., 2009; Chu et al., 2010; Rousk et al., 2010; Xiong et al., 2012). This method can generate one million bp reads with an average length of over 400 bp and can provide a much more detailed description of the microbial communities than traditional methods, such as cloning library, DGGE, PLFA, etc; as such, this method has been shown to be a very powerful technique in microbial ecology research (Roesch et al., 2007). In this study, using the bar-coded pyrosequencing technique, we examined the structure and diversity of the bacterial communities in 26 soils from the black soil zone of northeast China. The objectives of this study were 1) to reveal the bacterial community compositions of the black soils; 2) to examine which environmental factors were important in shaping the distribution of the bacterial community structures; and 3) to compare the differences and similarities of the bacterial communities in 26 black soils.

2. Materials and methods

2.1. Site selection and soil sampling

The soil nutrition, especially soil total C content, gradually changes along the black soil zone of northeast China (Zhang et al., 2007). In this study, based on the database of China Black Soil Ecology (http://www.blackland.csdb.cn), we purposely collected 26 farmland soil samples with different soil carbon contents across a wide range of the black soil zone of northeast China from September 24 to 29, 2012. Briefly, all soils were collected near the maturity growth stage of soybean or maize. At each site, the soil samples were randomly collected from 10 tillage layers (0–20 cm) within an area of approximately 100 m^2. The soil samples were composited and sieved through a 2-mm mesh to thoroughly homogenize and remove the roots, plant residues and stones. A portion of each soil sample was collected in a 50-mL centrifuge tube, placed in an ice-box and transferred to the laboratory. The tubes were archived at −80 °C until soil DNA extraction. The remaining soils were used to measure the microbial biomass (on field-moist soil) and were then air-dried to determine the soil physicochemical properties. The locations of the field sites and their soil properties are shown in Fig. S1 and Table 1, respectively.

2.2. Soil physicochemical property and microbial biomass determination

Soil pH was measured using a pH meter after shaking the soil water (1:5 *w*/*v*) suspension for 30 min. Soil moisture was measured gravimetrically. The soil total carbon (TC) and total nitrogen (TN) were determined using an Elemental analyzer (VarioEL III, Germany). The soil microbial biomass carbon (MBC) was estimated using the chloroform-fumigation-extraction methods, and the organic C in the extracts was determined using an automated TOC Analyzer (Shimadzu, TOC-V_{CPH}, Japan); a K_{EC} of 0.45 was used to convert the difference between the organic C extracted with 0.5 M K_2SO_4 from the chloroform fumigated and unfumigated soil samples (Vance et al., 1987).

2.3. Soil DNA extraction

DNA was extracted from the soil samples (0.5 g wet weight) with E.Z.N.A Soil DNA (OMEGA, USA) according to the manufacturer's instruction. The extracted DNA was diluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at −20 °C until use.

2.4. Bar-coded pyrosequencing

An aliquot of the extracted DNA from each sample was used as a template for amplification. The V1–V3 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 27F and 533R containing the A and B sequencing adapters (454 Life Sciences). The sequence of the forward primer (B-27F) was 5′-*CCT ATC CCC TGT GTG CCT TGG CAG TCC GACT* AGA GTT TGA TCC TGG CTC AG-3′, and the sequence of the reverse primer (A-533R) was 5′-*CCA TCT CAT CCC TGC GTG TCT CCG ACG ACT* NNN NNN NNN NNN TTA CCG CGG CTG CTG GCAC-3′. The sequences of the A and B adapters are shown in italics and are underlined, and the Ns represent a twelve-base sample specific barcode sequence. PCR reactions were performed in a 25 μL mixture containing 0.5 μL of each primer at 30 μmol L^{-1}, 1.0–1.5 μL of template DNA (10 ng), and 22.5 μL of Platinum PCR SuperMix (Invitrogen, Shanghai, China). The following thermal program was used for amplification: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by an extension at 72 °C for 10 min. Each sample was amplified in triplicate, and the PCR products were pooled and purified using the Agarose Gel DNA purification kit (TaKaRa). An equal amount of the PCR product from each sample was combined in a single tube to be run on a Roche FLX 454 pyrosequencing machine at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

2.5. Processing the pyrosequencing data

Considering the sources of errors described in previous studies that used 454 sequencing (Dohm et al., 2008), in this study, only sequences >200 bp in length, with an average quality score >25, without ambiguous base calls and with at least an 80% match to a

Table 1
Soil physical and chemical properties used for this study.

Sample	Location	Latitude and longitude	Crop	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	Moisture (%)	SMBC[a] (mg kg^{-1})	Number of phylotype[b]	Phylogenetic diversity[b]
CT1	Cangtu 1, Liaoning	42°50′N, 124°07′E	Maize	5.68	14.04	1.17	22	118	4000	181.70
CT2	Cangtu 2, Liaoning	43°05′N, 124°20′E	Maize	5.46	14.58	1.02	21	119	3816	159.24
LS	Lishu, Jilin	43°20′N, 124°28′E	Maize	6.02	11.77	0.99	25	64	4171	193.66
GZL	Gongzhuling, Jilin	43°26′N, 124°43′E	Maize	5.50	14.40	1.12	23	142	3375	137.69
CC	Changchun, Jilin	43°37′N, 125°34′E	Maize	4.95	15.59	1.26	21	111	2958	123.12
DH1	Dehui 1, Jilin	44°12′N, 125°33′E	Maize	4.79	17.45	1.44	22	123	2865	115.86
DH2	Dehui 2, Jilin	44°31′N, 125°45′E	Maize	4.56	14.26	1.30	22	178	2632	119.48
YS	Yushu, Jilin	44°53′N, 126°14′E	Maize	5.27	20.03	1.74	22	278	3428	129.65
FY	Fuyu, Jilin	45°06′N, 126°11′E	Maize	5.78	19.97	2.03	18	179	3774	155.97
SC	Shuangcheng, Heilongjiang	45°23′N, 126°22′E	Maize	6.53	17.02	1.68	23	201	3740	165.42
HRB	Harbin, Heilongjiang	45°41′N, 126°38′E	Soybean	6.57	26.36	1.69	23	319	3958	189.75
HL1	Hulan, Heilongjiang	46°06′N, 127°02′E	Maize	5.18	19.76	1.42	22	148	2794	93.49
BY	Bayan, Heilongjiang	46°23′N, 127°11′E	Maize	5.87	26.41	1.90	25	222	3492	116.39
SH	Suihua, Heilongjiang	46°41′N, 126°58′E	Maize	5.18	18.91	1.41	24	109	3207	101.22
SL	Suiling, Heilongjiang	47°13′N, 127°07′E	Maize	5.19	27.07	1.90	30	144	3085	101.40
HL	Hailun, Heilongjiang	47°27′N, 126°55′E	Maize	5.42	29.97	2.12	27	176	3289	113.83
BQ	Baiquan, Heilongjiang	47°35′N, 126°07′E	Maize	4.98	23.41	1.95	26	274	2816	110.77
KD	Kedong, Heilongjiang	48°09′N, 126°13′E	Soybean	5.41	32.03	2.45	24	323	3061	92.06
BA	Beian, Heilongjiang	48°09′N, 126°43′E	Soybean	6.10	53.53	4.25	40	595	3681	124.08
WC1	Wudalianchi 1, Heilongjiang	48°28′N, 126°15′E	Soybean	5.43	29.92	2.36	28	279	3298	101.55
WC2	Wudalianchi 2, Heilongjiang	48°52′N, 126°08′E	Soybean	5.39	36.76	3.06	35	292	2971	97.43
NH1	Nehe 1, Heilongjiang	48°41′N, 124°59′E	Maize	5.35	24.78	1.93	28	218	3327	130.41
NH2	Nehe 2, Heilongjiang	48°23′N, 124°55′E	Soybean	5.97	23.68	1.84	25	195	2945	97.44
NJ1	Nenjiang 1, Heilongjiang	49°08′N, 125°37′E	Maize	5.53	31.71	2.50	28	227	2770	84.61
NJ2	Nenjiang 2, Heilongjiang	49°26′N, 125°26′E	Wheat	5.17	37.23	2.96	33	439	3150	107.33
NJ3	Nenjiang 3, Heilongjiang	49°07′N, 125°13′E	Soybean	5.32	20.63	1.64	26	232	3147	113.61

[a] SMBC: soil microbial biomass carbon.
[b] Those data were calculated from 9000 sequences per soil sample. The number of phylotype was calculated by sequences at the 97% similarity level, and the phylogenetic diversity was estimated using Faith's index.

previously determined 16S rRNA gene sequence using the Ribosomal Database Project (RDP) pyrosequencing pipeline (http://pyro.cme.msu.edu/) were included in the subsequent analyses. The trimmed and unique sequences were used to define the number of operational taxonomic units (OTUs) at the 97% similarity level. To correct for survey effort (number of sequences analyzed per sample), we used a randomly selected subset of 9000 sequences per sample for subsequent community analysis. The differences in the overall community composition between each pair of samples were determined using the UniFrac metric (Lozupone and Knight, 2005), which provides a robust index of community distances because it integrates across levels of taxonomic resolution (Hamady and Knight, 2009). The analyses mentioned above were performed using the MOTHUR program (http://www.mothur.org). All sequences have been deposited in GenBank short-read archive SRA095901.

2.6. Statistical analysis

Statistical analyses were performed in a similar manner to Fierer and Jackson (2006), Lauber et al. (2008) and Chu et al. (2010). The relationships between the taxonomic diversity of the groups with the geochemical features were tested with linear regression analyses using SPSS 17.0 for Windows. Phylogenetic diversity was estimated using Faith's index, which incorporates the phylogenetic breadth across taxonomic levels (Faith, 1992; Faith et al., 2009). Pairwise UniFrac distances calculated for the total community analyses were visualized using non-metric multidimensional scaling (NMDS) plots as implemented in PRIMER v6 (Clarke and Warwick, 2001). BioEnv and canonical correspondence analysis (CCA) were also used to identify the abiotic factors that are most important to bacterial community composition, and these results were used to construct the soil property matrix for variation partitioning analysis in R v.2.8.1 with the vegan package. Meanwhile, the Cluster analysis of the bacterial communities based on the NMDS dissimilarity matrix was applied using the "picante" and "vegan" packages in the R environment (R Development Core Team, 2006).

3. Results

3.1. Soil physicochemical property and MBC

The general physicochemical characteristics and MBC of the 26 soil samples are summarized in Table 1. Soil pH varied from 4.56 to 6.57. Soil total C and total N varied from 11.77 to 53.53 g kg^{-1} and from 0.99 to 4.25 g kg^{-1}, respectively. Soil MBC ranged from 64 to 595 mg kg^{-1}. Pairwise geographic distance between the sampling sites ranged from 24.50 to 740.99 km (Table S1). There was no correlation between the soil pH and the geographic distances of the sampling sites ($P = 0.985$). In contrast, the total soil C ($r = 0.655$, $P < 0.001$), total soil N ($r = 0.554$, $P = 0.003$), and soil MBC ($r = 0.418$, $P = 0.034$) were significantly correlated with geographic distance, and these parameters were generally greater at higher latitudes than at lower latitudes (Table 1). The soil C was significant positively correlated with the soil N ($r = 0.971$, $P < 0.001$) and soil MBC ($r = 0.871$, $P < 0.001$).

3.2. Distribution of taxa and phylotypes

In total, we obtained 355,813 quality sequences from all 26 samples, and 9708–15,892 sequences were obtained per sample (mean = 13,685). The read lengths ranged from 200 to 586 bp, with an average of 477 bp. Of these sequences, 93.8% could be classified. When grouped at the 97% similarity level, there were 44,265 different phylotypes in all of the soils, with an average of 3992 phylotypes per sample. The dominant groups (relative abundance >5%) across all soil samples were *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*,

Bacteroidetes, *Chloroflexi*, *Gemmatimonadetes* and *Planctomycetes*, and these groups accounted for more than 78% of the bacterial sequences (Fig. 1 and Table S2). Groups of *Gammaproteobacteria*, *Verrucomicrobia*, *Nitrospirae*, *Firmicutes*, *Armatimonadetes*, *TM7*, *OD1*, *Fibrobacteres*, *Cyanobacteria*, *Elusimicrobia*, *WS3*, *SM2F11*, *Chlorobi*, and *OP3* were less abundant (relative abundance >0.1%) but were still found in most of the soils. Additionally, 12 rare groups were also identified in most of the soils (Table S2).

Soil pH closely correlated with the abundance of some dominant bacterial groups (Table 2). The relative abundance of *Gammaproteobacteria* ($r = 0.563$, $P = 0.003$), *Chloroflexi* ($r = 0.698$, $P < 0.001$) and *Nitrospirae* ($r = 0.756$, $P < 0.001$) were significantly positively correlated with soil pH, while *Alphaproteobacteria* ($r = -0.523$, $P = 0.006$), *Armatimonadetes* ($r = -0.538$, $P = 0.005$) and *Fibrobacteres* ($r = -0.593$, $P = 0.001$) were negatively correlated with soil pH (Fig. S2). Although the most abundant group of *Acidobacteria* had no significant correlation with soil pH, the relative abundance of *Acidobacteria* subgroups 1 and 3 decreased with increasing soil pH, while the relative abundance of *Acidobacteria* subgroups 4, 5, 6, 7 and 25 increased with soil pH (Fig. S3). Others bacterial groups had no significant correlation with soil pH.

Soil TC and TN contents were also shown to be closely related to the abundance of four dominant bacterial groups (Table 2). The abundance of *Actinobacteria* and *Verrucomicrobia* exhibited a highly significant positive correlation with TC ($r = 0.791$, $P < 0.001$ for *Actinobacteria*; and $r = 0.67$, $P < 0.001$ for *Verrucomicrobia*) and TN ($r = 0.727$, $P < 0.001$ for *Actinobacteria*; and $r = 0.618$, $P < 0.001$ for *Verrucomicrobia*), and the abundance of *Gammaproteobacteria* had a significant positive relationship with TC ($r = 0.448$, $P = 0.023$) but had no relationship with TN ($r = 0.358$, $P = 0.073$). The abundance of *Deltaproteobacteria* exhibited a markedly negative relationship with TC ($r = -0.533$, $P = 0.003$) and TN ($r = -0.551$, $P = 0.004$) (Fig. S4). The abundance of *Acidobacteria* and its subgroups had no relationship with the soil TC or TN contents, except *Acidobacteria* Gp7, which showed a significant positive relationship with TC ($r = 0.710$, $P < 0.001$) and TN ($r = 0.635$, $P < 0.001$). The soil C:N ratio was found to have no significant relationship with the abundance of all the dominant bacterial groups.

3.3. Bacterial community diversity

To compare the soil bacterial community diversity among all the soils, the same survey effort level of 9000 sequences were randomly selected from each sample in the sequencing library. We observed that bacterial community diversity was highly variable with respect to both phylogenetic diversity (ranged from 120 to 194) and phylotype richness (ranged from 2632 to 4171) in the 26 soils (Table 1). Pairwise analysis showed that the diversity of bacterial communities decreased with the increasing of geographic latitude (Fig. 2). Of the soil and the site characteristics that were considered, we found that soil pH was significantly positively correlated with both the phylotype richness ($r = 0.725$, $P < 0.001$) and the phylogenetic diversity ($r = 0.552$, $P = 0.003$); while, the TC ($r = -0.436$, $P = 0.026$) and TN ($r = -0.411$, $P = 0.037$) contents were negatively correlated with phylogenetic diversity; however, these characteristics were not significantly related with phylotype richness (Fig. 3). All other soil parameters were not related to bacterial diversity.

3.4. Bacterial community structure

The NMDS plots of unweighted and weighted pairwise UniFrac community distance ordinations clearly indicated that the bacterial community structures of the 26 soils were strongly influenced by soil pH (Mantel test $r = 0.429$, $P = 0.001$) (Fig. 4a and b), and the change in the bacterial communities along the pH gradient was confirmed using a regression analysis between the NMDS1 scores and soil pH (Fig. 5a). Additionally, the biplot of NMDS showed that the bacterial community structures were also influenced by soil TC content (Mantel test $r = 0.271$, $P = 0.012$) (Fig. 4c and d), and the changes in the bacterial communities along NMDS2 were closely correlated with soil TC contents (Fig. 5d). The CCA plots of the bacterial community structures also clearly showed that soil pH and soil TC were two longer arrows, and the direction of these two arrows was closely correlated with the *X* and *Y* axis, respectively (Fig. S5), which indicated that both soil pH and soil TC have strong effects on bacterial community structure.

The bacterial communities of the 26 soils were roughly clustered into four groups based on the NMDS dissimilarity matrix (Fig. 6). Group I consisted of five soils from the southern parts of the black soil zone, and the latitude of these locations ranged from 42°50′N to 44°12′N. Group II consisted of three soils with latitudes that ranged from 44°31′N to 45°06′N. Group III contained seven soils that were mainly collected in the middle of the black soil zone (exception of CT2), with latitudes ranging from 45°41′N to 47°27′N. Group IV contained 11 soils dominantly sampled from the northern

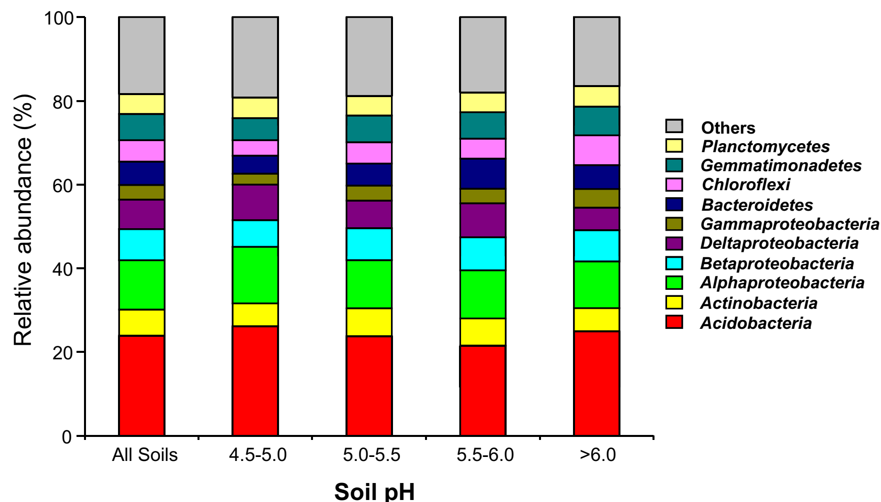

Fig. 1. Relative abundances of the dominant bacterial groups in all soils combined, and in soils separated according to pH categories. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified.

Table 2
The correlation (r) and significance (P) values of linear regressions between relative abundances of bacterial groups and soil pH, total C and N contents, and C/N ratio. Values in bold indicate significant correlations ($P < 0.01$).

Group	pH		TC		TN		C/N	
	r	P	r	P	r	P	r	P
Acidobacteria	−0.172	0.400	−0.233	0.252	−0.252	0.214	0.043	0.834
Actinobacteria	−0.014	0.945	**0.791**	<0.001	**0.727**	<0.001	−0.008	0.969
Alphaproteobacteria	**−0.523**	0.006	−0.169	0.410	−0.124	0.547	−0.194	0.343
Betaproteobacteria	0.294	0.145	0.312	0.121	0.299	0.137	0.109	0.596
Deltaproteobacteria	−0.223	0.274	**−0.533**	0.003	**−0.551**	0.004	0.037	0.858
Gammaproteobacteria	**0.563**	0.003	0.448	0.023	0.358	0.073	0.295	0.143
Bacteroidetes	0.327	0.103	0.264	0.193	0.277	0.17	−0.045	0.827
Chloroflexi	**0.698**	<0.001	−0.233	0.253	−0.21	0.304	−0.107	0.604
Gemmatimonadetes	0.698	0.047	0.283	0.161	0.309	0.124	−0.064	0.757
Planctomycetes	0.127	0.536	−0.004	0.986	0.019	0.925	−0.122	0.551
Armatimonadetes	**−0.538**	0.005	0.074	0.720	0.082	0.69	0.002	0.992
Fibrobacteres	**−0.593**	0.001	−0.114	0.580	−0.121	0.558	−0.005	0.979
Firmicutes	0.084	0.684	0.066	0.748	0.056	0.787	0.04	0.845
Nitrospirae	**0.756**	<0.001	−0.257	0.205	−0.258	0.204	−0.004	0.984
Verrucomicrobia	−0.247	0.224	**0.67**	<0.001	**0.618**	<0.001	0.291	0.149

parts of the black soil zone (exception of SC), and the latitude of these locations ranged from 47°35′N to 49°26′N. Thus, the bacterial community structures across the 26 soils were significantly correlated with the historical factor of geographic distance (Fig. 7), and the soils collected from the distant locations harbored more distinct bacterial communities than those collected in close proximity to each other.

3.5. Bacterial community links to soil properties and geographic distance

Variance partitioning analysis was conducted to quantify the relative contributions of the soil characteristics and geographic distance to the bacterial community structure (Xiong et al., 2012). A subset of soil environmental parameters of pH, TC, TN, and moisture was selected by the BioEnv procedure. The combination of the selected soil parameters and geographic distances showed a significant ($r = 0.405$, $P = 0.001$) correlation with the bacterial community structure. These variables explained 52.27% of the bacterial community variation, leaving 47.73% of unexplained variation. The geographic distance explained 14.75% of the bacterial community variation. Among the selected soil parameters, soil pH, TC, TN and moisture explained 15.31%, 7.88%, 7.43% and 6.90% of the bacterial community variation, respectively (Fig. 8). Therefore, the contemporary factors of soil characteristics were more important than the historical factor of geographic distance in determining the distribution of the black soil bacterial communities.

4. Discussion

4.1. Black soil properties

A previous study analyzing 1400 soil samples (0–20 cm) from the black soil zone indicated that the soil properties, including total C, total N, total P, and available N and K, decreased from north to south in the black soil zone in northeast China (Zhang et al., 2007). However, the soil pH and soil microbial biomass were not described in that paper. In this study, we collected 26 agricultural soil samples across a 741 km distance in the black soil zone, and we found that the soil C and N contents, as well as the MBC, greatly changed, and the highest positive relationship among soil C, soil N and soil MBC existed in the black soils. However, for the soil pH, the largest difference among the tested soils was only 2.01 pH units (Table 1). Therefore, before doing this study, we hypothesized that the soil C and N contents, rather than the soil pH might, have stronger influences in shifting the biogeographic distribution of soil bacterial communities in the black soil zone.

4.2. Bacterial community composition in the black soils

Although we obtained an average of 13,685 sequences per soil, the number of phylotypes (defined at the 97% sequence similarity level) still increased with the sequencing effort. This means that we still have not fully surveyed the bacterial composition within the individual soils, suggesting that the diversity of the bacterial

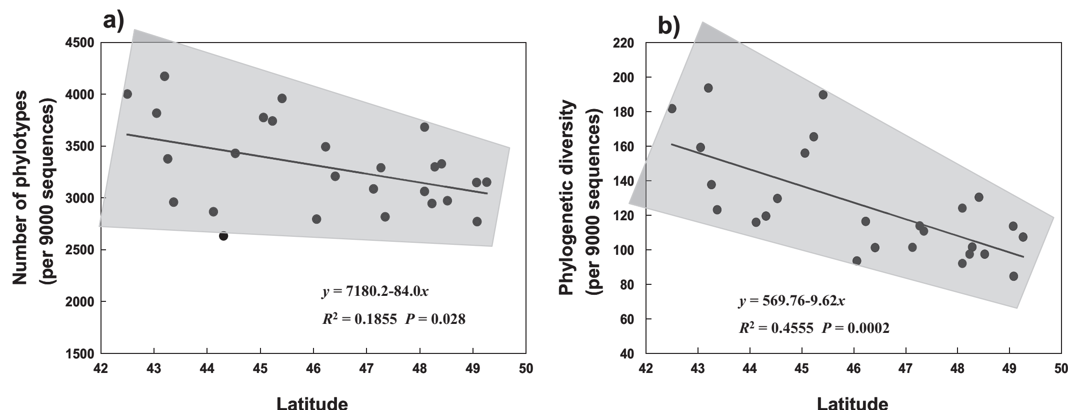

Fig. 2. The relationship between soil bacterial OTUs phylotype richness (a) or phylogenetic diversity (b) and the latitude of soil sampling locations.

Number of phylotypes (per 9000 sequences)
4500 4200 3900 3600 3300 3000 2700 2400
4 5 6 7
Soil pH
r = 0.725, P < 0.001

Phylogenetic diversity (per 9000 sequences)
200 180 160 140 120 100 80 60
4 5 6 7
Soil pH
r = 0.552, P = 0.003

Number of phylotypes (per 9000 sequences)
4400 4200 4000 3800 3600 3400 3200 3000 2800 2600 2400
0 10 20 30 40 50 60
Soil carbon content (g·kg^{-1})
r = -0.127, P = 0.538

Phylogenetic diversity (per 9000 sequences)
200 180 160 140 120 100 80 60
0 10 20 30 40 50 60
Soil carbon content (g·kg^{-1})
r = -0.436, P = 0.026

Number of phylotypes (per 9000 sequences)
4400 4200 4000 3800 3600 3400 3200 3000 2800 2600 2400
0 1 2 3 4 5
Soil nitrogen content (g·kg^{-1})
r = -0.125, P = 0.544

Phylogenetic diversity (per 9000 sequences)
200 180 160 140 120 100 80 60
0 1 2 3 4 5
Soil nitrogen content (g·kg^{-1})
r = -0.411, P = 0.037

Fig. 3. The relationship between soil pH, soil carbon content, soil nitrogen content and bacterial OTUs phylotype richness and phylogenetic diversity. The communities were randomly sampled at the 9000 sequences level.

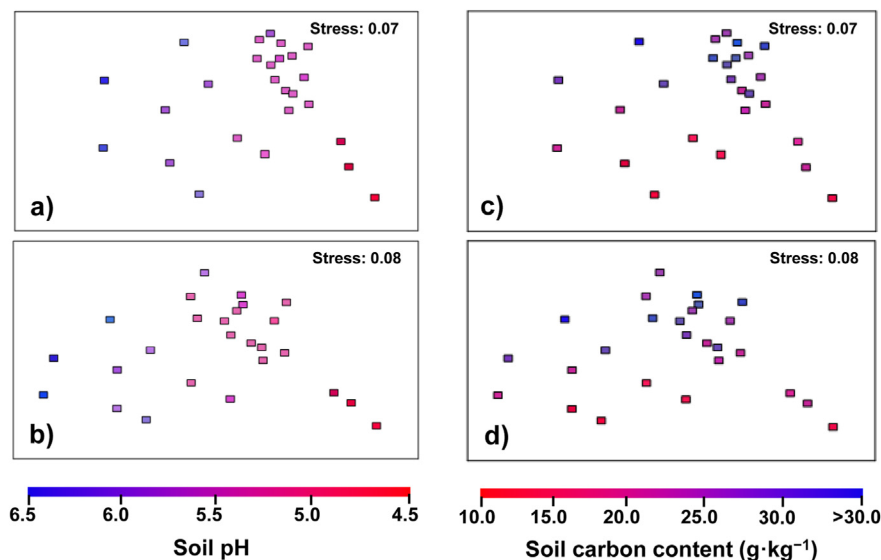

Fig. 4. Bacterial community compositional structure in black soils as indicated by non-metric multi-dimensional scaling plots of unweighted (a and c) and weighted (b and d) pairwise UniFrac community distances between sites. Sites have been colour-coded to soil pH gradient (a and b) and soil total carbon gradient (c and d).

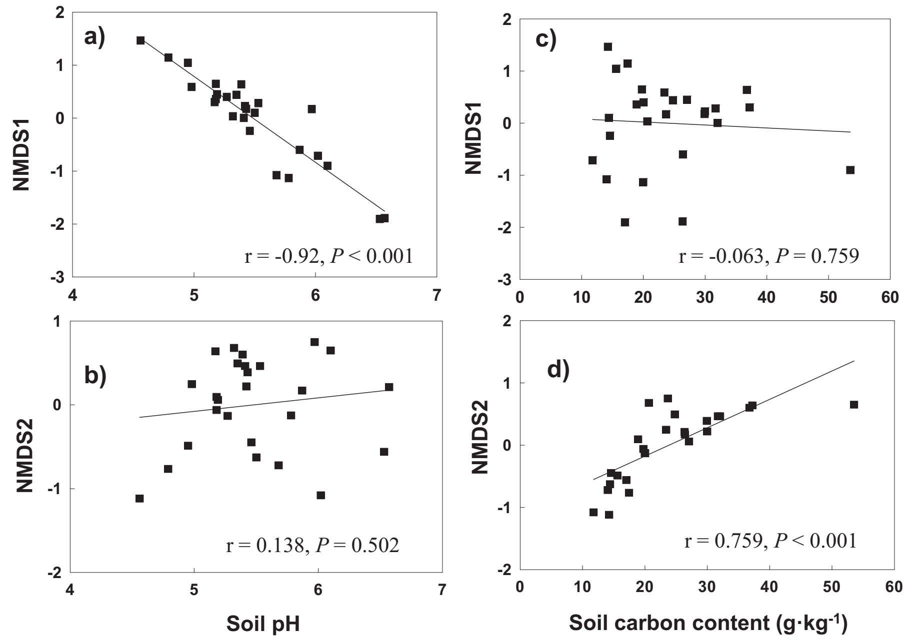

Fig. 5. Pairwise regression between NMDS1 scores and soil pH (a) and soil carbon content (c), and between NMDS2 scores and soil pH (b) and soil carbon content (d).

community in black soil is very high. Because most soil bacterial taxa are rare (Elshahed et al., 2008), it is impossible to detect the full extent of bacterial diversity in a single soil, even if a full pyrosequencing was conducted (Roesch et al., 2007; Fulthorpe et al., 2008). Therefore, the pyrosequencing depth of this study is suitable for the purpose of this research.

All of the bacterial communities in the black soils were dominated by seven major groups (*Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, and *Planctomycetes*). The dominant phyla *Acidobacteria*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* observed in this study roughly corresponded with 88 soils across North and South America as

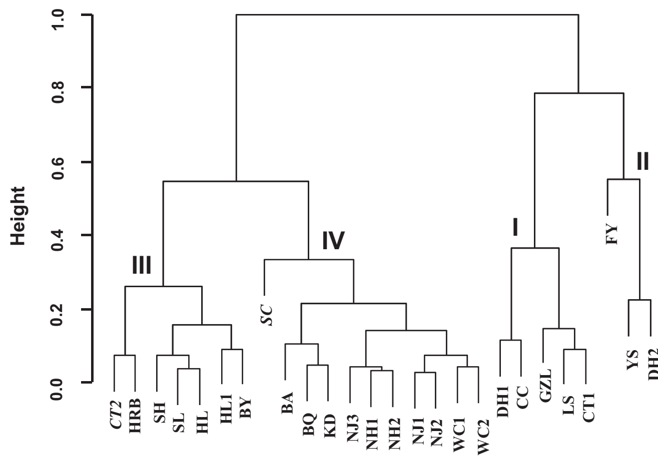

Fig. 6. Cluster analysis of bacterial communities based on NMDS (non-metric multidimensional scaling) dissimilarity matrix. CT1 and CT2 represent locations of Cangtu 1 and Cangtu 2 from Liaoning province, respectively; LS, GZL, CC, DH1, DH2, YS, and FY represent locations of Lishu, Gongzhuling, Dehui 1, Dehui 2, Yushu, and Fuyu from Jilin province, respectively; SC, HRB, HL1, BY, SH, SL, HL, BQ, KD, BA, WC1, WC2, NH1, NH2, NJ1, NJ2, and NJ3 represent locations of Shuangcheng, Harbin, Hulan, Bayan, Suihua, Suiling, Hailun, Baiquan, Kedong, Beian, Wudalianchi 1, Wudalianchi 2, Nehe 1, Nehe 2, Nenjiang 1, Nenjiang 2, and Nenjiang 3 from Heilongjiang province, respectively.

reported by Lauber et al. (2009) and were also consistent with 29 soils observed in the Arctic (Chu et al., 2010). Whereas, the relative average abundance of *Chloroflexi*, *Gemmatimonadetes* and *Planctomycetes* in the black soils was nearly 5% (Table S2), which is five times higher than the abundances of those phyla reported by Lauber et al. (2009) and Chu et al. (2010) (less than 1%). It should be noted that the samples involved in the studied by Lauber et al. (2009) and Chu et al. (2010) were collected from nonagricultural soils and the samples of this study were from agricultural soils, the differences of relative abundances of bacterial groups in this study with those reported in pristine ecosystems, suggesting that the bacterial community compositions in black soils might be altered by agricultural practices. More researches to confirm this suggestion by comparison of microbial communities between pristine and agricultural black soils need to be conducted in the future.

4.3. Effects of soil pH

Soil pH has been recently documented in various soils as the major factor in determining the soil bacterial diversity and composition. For example, soil pH has been shown to influence bacterial communities in soils across North and South America (Fierer and Jackson, 2006; Lauber et al., 2009), British soils (Griffiths et al., 2011), Arctic soils (Chu et al., 2010), Changbai Mountain soils (Shen et al., 2013), and the Hoosfield acid strip soils (Rousk et al., 2010). It should be noted that the samples used in these studies were different soil types and the soil pH changed greatly, or the samples were collected from one soil type that had large changes in soil pH but minimal variation in other soil parameters (Rousk et al., 2010). These studies raise the question of whether soil pH still acts as the dominant factor in determining bacterial community in a type of soil where the other soil properties change more than the soil pH. In this study, although the biggest difference in soil pH was only 2.01 pH units and large changes in soil C and N contents were detected in the 26 soils, we still observed that the bacterial community composition, phylotype richness and phylogenetic diversity were significantly correlated with soil pH (Table 2 and Fig. 3). Thus,

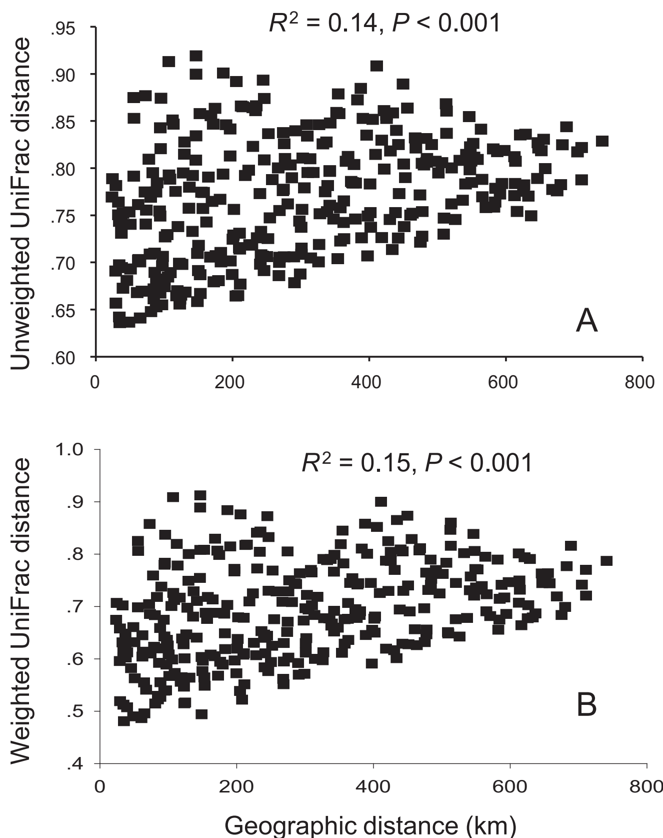

Fig. 7. Relationships between geographic distances and bacterial community distances as indicated by the unweighted (A) and weighted (B) pairwise UniFrac differences in phylotype composition between sites.

our results further emphasized that soil pH plays an important role in shifting the bacterial community composition in black soils.

The effects of soil pH on the relative abundance of some bacterial groups in this study are different from others studies. For example, the relative abundance of *Acidobacteria* has often been observed to increase toward lower pHs (Männistö et al., 2007; Jones et al., 2009; Lauber et al., 2009; Dimitriu and Grayston, 2010; Chu et al., 2010; Rousk et al., 2010; Shen et al., 2013). However, this trend was not observed in this study. We ascribed this disagreement to the differential responses of the *Acidobacteria* subgroups to soil pH. The relative abundances of *Acidobacteria* subgroups 1 and 3 were negatively correlated with soil pH in this study (Fig. S3) and in other reports (Jones et al., 2009; Rousk et al., 2010). In contrast, *Acidobacteria* subgroups 4, 5, 6, and 7 were positively correlated with higher soil pHs in this study (Fig. S3) and in the study conducted by Rousk et al. (2010). Thus, the positive or negative responses of the *Acidobacteria* subgroups with soil pH result in irregular changes in the abundance of *Acidobacteria* in the black soils. The positive relationship between the abundance of *Actinobacteria* and *Bacteroidetes* and soil pH have been reported in different soils (Lauber et al., 2009; Chu et al., 2010) but were not observed in black soils in this study. Furthermore, the relationship between the relative abundance of *Alphaproteobacteria* and soil pH was significantly negative in black soils and Changbai Mountain soils (Shen et al., 2013) but was positive in soils across North and South America (Lauber et al., 2009) and the Arctic (Chu et al., 2010). Additionally, several bacterial groups, such as *Gammaproteobacteria*, *Chloroflexi*, *Armatimonadetes*, *Fibrobacteres* and *Nirtrospirae*, were observed as positively or negatively correlated with soil pH in this study (Fig. S2), and those relationships had not been previously discerned. These findings indicated that the impact of soil pH on the distribution of bacterial groups in black soils was different from other soils, suggesting that there are other soil parameters, such as soil C content, that contributed to the bacterial community compositions in black soils.

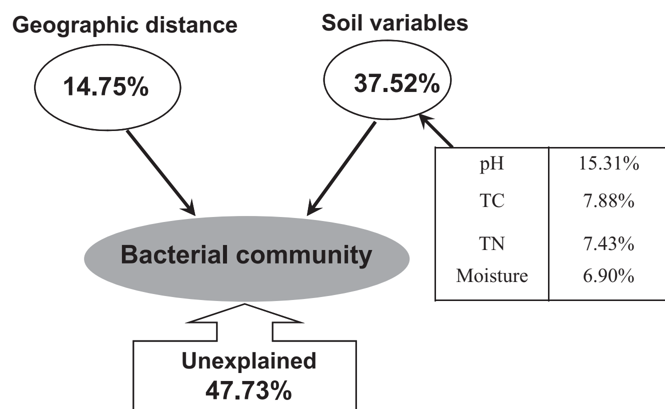

Fig. 8. Variation partition analysis of the effects of geographic distance and sediment variables on the phylogenetic structure of bacterial communities.

4.4. Effects of soil C content

Although pH has been found to be the prevailing environmental factor in shaping soil bacterial community compositions, other factors were also shown to mediate the geographic distribution of the microbial communities in various environments. For example, estuarine bacterioplankton communities change along a salinity gradient (Crump et al., 2004), cyanobacterial communities in hot springs shift with temperature (Ward et al., 1998) and the bacterial structures in hypersaline soils and sediment soils are significantly correlated with pH, organic carbon and phosphorus contents (Hollister et al., 2010). In this study, soils with distinctly different total C contents within the black soil zone were sampled, and we expected to reveal the influence of soil C content on the bacterial community compositions. Given that the soil total C and N contents in the black soils are significantly positively correlated; therefore we just discussed the relationship between soil total C and bacterial community structures in this paper.

Several small scale studies, such as within a field treated with different fertilizers, confirmed that soil bacterial communities changed with soil C contents (Cookson et al., 2005; Eilers et al., 2010; Shen et al., 2010). Because the 'paradox of scale' exists in the study of bacterial biogeography, the factors involved in shifting bacterial distribution patterns within a small scale may not be necessarily important in the large scale (Ganderton and Coker, 2005; Fierer, 2008). In this study, the NMDS plots of the soil samples indicated that the changes of soil bacterial community compositions along NMDS1 and NMDS2 were significant closely with the soil pH and soil C gradients, respectively (Fig. 5), suggesting that soil C content is another important parameter in determining the black soil bacterial community structures.

Fierer et al. (2007) collected 71 soil samples from a wide range of ecosystems across North America and found that the C mineralization rate was the best predictor of bacterial phylum-level abundances. They found a negative correlation between *Acidobacteria* abundance and C mineralization rates ($P < 0.001$) and positive correlations between the abundances of *Betaproteobacteria* and *Bacteroidetes* and C mineralization rates ($P < 0.001$). In this study, we did not measure the C mineralization rates in the 26 soils;

however, we did find that the relative abundances of four bacterial groups, *Actinobacteria*, *Verrucomicrobia*, *Gammaproteobacteria* and *Deltaproteobacteria*, had significantly positive or negative relationships with soil C contents in black soils (Table 1). Recently, Sul et al. (2013) discovered that SOC was the most important factor to explain the differences in the soil bacterial community structures in a tropical agricultural soil. They found that *Actinobacteria* and *Acidobacteria* subgroups 1 and 7 were more abundant in the low SOC plots, while *Acidobacteria* subgroups 4 and 6 were more abundant in the higher SOC plots. In the black soil, the inorganic C content is negligible; thus, the soil C content is equal to its SOC content. We found that *Actinobacteria* was more abundant in the higher C content soils (Table 2, Fig. S4), and the relationship between the abundance of *Acidobacteria* subgroups and soil C content was not correlated. Therefore, our results were inconsistent with the findings reported by Sul et al. (2013). We speculated that the different responses of the bacterial groups to soil C contents between the two studies might be related to the soils. The black soils used in this study are located in the temperate zone and have relatively higher soil C contents (1.18%–5.35%). While, the soil used by Sul et al. (2013) is classified as Haplic Lixisols located in the tropical zone and has a low SOC content (0.80%–1.67%). Thus, the response of the soil bacteria to soil C contents is not uniform, and it may vary depending on the soil type or the range of soil C contents.

4.5. Biogeographical distribution of bacterial communities in black soils

Environmental heterogeneity (contemporary factor) and dispersal limitation (historical factor) are two major processes in determining the biogeographical distribution patterns of macroorganisms (Ganderton and Coker, 2005; Lomolino et al., 2006). However, for microorganisms, the observed results varied between studies. Some studies showed that the dispersal limitation was not the primary driver of biogeographical patterns, and bacterial communities in neighboring habitats might be more dissimilar than those in remote habitats (Fenchel et al., 1997; Finlay and Clarke, 1999; Finlay, 2002; Chu et al., 2010). However, other studies indicated that soil bacterial communities were driven both by geographic distance and soil pH (Ge et al., 2008; Griffiths et al., 2011; Xiong et al., 2012). In this study, we found that 26 soil samples were clustered into four groups irrespective of whether the soils used for growing maize, soybean or wheat, and the soils within each individual group were collected closely (Fig. 6). Furthermore, a significant correlation was observed between the geographic distances and the bacterial community distances (Fig. 7). These findings suggested that the influence of crop on the black soil bacterial community is minor, and the soil bacterial communities in the black soil zone are distributed geographically. Similar to the results of Xiong et al. (2012), the geographic distance and selected four soil parameters (pH, TC, TN and moisture) explained 14.75% and 37.52% of the variation of the soil bacterial community, respectively; however, there was a large portion of the variation that was unexplained in this study (Fig. 8), suggesting that there are many unmeasured factors, such as soil temperature, soil nutrient availability and soil texture, that can influence bacterial community compositions.

The latitudinal diversity gradient is another fundamental biogeographical distribution pattern for a wide range of plant and animal taxa, and the diversity of those macroorganisms tends to increase as the latitude decreases (Lomolino et al., 2006). However, few studies have tested whether microorganisms also exhibit the latitudinal diversity gradient. Hillebrand and Azovsky (2001) hypothesized that the strength of the latitudinal gradient was positively correlated with organism size, and microorganisms, such as diatoms, are excluded from the latitudinal diversity gradient. In contrast, Fuhrman et al. (2008) reported that the diversity of the marine bacterial community decreased with increases in latitude. Other studies have found that soil bacterial diversity has no latitudinal diversity gradient (Fierer and Jackson, 2006; Chu et al., 2010). It should be noted that the samples involved in those studies were collected from broad areas with different soil types; thus, the latitudinal diversity gradient of the soil bacterial community may have been concealed by distinct soil heterogeneity. In this study, we focused on one soil type—black soils, and observed that the bacterial community structures varied spatially from the south to the north in the black soil zone. Pairwise correlation analysis showed that the bacterial diversity, as estimated by phylotype richness or phylogenetic diversity, within 9000 sequences in each soil samples presented a significant negative relationship with the latitude of the soil sampling locations (Fig. 2). This finding suggested that the bacterial latitudinal diversity gradient might exist in the black soil zone. Since the soil C contents and soil temperature are closely correlated with geographic latitude in black soil zone, we still do not know which factors lead to the occurrence of this bacterial latitudinal diversity gradient. Further detailed experiments, such as outdoor soil column moving experiments or indoor soil incubation experiments using different C contents soils, should be considered to confirm this finding in the future.

In conclusion, this study indicated that the bacterial community distributions in the black soil zone of northeast China were mainly determined by both soil pH and soil C content, and the effect of pH was stronger than that of the soil C content. Additionally, the results also showed that the geographic distance was another factor that contributed to the variation of the soil bacterial community compositions, and a relatively high diversity of the bacterial community was observed at lower latitudes. These findings suggested that, similar to soil nutrition, the bacterial communities were also spatially distributed in the black soil zone of northeast China.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.12.014.

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