Annals of Microbiology (2019) 69:1461–1473
https://doi.org/10.1007/s13213-019-01529-9

ORIGINAL ARTICLE

Soil pH is the primary factor driving the distribution and function of microorganisms in farmland soils in northeastern China

Cheng-yu Wang[1] · Xue Zhou[1] · Dan Guo[1] · Jiang-hua Zhao[1] · Li Yan[1] · Guo-zhong Feng[1] · Qiang Gao[1] · Han Yu[2] · Lan-po Zhao[1]

Received: 26 May 2019 / Accepted: 3 November 2019 / Published online: 19 December 2019

Abstract

Purpose To understand which environmental factors influence the distribution and ecological functions of bacteria in agricultural soil.
Method A broad range of farmland soils was sampled from 206 locations in Jilin province, China. We used 16S rRNA gene-based Illumina HiSeq sequencing to estimated soil bacterial community structure and functions.
Result The dominant taxa in terms of abundance were found to be, Actinobacteria, Acidobacteria, Gemmatimonadetes, Chloroflexi, and Proteobacteria. Bacterial communities were dominantly affected by soil pH, whereas soil organic carbon did not have a significant influence on bacterial communities. Soil pH was significantly positively correlated with bacterial operational taxonomic unit abundance and soil bacterial α-diversity ($P<0.05$) spatially rather than with soil nutrients. Bacterial functions were estimated using FAPROTAX, and the relative abundance of anaerobic and aerobic chemoheterotrophs, and nitrifying bacteria was 27.66%, 26.14%, and 6.87%, respectively, of the total bacterial community. Generally, the results indicate that soil pH is more important than nutrients in shaping bacterial communities in agricultural soils, including their ecological functions and biogeographic distribution.

Keywords Agricultural soil · Soil bacterial community · Bacterial diversity · Bacterial biogeographic distribution · Driving factor

Introduction

With recent developments in high-throughput sequencing technology, research in soil microbiology has also been able to well understand the important interface between environment and life sciences (Liu et al. 2014). The massive levels of soil microbial diversity are considered to drive element cycling (Delgado-Baquerizo et al. 2017a; Delgado-Baquerizo et al. 2017b), which exchanges material and energy and links the atmosphere, hydrosphere, lithosphere, and biosphere. This is important for the healthy development of soil and helps to maintain the sustainable development of soil ecosystems (Constancias et al. 2015; Delgado-Baquerizo et al. 2017a). As much as 99% of soil microbial species and their functions remain unknown, so they are known as Earth's "microbial dark matter" Lloyd et al., 2018). These microorganisms and the complex soil environment, known as the soil microbiome, are core resources in industrial and agricultural production, medicine and health, and environmental protection, and have recently received attention because of their strategic importance for improving research and technology in these areas.

Diversity, geographical distribution, and the metabolic activity of soil microorganisms are major factors shaping farmland ecosystems. Soil microbes are important decomposers and have multiple ecological and environmental functions (Nelson et al. 2016; Delgado-Baquerizo et al. 2017a; Bahram et al. 2018; Dai et al. 2018; Louca et al. 2018). They directly participate in processes involved in plant nutrient acquisition and soil nutrient cycling, such as the decomposition and accumulation of organic matter in the soil and nitrogen transformation, including biological nitrogen fixation, which are closely related to microbial activity. This was also the focus of traditional soil microbiology research,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-019-01529-9) contains supplementary material, which is available to authorized users.

✉ Han Yu
yuhan991225@126.com

[1] College of Resources and Environmental Sciences, Jilin Agricultural University, Changchun 130118, People's Republic of China

[2] College of Agriculture, Jilin Agricultural University, No. 2888 Xincheng Street, Changchun 130118, People's Republic of China

including element transformation rates, nutrient use, and the relationships between the activities of soil enzymes and their functional genes (Gul and Whalen 2016; Denk et al. 2017; Kuypers et al. 2018; Maron et al. 2018). However, owing to factors such as soil heterogeneity, resource diversity, and niche differentiation, soil microbial community composition and functions can be different in different habitats.

Although numerous previous studies have demonstrated the key role of microbial diversity in soil functions and ecosystem services under different environmental conditions (Liu et al. 2014; Delgado-Baquerizo et al. 2016; Delgado-Baquerizo et al. 2017c), little is known about the variation and determining factors of bacterial diversity in agricultural landscapes (Hanson et al. 2013). Jilin Province produces most of China's grain (Wang et al. 2011). The soil physicochemical properties and microbial diversity have changed in some areas owing to the overuse of pesticides and fertilizers, including the application of organic manure. For the present study, we investigated bacterial community diversity and functions in agricultural soil from Jilin province. We also provide insights into the distribution of soil microorganisms in these ecosystems. This research provides a deeper understanding of these soil microbial resources and improves the regulation and control of these systems for more sustainable use and development.

Material and methods

Soil sampling strategy

Soil samples were randomly collected from Agricultural soil in Antu, Changling, Da'an, Dehui, Dongfeng, Dongliao, Dunhua, Fusong, Fuyu, Gongzhuling, Huadian, Huinan, Ji'an, Jiangyuan, Jiaohe, Jingyu, Jiutai, Linjiang, Lishu, Liuhe, Meihekou, Panshi, Qianguo, Shuangliao, Shuangyang, Shulan, Songyuan, Taonan, Taobei, Tonghua, Wangqing, Yitong, Yushu, and Zhenlai, Jilin Province, China, including 206 sites (Fig. 1). All sites were described including geographic coordinates, soil types (alluvial, brown, black, chestnut, chernozem, dark brown, lessive, meadow, saline, and sandy, according to Chinese soil classification system) and the soil site environment and vegetation (corn, rice, soybean, ginseng, peanut, apple-pear, or sunflower) (Table S1). At each of the 206 sites, sampling was arranged in five randomized blocks with three replicates fully randomized within each block, giving a total of 15 soil samples. All samples of each block were taken from the topsoil (0–30 cm) and fully mixed to form a composite sample. Any plant residues, such as roots and leaves, were removed. Some of each composite soil sample were stored at − 80 °C to extract total soil DNA for high-throughput sequencing, and the rest of each samples was gently air-dried and used for physicochemical measurements. Physicochemical parameters, e.g., pH, soil organic C (SOC), and available N, P, and K were measured for each composite sample according to the methods of previous studies (Sun et al. 2015; Qi et al. 2017).

Molecular characterization of soil microbial communities

DNA was extracted from 0.25 g of each block of the 206 sites by using the MoBio PowerSoil DNA extraction kit and following manufacturer's instructions (MoBio Laboratories, Carlsbad, CA, USA). Purified soil DNA from five blocks of one site was fully mixed and then sequenced. The high-throughput pyrosequencing of the 16S rRNA gene was conducted on the Illumina HiSeq platform to determine soil microbial community composition as per previous studies (Cao et al. 2015; Zarraonaindia et al. 2015; Xu et al. 2017). Raw sequence data were quality filtered and analyzed using QIIME v 1.7.0 (Caporaso et al. 2010; Edgar et al. 2011). High-quality representative sequences for each operational taxonomic units (OTUs) were assigned using UCLUST with 97% sequence identity (Edgar et al. 2011). The V3–V4 of the 16S rRNA gene was amplified following standard protocols (Chen et al. 2016; Xu et al. 2017; Yao et al. 2017; Zhang et al. 2017). The sequencing was carried out on an Illumina HiSeq platform at Personal Biotechnologies Co., Ltd., Shanghai, China. Based on the 16S rDNA sequences, the online FAPROTAX program was used to analyze the metagenome functions of the bacterial communities according to the methods of previous research (Louca et al. 2016), which is more informative than a purely taxonomic community structure approach.

Data analysis

The sequencing results were clustered based on 97% similarity by UCLUST in QIIME (version 1.8.0) to determine operational taxonomic units (OTUs). OTUs were classified according to the Silva (Bacteria) taxonomy database annotations. Several indices, including Simpson, Shannon, ACE, and Chao1, were calculated from these OTU tables using the Mothur software (version 1.31.2). We also determined the diversity of dominant OTUs (> 5% of abundance across all samples). Using the R program, significantly different taxa were analyzed by constructing a PLS-DA discriminant model based on a species abundance matrix and sample grouping data. The variable importance in projection (VIP) coefficients of each species were calculated (VIP > 1; the higher the value, the greater the contribution of the species to inter-group differences). Correlations of occurrence patterns of the top 50 dominant genera according to a connection stands for a strong (Spearman's $\rho > 0.6$) and significant ($P < 0.01$) correlation were visualized as a network using Cytoscape (http://www.cytoscape.org/) (Shannon et al. 2003). The heatmap.2 package of R (version 3.1.1) was used to conduct the clustering analysis (Zhang et al. 2017). Pearson's correlation and Spearman's

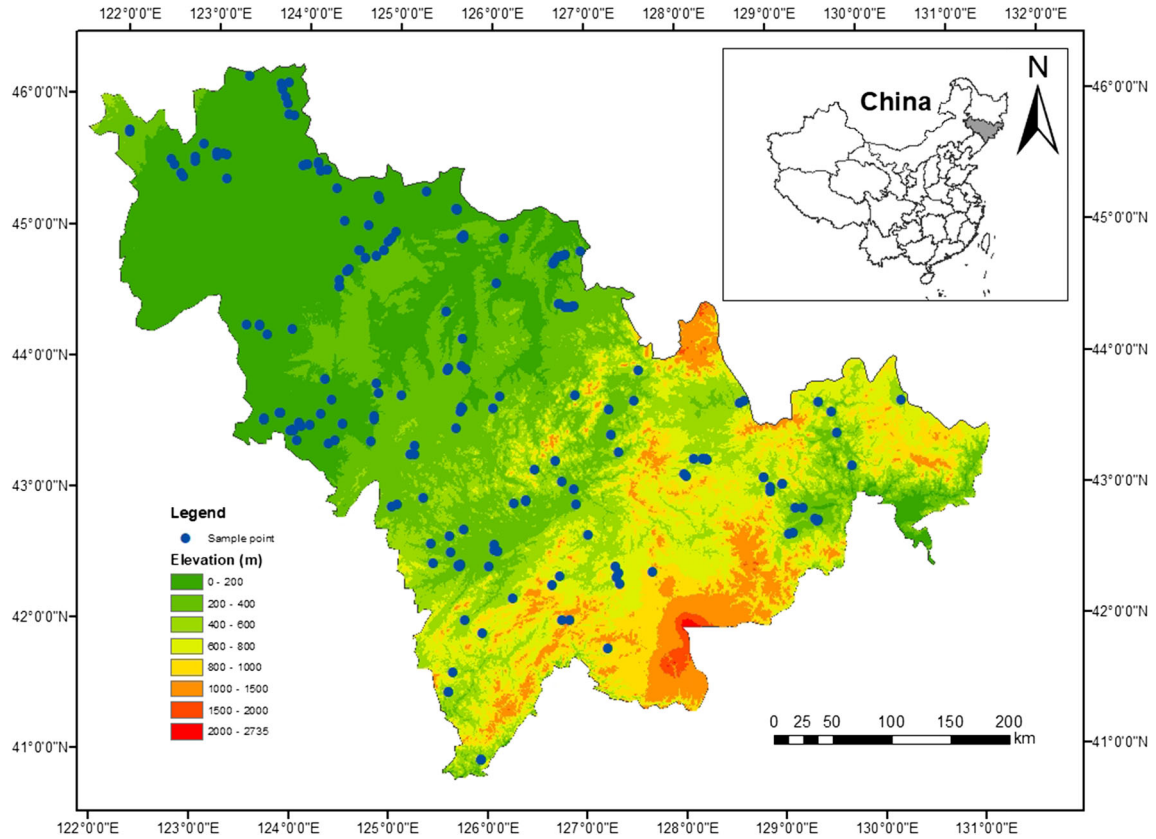

Fig. 1 Map of the agricultural soil sampling sites. The map (including the inset map of china) was generated by Guo Dan using ArcGIS 10.0 (http://www.esrichina.com.cn/softwareproduct/ArcGIS/)

correlation visual analyses were performed in R with the ggpairs function of the GGally package (version 1.4.0, https://cran.r-project.org/web/packages /GGally/index.html) (Emerson et al. 2012) and the corr.test function of the corrplot package (version 1.6.6, https://cran.r-project.org/web/packages/corrplot/index.html).

Results

Environmental and physicochemical analyses

In the present study, all soil samples were with long-term agricultural cultivation from Jilin Province in northern China (Table S1) and clustered into eight soil categories: black (lessive 20.29%; chernozem 18.84%; black 14.01%), dark brown (20.29%), sandy (9.18%), saline (5.31%), alluvium (4.35%), meadow (4.35%), brown (1.93%), and chestnut (0.97%), with acidic pH (median 6.36, Table S1). As shown in Fig. 2 and Table S2, soil pH was significantly negatively correlated with SOC, available N, and available P ($r = -0.331$, -0.333, and -0.295, respectively; $P < 0.001$). However, the longitude of the site from which the samples were taken was significantly positively correlated with soil nutrient content. There was also a significantly negative correlation between SOC content and soil pH ($r = -0.33$, $P < 0.001$).

Characterizing soil microbial communities

In total, we obtained 7,580,795 quality sequences (27,023–53,684 sequences per soil sample, mean = 36,800). The read lengths ranged from 136 to 464 bp, with an average of 314 bp. From these sequences, 2018–7240 OTUs (mean = 4974 ± 872) representing bacteria were recovered from the 206 soil samples. Such wide variation may have resulted from our extensive sampling strategy, which included various farmland soil types and vegetation to be compared. As shown in Fig. 2 and Table S2, in the soil with long-term agricultural cultivation, soil pH was significantly positively correlated with number of bacterial OTUs ($r = 0.216$, $P < 0.01$) and soil bacterial α-diversity (Simpson index, Shannon index, Chao1, and ACE, r were 0.165, 0.18, 0.169, and 0.144, respectively, $P < 0.05$). Interestingly, we also found a negative correlation between soil nutrient content (including SOC, available N, P, and K) and soil microbial community composition (including OTUs and soil bacterial α-diversity index) (Fig. 2). Soil SOC was significantly negatively correlated with number of bacterial OTUs ($r = -0.15$, $P < 0.05$). Available N and P were significant negatively correlated with Simpson index ($r = -$

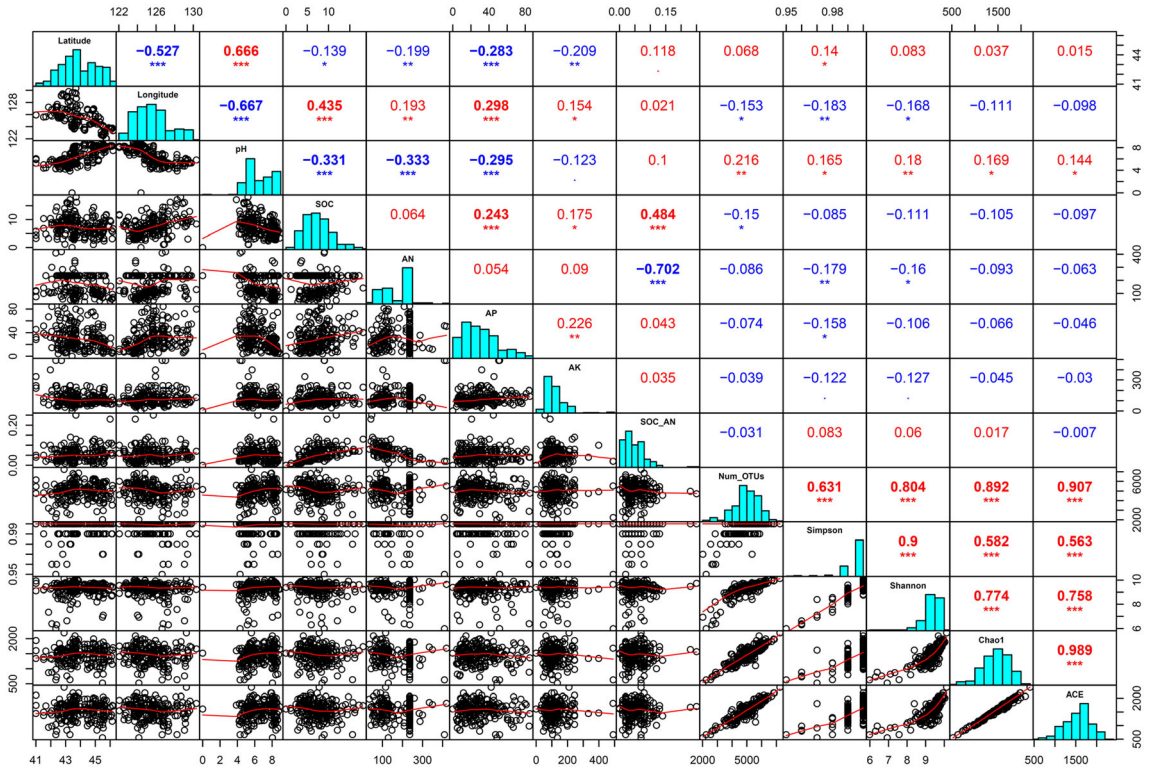

Fig. 2 Pairwise Pearson's correlation analyses between each of the two soil characteristics. Red and blue indicate positive and negative correlation, respectively. The figures demonstrate the scale of correlation. Lat Latitude, Lng Longitude, SOC Soil Organic Carbon, AN Available N, AP Available P; AK Available K, MMB Molecular Microbial Biomass. Num_OTUs are the numbers of bacterial OUTs, respectively. Simpson, Shannon, Chao1, and ACE are the α-diversity index. *Significant level (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

0.18, $P < 0.01$ and $r = -0.16$, $P < 0.05$, respectively). Available N was also significant negatively correlated with Shannon index ($r = -0.16$, $P < 0.05$) (Fig. 2).

As shown in Fig. 2, the latitude of the site from which the samples were taken was significantly positively correlated with soil pH ($r = 0.67$, $P < 0.001$) and showed a positive correlation with the number of OTUs ($r = 0.07$, $P > 0.05$) and within-habitat bacterial diversity (Simpson index, $r = 0.14$, $P < 0.01$). However, it was significantly negatively correlated with SOC, available N, P, and K ($r = -0.14$, $P < 0.05$; $r = -0.20$, $P < 0.01$; $r = -0.28$, $P < 0.001$; and $r = -0.21$, $P < 0.01$, respectively).

Bacterial community structure

All soils were classified according to soil pH showing bacterial community structures in the surface soils (Fig. 3). Diverse bacterial abundances were found at different samples. OTUs were affiliated with 38 bacterial phyla, with the dominant bacterial phyla (relative abundance > 1%) across all soil samples that were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, and Gemmatimonadetes, with relative abundances of 16.23–50.26%, 7.21–59.28%, 4.67–33.27%, 4.03–17.86%, and 3.28–10.80%, respectively (Fig. 3). The relative abundances of the minor phyla, including Cyanobacteria, Saccharibacteria, Planctomycetes, Latescibacteria, Ignavibacteriae, Tectomicrobia, Parcubacteria, Armatimonadetes, Chlorobi, Aminicenantes, Nitrospinae, Microgenomates, Deinococcus-Thermus, Elusimicrobia, Fibrobacteres, Fusobacteria, Lentisphaerae, and Chlamydiae, were all < 1%. In addition, some sequences could not be classified to known bacteria, and these had relative abundances from 0 to 2.81%.

Dominant microbial groups

Using the GraPhlAn visual tool (Asnicar et al. 2015), a hierarchical tree was constructed for the composition of all samples at each classification level. Each classification unit was distinguished by a different color, and their abundance was reflected by the size of the node. Compared to MEGAN, GraPhlAn trees provide a method for rapidly discovering the dominant microbial groups from complex community data. A phylogenetic tree of all samples was constructed basing on GraPhlAn. Across the phylogenetic tree, the dominant taxa (> 5% of total abundance across all samples) belonged to five phyla (Actinobacteria, Acidobacteria, Gemmatimonadetes, Chloroflexi and Proteobacteria), eight classes (Actinobacteria, Themoleophilia, Gemmatimonadetes, Anaerolineae, Deltaproteobacteria, Alphaproteobacteria,

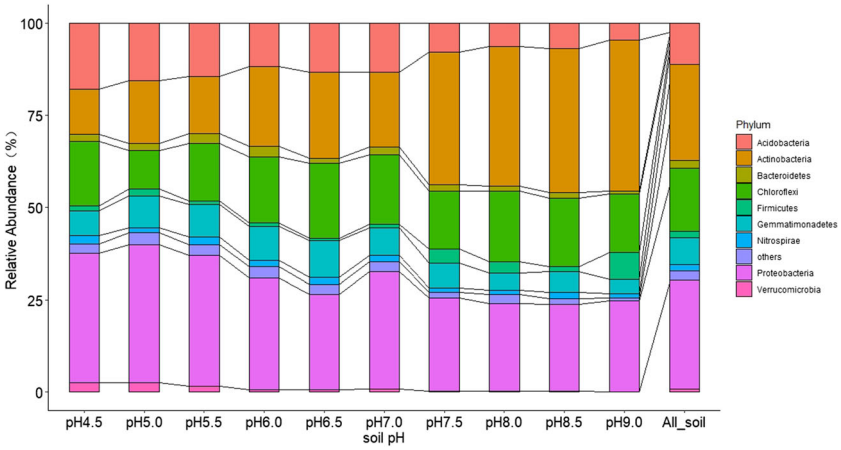

Fig. 3 Relative abundance of the bacterial group, according to pH. pH 4.5: pH ≤ 4.5 (n = 12), pH 5.0: 4.5 < pH ≤ 5.0 (n = 13), pH 5.5: 5.0 < pH ≤ 5.5 (n = 41), pH 6.0: 5.5 < pH ≤ 6.0 (n = 30), pH 6.5: 6.0 < pH ≤ 6.5 (n = 11), pH 7.0: 6.5 < pH ≤ 7.0 (n = 17), pH 7.5: 7.0 < pH ≤ 7.5 (n = 11),, pH 8.0: 7.5 < pH ≤ 8.0 (n = 25), pH 8.5: 8.0 < pH ≤ 8.5 (n = 38), pH 9.0: 8.5 < pH < 9.0 (n = 8)

Betaproteobacteria and Gammaproteobacteria), five orders (Micrococcales, Galellales, Gemmatimonadetes, Anaerolineales, and Rhizobiales), and two families (Gemmatimonadaceae and Anaerolineaceae) (Fig. 4 and Table S3). Bacterial communities differed across soil pH value. The relative abundance of the dominant phylum, Actinobacteria sequences decreased with decreasing pH and was higher in higher pH; in contrast, Proteobacteria and Acidobacteria sequences increased with decreasing soil pH value (Table S3). The relative abundance of Actinobacteria in the farmland soil from alkaline to acidic soils decreased from 40.63 to 14.86% (r = 0.31, P < 0.001) and that of Proteobacteria and Acidobacteria increased from 24.58 to 36.52% (r = −0.43, P < 0.001) and from 4.72 to 16.43% (r

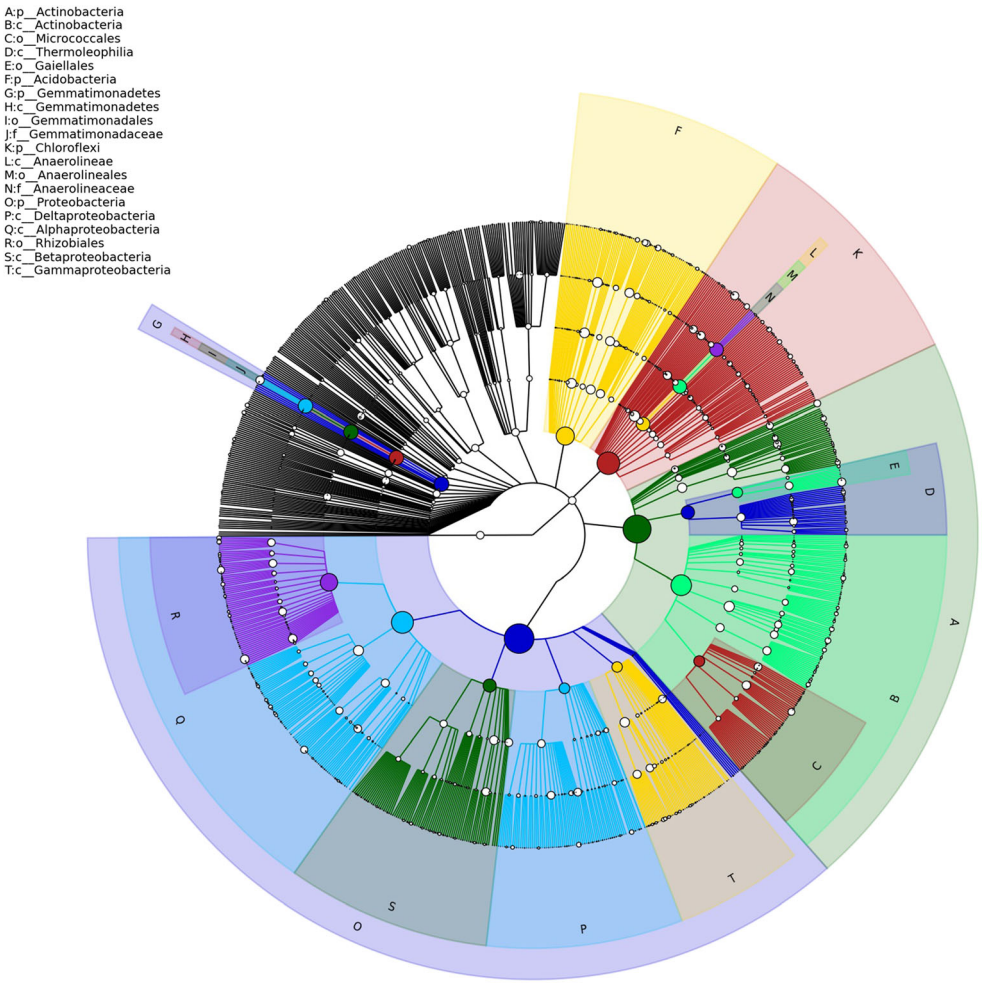

Fig. 4 Phylogenetic tree of all samples based on GraPhlAn. The phylogenetic tree showed the hierarchical relationship of all taxonomy units (represented by nodes) from phylum to genus (arranged from the inner circle to the outer circle sequentially) in all samples. The size of the nodes corresponded to the average relative abundance of the taxonomy. The top 20 taxonomy units will also be identified by letters in the graph. From the outer layer to the inner layer, the shadow color on the letter is the same as the color of the corresponding node

= 0.31, $P < 0.001$), respectively. Notably, a large diversity of phylum Gemmatimonadetes was detected (Table S3). The relative abundance of Gemmatimonadetes sequences was the highest (9.77%) at $5.5 < pH \leq 6.0$ level samples and decreased with changing soil pH value. Class Alphaproteobacteria and order Rhizobiales were the dominant group across all soil samples and were primarily observed in the acidic soils. Class Actinobacteria was the dominant group across all soil samples and was primarily observed in the alkaline soils. Class Thermoleophilia was primarily observed in the neutral and alkaline soils.

According to the composition and sequence distribution of each sample at each classification level, the differences in abundance at each taxonomic level between two or more samples (groups) were compared one by one, and the significant differences were evaluated by statistical test to determine the top 20 groups in terms of differences among samples. As shown in Fig. 5, these were Acidobacteria, Actinobacteria, Aminicenantes, Armatimonadetes, Chlamydiace, Chlorobi, Deinococcus-Thermus, Elusimicrobia, Euryarchaeota, FBP, Fibrobacteres, GAL15, Gemmatimonadetes, Latescibacteria, Parcubacteria, Proteobacteria, Saccharibacteria, TM6_(Dependentiae), WS1, and WWE3.

Network analysis of co-occurrence patterns among dominant genera

As Fig. 6 shows, Proteobacteria (47.5%), Actinobacteria (45.0%), Gemmatimonadetes (2.5%), Chloroflexi (2.5%), and Bacteroidetes (2.5%) accounted for more links in the soil habitats of farmland. *Roseiflexus* (phylum: Chloroflexi) co-occurred with *Streptomyces* and *Pseudonocardia*, *Sediminibacterium* (phylum: *Bacteroidetes*) co-occurred with *Methylobacterium*, *Gemmatimonas*, *Acinetobacter*, *Ochrobactrum*, and *Mesorhizobium*. Whereas *Gaiella* and *Sphingomonas* had less links in network, and they co-occurred only with *Rubrobacter* and *Rhodanobacter*, respectively. In contrast, Actinobacteria (including genera *Acidothermus*, *Blastococcus*, *Geodermatophilus*, *Iamia*, *Streptomyces*, *Solirubrobacter*, *Dactylosporangium*, *Rubrobacter*, and *Pseudonocardia*) and Proteobacteria (including genera *Pseudolabrys*, *Rhizomicrobium*, *Anaeromyxobacter*, *Reyranella*, *Sideroxydans*, *Skermanella*,

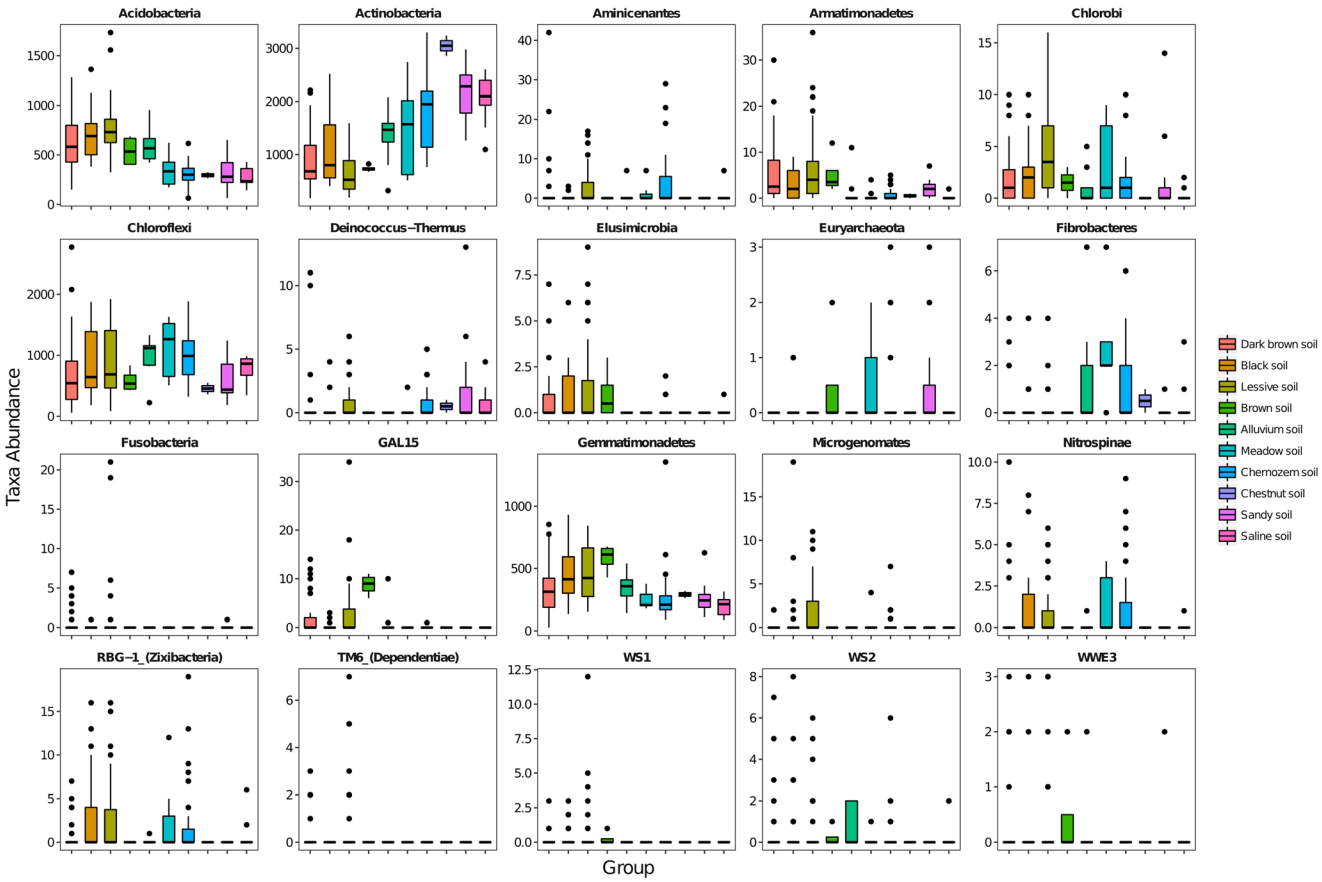

Fig. 5 Abundance distribution maps of the most significant difference between samples. The abscissa is the first 20 taxonomy with the most significant difference, and the ordinate is the sequence number of each of taxonomy in each sample. The box line border represents the interquartile range (IQR), the horizontal line represents the median, and the upper and lower tentacles represent 1.5 times the IQR range beyond the upper and lower quartiles, respectively. Black circle represents the extreme value beyond the range

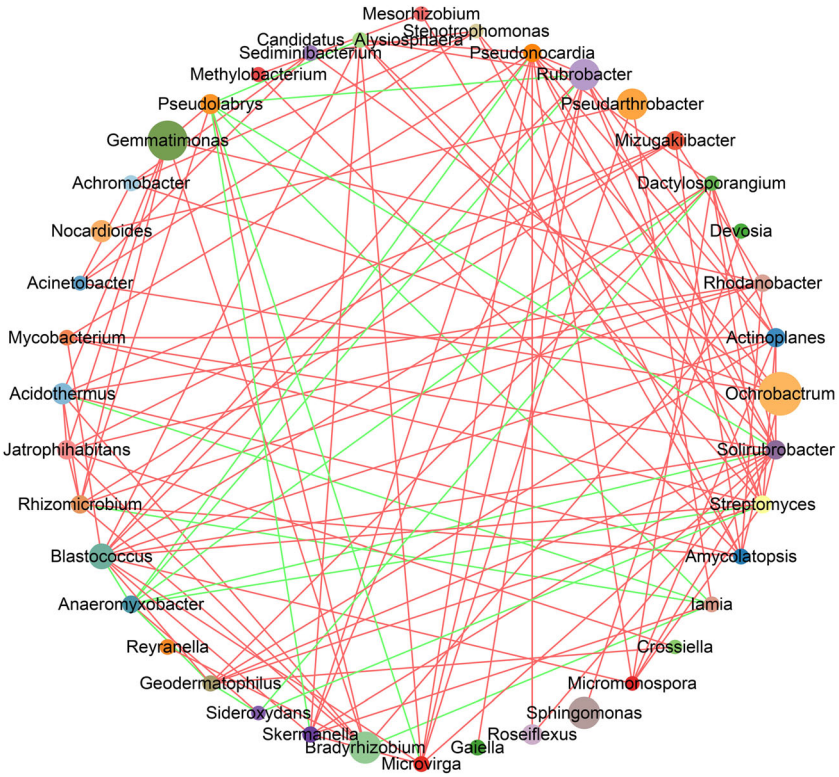

Fig. 6 Network analysis of co-occurring patterns among dominant genus based on the Spearman's $\rho > 0.6$ and $P < 0.01$. Nodes represented dominant genera and identified by different colors. The connections between nodes indicate the correlation between the two genera, red lines indicate positive correlation, and green lines indicate negative correlation. The more connections, the more association is associated with other members of the community

and *Microvirga*) were mutually exclusive in the soil habitats. Their abundance in soil was negatively correlated.

Effects of environmental factors on community composition

Relationships between environmental factors and geographical position on bacterial distribution and community composition based on OTUs were portrayed with biplots using redundancy analysis (RDA). As shown in Fig. 7, the two axes explained 5.54% and 1.11% of the variance in bacterial composition. Among all the examined soil characteristics, pH, latitude, longitude, and available N were found to have significant effects ($P < 0.05$) on the farmland bacterial community.

As Fig. 8 shows, latitude and soil pH were significantly positively correlated with the abundance of Actinobacteria (dominant bacteria in all samples and significantly different among all samples; $r = 0.51$ and 0.61, respectively; $P < 0.001$). Latitude and soil pH were also significantly negatively correlated with the abundance of Proteobacteria ($r = -0.41$ and -0.42, respectively; $P < 0.001$).

As the dominant microorganism and the significant difference microorganism, Acidobacteria responded to environmental factors that also showed negative opposite results ($r = -0.48$ and -0.62, respectively; $P < 0.001$). In all bacterial groups, not only the dominant microbial groups but also the significantly different microbial groups that were negatively correlated with soil pH showed strong nutritional preferences

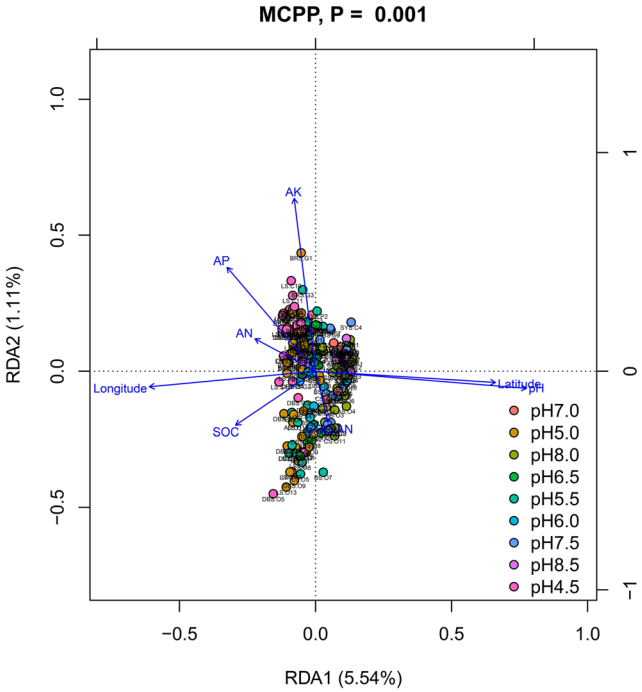

Fig. 7 Redundancy analysis of the bacterial community compositions in the soil collected from farmland. Soil samples with pH ≤ 4.5, 4.5 < pH ≤ 5.0, 5.0 < pH ≤ 5.5, 5.5 < pH ≤ 6.0, 6.0 < pH ≤ 6.5, 6.5 < pH ≤ 7.0, 7.0 < pH ≤ 7.5, 7.5 < pH ≤ 8.0, 8.0 < pH ≤ 8.5, and 8.5 < pH < 9.0 were labeled as pH 4.5, pH 5.0, pH 5.5, pH 6.0, pH 6.5, pH 7.0, pH 7.5, pH 8.0, pH 8.5, and pH 9.0, respectively

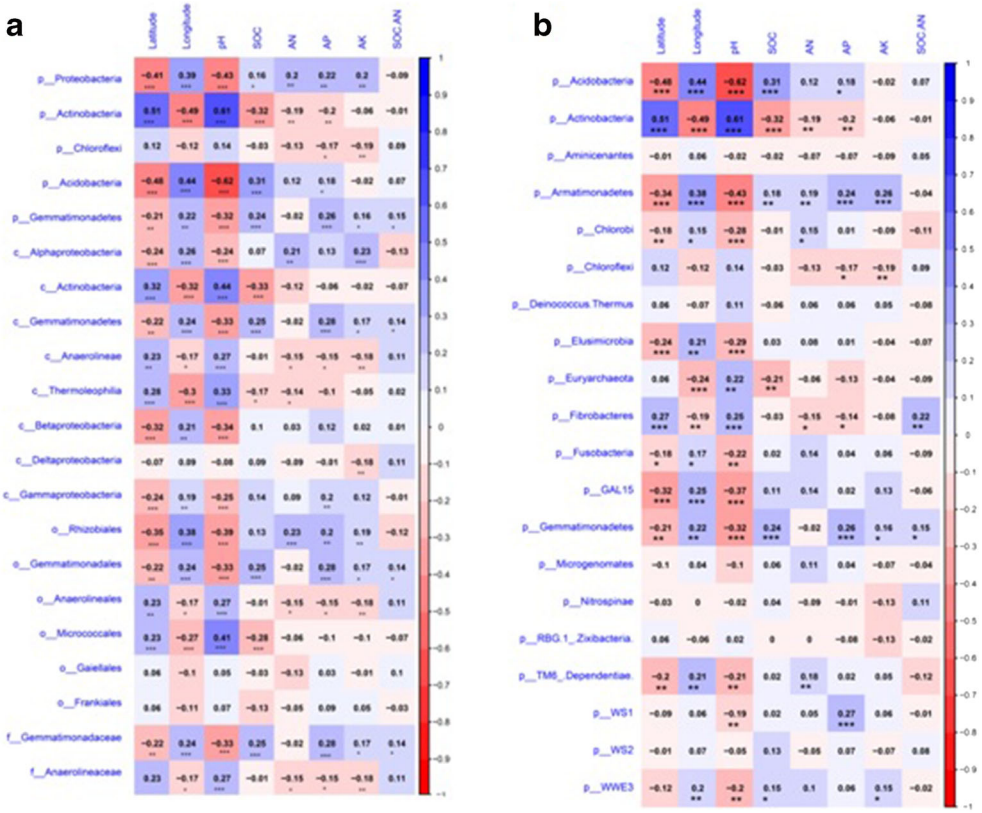

Fig. 8 The correlation analysis between soil environmental factors and bacterial relative abundance. **a** The correlation analysis between soil environmental factors and dominant bacterial relative abundance at phylum level. **b** The correlation analysis between soil environmental factors and significant different bacterial relative abundance at phylum level. The numbers display the Pearson's correlation coefficient (r). Blue and red indicate positive and negative correlation, respectively. The color density and numbers reflect the scale of correlation. *Significant level (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

(Fig. 8). The results indicate that both bacterial abundance and diversity were independent of soil pH and latitude of sampling sites.

Ecological functional diversity of bacteria in farmland soils

According to the classification results from the 16S sequences in all soils, a total of 64 functional groups were obtained using the FAPROTAX tool to annotate microbial community functions. These functional groups included 3836 OTUs, accounting for 26.42% of all OTUs (total of 14,519 records). The grouping of functions with relatively high abundance (> 1.0%) is shown in Fig. 9. As shown in Fig. 9a, microbial functional groups were divided into three groups according to pH (pH ≤ 5.0, 5.0 < pH ≤ 7.0, and pH > 7.0). The abundance of bacterial community members in soil with 5.5 < pH ≤ 7.0 was greatly different among samples (Fig. 3), but their ecological functions were similar. In soil with pH ≤ 5.0 and pH > 7.0, the ecological functions of bacterial communities were significantly different from all other soils.

As Fig. 10 and Tables S4 and S5 show, microorganisms in soil drove different soil functions, and each soil bacteria plays diverse and critical roles in these ecosystem services. In soil, for dominantly and significantly different bacteria, the ecological functions with the correlation coefficient greater than 0.9 with microbial abundance include nitrogen fixation, aromatic compound degradation, cellulolysis, and ligninolysis. Bacteria under phylum Gemmatimonadetes play a role in ecological function of nitrogen fixation. The bacteria, such as order Micrococcales, play a role in ecological function of aromatic compound degradation. The group includes the bacteria, such as order Rhizobiales and genera Corynebacterium 1, Blastochloris, and Cetobacterium which play a role in ecological function of cellulolysis. Order Gaiellales and genus Algoriphagus play a role in ecological function of ligninolysis. As Fig. 10 shows, for bacteria with abundance greater than 1% at family and genus level, soil bacteria are obviously divided into two groups according to the ecological function.

As Fig. 11 shows, pH was significantly negatively correlated with nitrification ($r = -0.22$, $P < 0.01$), aerobic ammonia oxidation ($r = -0.24$, $P < 0.001$), aerobic nitrite oxidation ($r = -0.14$, $P < 0.01$), nitrogen fixation ($r = -0.48$, $P < 0.001$), ureolysis ($r = -0.48$, $P < 0.001$), nitrite respiration ($r = -0.34$, $P < 0.001$), nitrate respiration ($r = -0.39$, $P < 0.001$), and denitrification ($r = -0.34$, $P < 0.001$), and was significantly positively correlated with nitrate reduction ($r = 0.41$, $P < 0.001$). In 206 soil samples, two records of plant pathogens were detected, all of which were *Staphylococcus aureus* (Ravn et al. 1989). As Fig. 10 shows that plant pathogen was negatively correlated with soil pH, so we inferred that soil acidification might increase the incidence of plant ($r = -0.08$, $P > 0.05$).

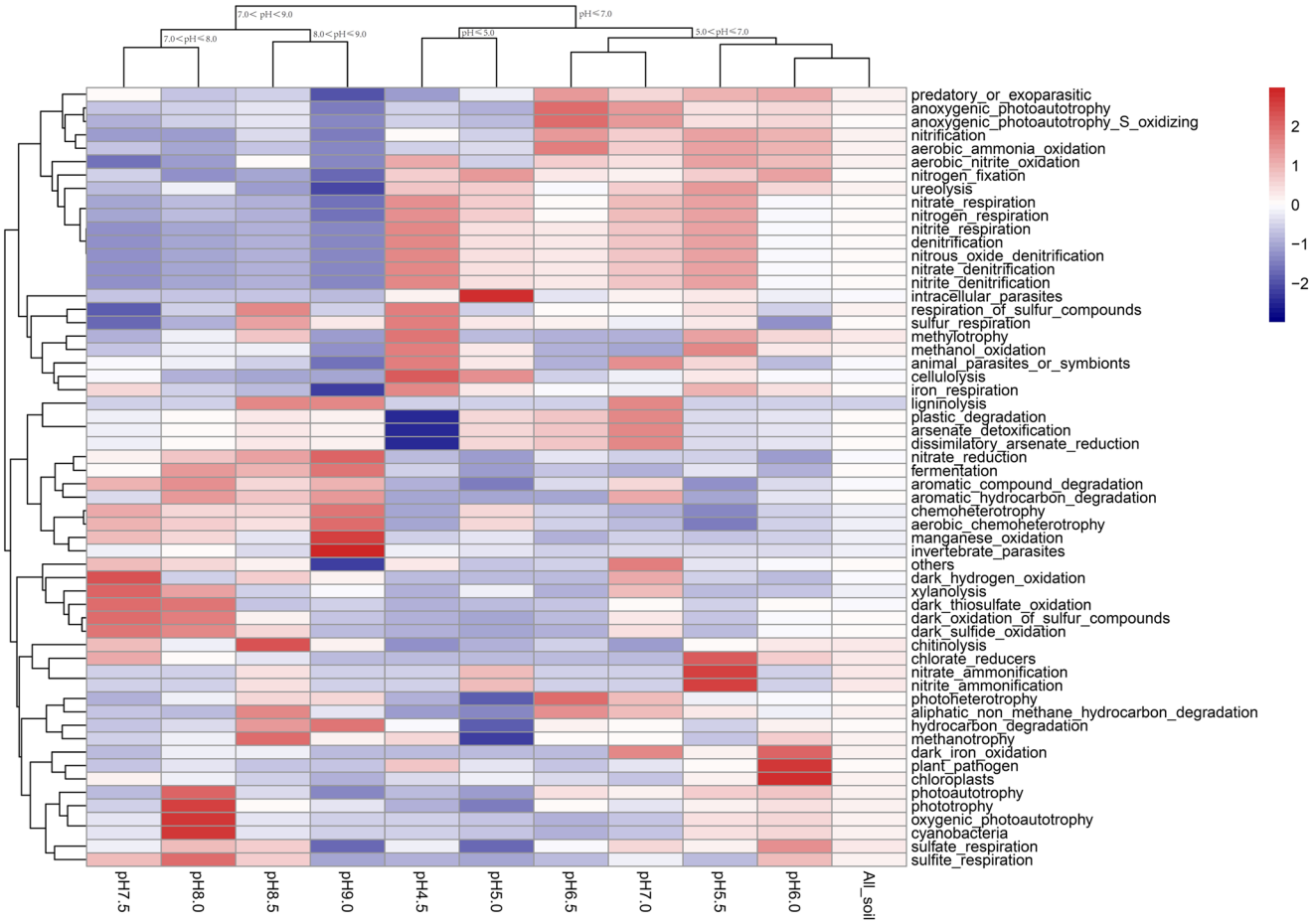

Fig. 9 Heatmap of the bacterial ecological functions according to pH. pH 4.5: pH ≤ 4.5 (n = 12), pH 5.0: 4.5 < pH ≤ 5.0 (n = 13), pH 5.5: 5.0 < pH ≤ 5.5 (n = 41), pH 6.0: 5.5 < pH ≤ 6.0 (n = 30), pH 6.5: 6.0 < pH ≤ 6.5 (n = 11), pH 7.0: 6.5 < pH ≤ 7.0 (n = 17), pH 7.5: 7.0 < pH ≤ 7.5 (n = 11), pH 8.0: 7.5 < pH ≤ 8.0 (n = 25), pH 8.5: 8.0 < pH ≤ 8.5 (n = 38), pH 9.0: 8.5 < pH < 9.0 (n = 8). Others are the ecological function of human pathogens

Discussion

Bacteria are dominant organisms in soil habitats in terms of diversity, biomass, and their effects on basic soil processes (Bahram et al. 2018). Their spatial patterns and community compositions in agricultural soils have not yet been well documented. Some recent studies of soil microbial biogeography and long-term fertilization experiments have highlighted the major contribution of soil pH as drivers of microbial community (Fierer and Jackson 2006; Constancias et al. 2015; Liu et al. 2014). Here, we examined the bacterial community structure and its distribution among 206 sites distributed across a major grain producing area of northeastern China. As shown in Fig. 2 and Table S2, we did not find a significant relationship between the characters of soil microbial communities and soil nutrient content. Conversely, we found significant positive correlation between characters of soil microbial communities and pH. Soil pH was found to be the main driving factor of bacterial community structure in agricultural soils, which agrees with previous research (Fierer and Jackson 2006; Liu et al. 2014; Constancias et al. 2015; Zhou et al. 2015; Yashiro et al. 2016; Wang et al. 2018). In the present study, the results showed that bacterial composition was similar in different soil samples at phyla level; relative abundance was different. The dominant phyla (relative abundance > 5%) were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, and Gemmatimonadetes, which were observed in this study across all soil samples that roughly represent the 26 soil types across the black soils of northeastern China as reported by Liu et al. (Liu et al. 2014), whereas the relative abundance of Planctomycetes in the soil samples was < 1%, which is similar to that found in previous studies (Chu et al. 2010), but over five times lower than the abundance reported by Liu et al. and Ding et al. (Liu et al. 2014; Ding et al. 2016).

Long-term excessive application of chemical fertilizers is generally considered to accelerate soil acidification (Ju et al. 2007; Zhou et al. 2015). Tectomicrobia and Actinobacteria produce the most bacteriogenic drugs. However, most of them have not been cultured, so their potential for drug production remains unknown and they may represent a potential resource for drug development (Jaspars and Challis 2014; Wilson et al.

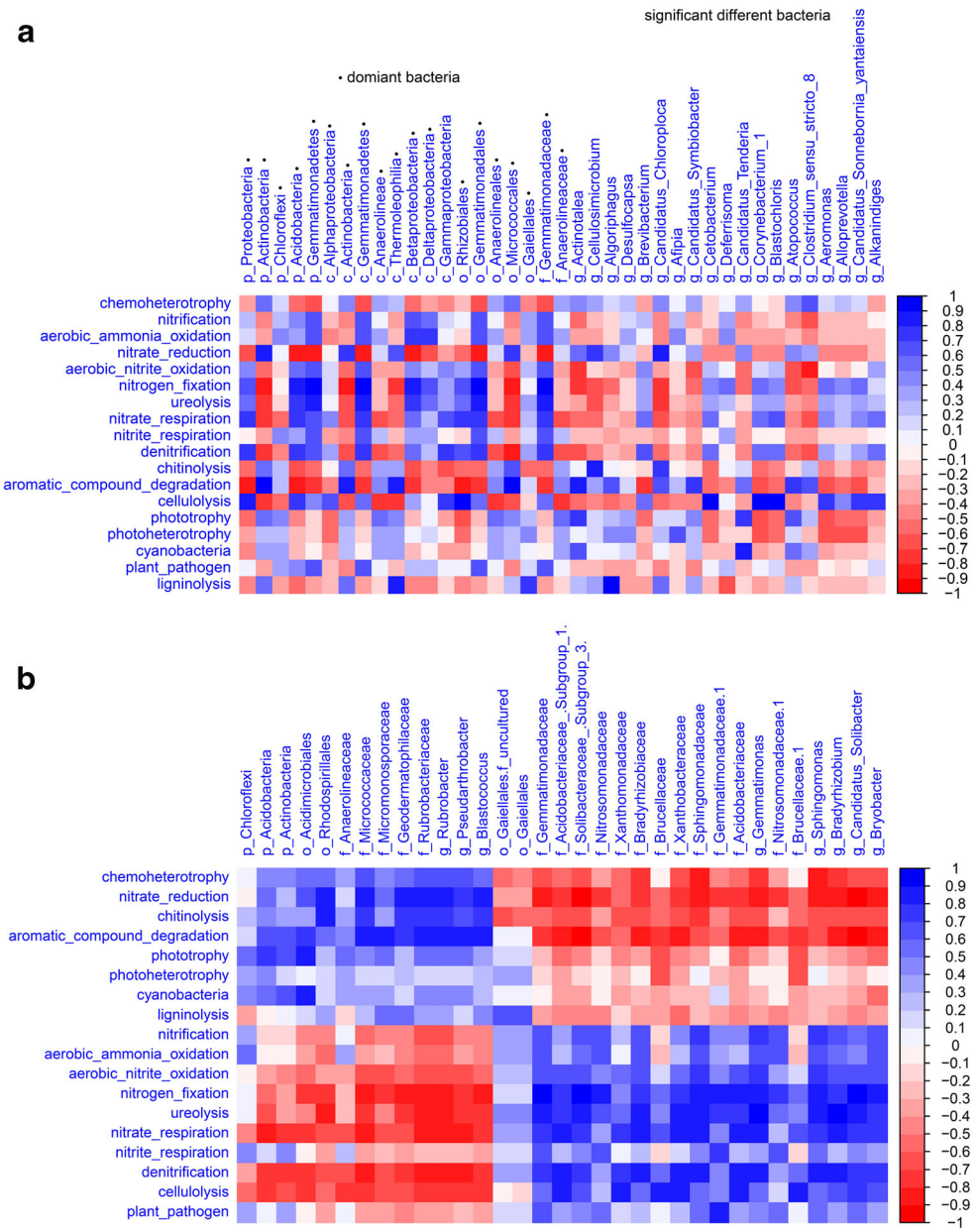

Fig. 10 The correlation analysis between bacteria and ecological functions. **a** The correlation analysis between dominant and significant different bacteria and ecological functions. **b** The correlation analysis between bacteria at family and genus level and ecological functions

2014). They may also play an important role in maintaining soil health. However, long-term agricultural production inputs many chemical nutrients into the soil, resulting in "eutrophication," which leads to soil acidification and decreases the diversity of Actinobacteria in the soil (Fig. 8). At the same time, compared with other soil bacteria, Acidobacteria showed a strong nutritional preference, while under the condition of long-term chemical fertilizer application leading to soil acidification, the abundance and diversity of phylum Acidobacteria increased; however, only Acidobacteria was positively correlated with soil pH reported by Wang et al. (Wang et al. 2018). Fibrobacteres is an important cellulose-degrading bacteria (Ransom-Jones et al. 2012; Jewell et al. 2013). It was found to be positively correlated with pH in our study, suggesting that bacterial community diversity having the ecological function of cellulose decomposition will also be affected by pH. However, Delgado-Baquerizo's researching team thought that bacterial diversity and composition were primarily driven by variation in soil nutrients at a regional scale (Delgado-Baquerizo et al. 2017b). Our results indicated that pH rather than soil nutrients was the main driving force for the structural diversity and abundance of soil microbial communities, which does not agree with the results of previous studies (Delgado-Baquerizo et al. 2017b) but agrees with the results of Wang et al. (Wang et al. 2018). Our results also showed that soil bacterial community composition varied according to latitude and that high-diversity bacterial communities were found in high-latitude areas. In

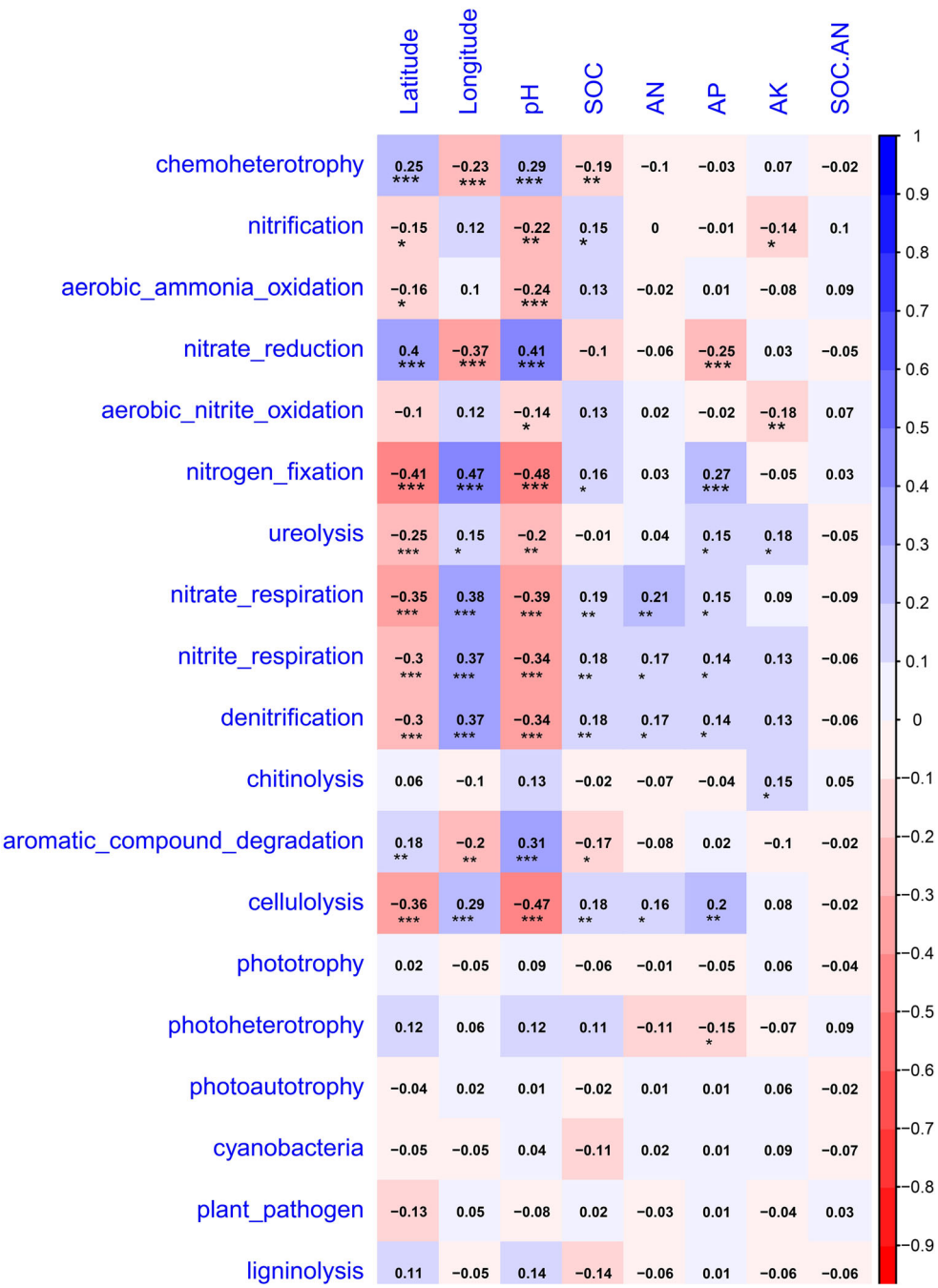

Fig. 11 The correlation analysis between environmental factors and ecological functions of bacteria. The numbers display the Pearson's correlation coefficient (r). Blue and red indicate positive and negative correlation, respectively. The color density and numbers reflect the scale of correlation. *Significant level (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

aggregate, these findings suggested that, similar to pH, the bacterial communities in the study area varied according to spatial distribution.

Mounting evidence indicates that, in natural environments such as oceans and soils, the functional composition of microbial communities, rather than taxonomic composition, is closely related to environmental factors (Nelson et al. 2016; Gibbons 2017; Louca et al. 2017). The ecological functions of microorganisms living in similar environments are more similar, but the composition of microbial species performing the functions may be greatly different (Gibbons 2017). This suggests that, in addition to revealing which microorganisms are present in the environment, it is particularly important to reveal the functional profile of microbial communities (Nelson et al. 2016; Gibbons 2017; Louca et al. 2017). Our findings suggest that the soil microbial functional structure was strongly shaped by local geochemistry conditions. The content of soil organic carbon had an important effect on soil pH, but the mechanisms underlying SOC mineralization by soil microorganisms due to pH change have been little studied. According to our analysis of ecological functions, we also discovered distinct pH controls on microbial mechanisms of

carbon decomposition, which leads to carbon loss through increased cellulolysis ($r = -0.47$, $P < 0.001$), and the higher cellulolysis activity promoted the accumulation of SOC ($r = 0.18$, $P < 0.01$) (Fig. 10). However, under low pH conditions, decomposition is faster than accumulation, and therefore SOC accumulation decreases. This is consistent with the observed correlation between pH and SOC ($r = -0.33$, $P < 0.001$; Fig. 2). Therefore, SOC mineralization was directly affected by microbial diversity and indirectly affected by soil pH. This suggests that increasing in soil pH conveys benefits in terms of SOC accumulation. These results also suggest that soil microbial communities accelerated the transformation of SOC after applying excess fertilizer that changed the soil pH, and soil microorganisms had more important effects on SOC mineralization in acidic soils than alkaline soil.

Soil microbial activity and diversity are not only a part of ecosystem function but also an inseparable part (Nannipieri et al. 2003; Delgado-Baquerizo et al. 2017a, 2017b, 2017c). We believe that soil microbial is essential for ecosystem functioning under steady conditions. However, it does not necessarily suggest that all microbial species will play a similar role. As shown in Fig. 10, soil microorganisms can be divided into different functional groups according to their functions. Different functional groups of microorganisms maintain the functions of soil, and there is functional complementarity between species in specific functional group. Our results suggest that a reduction in any group of species has little effect on soil ecosystem function overall processes in soil because other bacteria can take on its function, which is consistent with results of many references (Torsvik and Øvreås 2002; Nannipieri et al. 2003; Delgado-Baquerizo et al. 2017a).

Conclusion

The ultimate researching goal of microbial diversity is to understand who is where, with whom, doing what, why, and when. To answer such questions, reliable, reproducible, quantitative, and statistically valid experimental information on community-wide spatial is needed. Here, we used the high-throughput sequencing method to analyze the diversity of bacterial community in the farmland soil of Jilin Province. Our results show that each sample is composed of low abundance species and high abundance species, and has obvious environmental specificity, at the level of 16S rRNA. Of all environmental factors, soil pH was found to be more important than nutrient content for shaping bacterial communities in agricultural soil in terms of ecological function and bio-geographic distribution. Environmental conditions, especially chemical conditions, change the diversity of microorganisms in habitats. Due to the different ecological functions of each microorganism, the transformation and storage of nutrient elements in habitats are accompanied by the changes of microbial composition and ecological functions. In general, habitat conditions are the main factors affecting microbial diversity and its distribution, and the change of microbial diversity is the driving force of nutrient element transformation and storage in the habitat.

Funding information The authors received a financial grant from the National Key Research and Development Program of China (2018YFD0201300), the Natural Science Foundation of Jilin province, China (Subject to guide project, No. 20170101004JC), Program of Innovation and Entrepreneurship for Undergraduates, the Natural Science Foundation of Jilin Province, China (No. 20170101077JC), and the National Key Technology R&D Program of China (No. 2016YFD0300807).

References

Asnicar F, Weingart G, Tickle TL, Huttenhower C, Segata N (2015) Compact graphical representation of phylogenetic data and metadata with GraPhlAn. PeerJ 3:e1029

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Polme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P (2018) Structure and function of the global topsoil microbiome. Nature 560(7717):233–237

Cao J, Ji D, Wang C (2015) Interaction between earthworms and arbuscular mycorrhizal fungi on the degradation of oxytetracycline in soils. Soil Biol Biochem 90:283–292

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):2

Chen H, Hu HY, Chen QQ, Shi ML, Jin RC (2016) Successful start-up of the anammox process: influence of the seeding strategy on performance and granule properties. Bioresour Technol 211:594–602

Chu H, Fierer N, Lauber CL, Caporaso JG, Knight R, Grogan P (2010) Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. Environ Microbiol 12(11):2998–3006

Constancias F, Terrat S, Saby NP, Horrigue W, Villerd J, Guillemin JP, Biju-Duval L, Nowak V, Dequiedt S, Ranjard L, Chemidlin Prevost-Boure N (2015) Mapping and determinism of soil microbial community distribution across an agricultural landscape. Microbiologyopen 4(3):505–517

Dai G, Ma T, Zhu S, Liu Z, Chen D, Bai Y, Chen L, He J-S, Zhu J, Zhang Y, Lü X, Wang X, Han X, Feng X (2018) Large-scale distribution of

molecular components in Chinese grassland soils: the influence of input and decomposition processes. J Geophys Res Biogeosci 123(1):239–255
Delgado-Baquerizo M, Eldridge DJ, Ochoa V, Gozalo B, Singh BK, Maestre FT (2017a) Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. Ecol Lett 20(10):1295–1305
Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, Berdugo M, Campbell CD, Singh BK (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat Commun 7:10541
Delgado-Baquerizo M, Reich PB, Khachane AN, Campbell CD, Thomas N, Freitag TE, Abu Al-Soud W, Sorensen S, Bardgett RD, Singh BK (2017b) It is elemental: soil nutrient stoichiometry drives bacterial diversity. Environ Microbiol 19(3):1176–1188
Delgado-Baquerizo M, Trivedi P, Trivedi C, Eldridge DJ, Reich PB, Jeffries TC, Singh BK, Bennett A (2017c) Microbial richness and composition independently drive soil multifunctionality. Funct Ecol 31:2330–2343
Denk TRA, Mohn J, Decock C, Lewicka-Szczebak D, Harris E, Butterbach-Bahl K, Kiese R, Wolf B (2017) The nitrogen cycle: a review of isotope effects and isotope modeling approaches. Soil Biol Biochem 105:121–137
Ding J, Jiang X, Ma M, Zhou B, Guan D, Zhao B, Zhou J, Cao F, Li L, Li J (2016) Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China. Appl Soil Ecol 105:187–195
Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200
Emerson JW, Green WA, Schloerke B, Crowley J, Cook D, Hofmann H, Wickham H (2012) The generalized pairs plot. J Comput Graph Stat 22(1):79–91
Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. PANS 103(3):626–631
Gibbons SM (2017) Microbial community ecology: function over phylogeny. Nature Ecol Evol 1(1):0032
Gul S, Whalen JK (2016) Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biol Biochem 103:1–15
Hanson AC, Horner-Devine MC, Martiny JBH, Fuhrman JA (2013) Microbial biogeography. J Biogeogr 33:199–200
Jaspars M, Challis G (2014) Microbiology: a talented genus. Nature 506(7486):38–39
Jewell KA, Scott JJ, Adams SM, Suen G (2013) A phylogenetic analysis of the phylum Fibrobacteres. Syst Appl Microbiol 36(6):376–382
Ju XT, Kou CL, Christie P, Dou ZX, Zhang FS (2007) Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. Environ Pollut 145(2):497–506
Lloyd KG, Steen AD, Ladau J, Yin J, Crosby L (2018) Phylogenetically novel uncultured microbial cells dominate earth microbiomes. Appl Environ Sci 3(5):1–12
Kuypers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16(5):263–276
Liu J, Sui Y, Yu Z, Shi Y, Chu H, Jin J, Liu X, Wang G (2014) High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China. Soil Biol Biochem 70:113–122
Louca S, Jacques SMS, Pires APF, Leal JS, Gonzalez AL, Doebeli M, Farjalla VF (2017) Functional structure of the bromeliad tank microbiome is strongly shaped by local geochemical conditions. Environ Microbiol 19(8):3132–3151
Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. Science 353(6305):6
Louca S, Polz MF, Mazel F, Albright MBN, Huber JA, O'Connor MI, Ackermann M, Hahn AS, Srivastava DS, Crowe SA, Doebeli M, Parfrey LW (2018) Function and functional redundancy in microbial systems. Nat Ecol Evol 2(6):936–943
Maron PA, Sarr A, Kaisermann A, Leveque J, Mathieu O, Guigue J, Karimi B, Bernard L, Dequiedt S, Terrat S, Chabbi A, Ranjard L (2018) High microbial diversity promotes soil ecosystem functioning. Appl Environ Microbiol 84(9):e02738–e02717
Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54(12):655–670
Nelson MB, Martiny AC, Martiny JB (2016) Global biogeography of microbial nitrogen-cycling traits in soil. PNAS 113(29):8033–8040
Qi D, Wieneke X, Zhou X, Jiang X, Xue P (2017) Succession of plant community composition and leaf functional traits in responding to karst rocky desertification in the Wushan County in Chongqing, China. Community Ecol 18(2):157–168
Ransom-Jones E, Jones DL, McCarthy AJ, McDonald JE (2012) The Fibrobacteres: an important phylum of cellulose-degrading bacteria. Microb Ecol 63(2):267–281
Ravn HW, Andary C, Kovács G, Mølgaard P (1989) Caffeic acid esters as in vitro inhibitors of plant pathogenic bacteria and fungi. Biochem Syst Ecol 17(3):10
Shannon P, Andrew M, Owen O, Nitin SB, Jonathan TW, Daniel R, Nada A, Benno S, Trey I (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):7
Sun R, Zhang X-X, Guo X, Wang D, Chu H (2015) Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol Biochem 88:9–18
Torsvik V, Øvreås L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5:240–245
Wang M, Li Y, Ye W, Bornman JF, Yan X (2011) Effects of climate change on maize production, and potential adaptation measures: a case study in Jilin Province, China. Clim Res 46(3):223–242
Wang Q, Wang C, Yu W, Turak A, Chen D, Huang Y, Ao J, Jiang Y, Huang Z (2018) Effects of nitrogen and phosphorus inputs on soil bacterial abundance, diversity, and community composition in Chinese fir plantations. Front Microbiol 9:1–10
Wilson MC, Mori T, Ruckert C, Uria AR, Helf MJ, Takada K, Gernert C, Steffens UA, Heycke N, Schmitt S, Rinke C, Helfrich EJ, Brachmann AO, Gurgui C, Wakimoto T, Kracht M, Crusemann M, Hentschel U, Abe I, Matsunaga S, Kalinowski J, Takeyama H, Piel J (2014) An environmental bacterial taxon with a large and distinct metabolic repertoire. Nature 506(7486):58–62
Xu P, Wang J, Hong F, Wang S, Jin X, Xue T, Jia L, Zhai Y (2017) Melatonin prevents obesity through modulation of gut microbiota in mice. J Pineal Res 62(4):e12399
Yao Q, Liu J, Yu Z, Li Y, Jin J, Liu X, Wang G (2017) Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China. Appl Soil Ecol 113:11–21
Yashiro E, Pinto-Figueroa E, Buri A, Spangenberg JE, Adatte T, Niculita-Hirzel H, Guisan A, van der Meer JR (2016) Local environmental factors drive divergent grassland soil bacterial communities in the Western Swiss Alps. Appl Environ Microbiol 82(21):6303–6316
Zarraonaindia I, Owens SM, Weisenhorn P, West K, Hampton-Marcell J, Lax S, Bokulich NA, Mills DA, Martin G, Taghavi S, van der Lelie D, Gilbert JA (2015) The soil microbiome influences grapevine-associated microbiota. MBio 6(2):e02527–e02514
Zhang Z-Z, Hu H-Y, Xu J-J, Shi Z-J, Deng R, Ji Z-Q, Shi M-L, Jin R-C (2017) Effects of inorganic phosphate on a high-rate anammox system: performance and microbial community. Ecol Eng 101:201–210
Zhou J, Guan D, Zhou B, Zhao B, Ma M, Qin J, Jiang X, Chen S, Cao F, Shen D, Li J (2015) Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. Soil Biol Biochem 90:42–51