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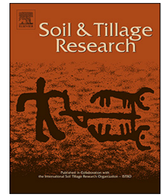

Organic and inorganic fertilizers respectively drive bacterial and fungal community compositions in a fluvo-aquic soil in northern China

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ABSTRACT

Soil microbes play vital roles in energy flow and nutrient cycling and, thus, are important for agricultural production. A better understanding of the complex responses of microbial communities to various organic and inorganic fertilization regimes is critical for sustainable development of agroecosystems. Changes in bacterial and fungal abundance and diversity in fluvo-aquic soil in Northern China were studied under 38-year long-term fertilization strategies: four chemical-fertilization strategies (i.e., no fertilizer, N, NP, or NPK), with or without manure amendment, were investigated by high-throughput sequencing and quantitative polymerase chain reaction-based amplification of bacterial 16S rRNA and fungal internal transcribed spacer (ITS) rRNA genes. Chemical fertilizer plus manure addition clearly increased the soil fertility and was recommended for further optimization of fertilization patterns. Both principal component analysis and partial least-square discriminant analysis showed greater impacts of manure addition than chemical fertilizer on bacterial community distributions, whereas fungal communities were more sensitive to inorganic fertilizer. The linear discriminant analysis effect size method revealed that the number of responding microbes (microbes significantly affected by various fertilizations) in bacterial communities in manure-treated soils was markedly higher than that in chemical fertilizer-treated soils, whereas those of fungal communities showed the opposite trend. In addition, redundancy analysis further illustrated the primary importance of organic matter in shaping community distributions of bacteria, rather than in driving fungal community patterns. These results suggested that organic and inorganic fertilizers, respectively, dominated in shaping bacterial and fungal community distributions in fluvo-aquic soils.

1. Introduction

Soil microorganisms play vital roles in the maintenance of soil health, productivity, and sustainability, and thus crop production, because of their contributions to soil nutrient transformation, formation and decomposition of organic matter, and stabilization of soil aggregates (Van Der Heijden et al., 2008; Zhao et al., 2014; Singh, 2016; Pan et al., 2018a). Therefore, a profound understanding of soil microbial ecology has become increasingly recognized and emphasized because of the ecological and economic importance of microorganisms (Mele and Crowley, 2008).

Due to the increasing concerns regarding food demands and the scarcity of land available for agriculture, high amount of fertilizer is implemented to increase crop productivity (Inselsbacher et al., 2010). The application of organic and inorganic fertilizers can cause detectable changes in nutrient availability to plants, as well as in the diversity and function of microorganisms (Marschner et al., 2003). For example, long-term field experiments involving polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) demonstrated the importance of balanced fertilization in promoting the activities of bacterial communities (Chu et al., 2007a,b; Luo et al., 2015). Nitrogen (N) is regarded as a key limiting factor for soil microbes, and N amendment can alter the activity and diversity of soil organisms (Sarathchandra et al., 2001; Xu et al., 2017; Liu et al., 2019). Organic fertilization exerted no effects on the fungal biomass, while increasing the bacterial biomass by increasing soil organic carbon (C_{org}) and C/N ratio (Crecchio et al., 2001; Marschner et al., 2003). In addition, manure application promoted soil bacterial communities by enhancing root exudates and also improved plant growth (Bittman et al., 2005). Suzuki et al. (2009) suggested that fungal communities responded more

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sensitively than bacterial communities both to inorganic fertilization and to organic fertilization, while the growth of bacterial communities was related more to the soil type than these fertilization regimes. In contrast, Lazcano et al. (2013) proposed that bacterial growth was especially affected by the fertilizer type, whereas that of fungi responded to the amount of fertilizer. Changes in organic matter compositions can result in a succession of microorganisms such as those detected by Ponge (1991) for fungal successions during the decomposition of pine needles. The fertilizers of N and P tended to inhibit arbuscular mycorrhizae (Kabir et al., 1997; Bethlenfalvay et al., 1999). Different fertilization regimes exerted the greatest effects on bacterial and fungal community structures from a management perspective, leading to temporal increases in total culturable bacterial counts (Girvan et al., 2004; David et al., 2014).

Given the differential responses of fungi and bacteria to manure and chemical fertilizers, as well as the vital importance of microbes in soils, additional research of the microbial species supported by different fertilization methods in agroecosystems is the key for gaining a more complete understanding of the soil microbial ecology in intensively managed agricultural soils. High-throughput sequencing technology provides compelling evidence for characterizing the microbial diversity of environmental ecosystems at unprecedented levels of coverage and precision (David et al., 2014). The technique has been used to provide holistic insights into species and functional diversities of microbial communities in soil systems (Frindte et al., 2019; Averill et al., 2019). For example, organic fertilization tended to favor the growth of copiotrophic microorganisms (Marschner et al., 2003; Wang et al., 2017a,b). Long-term chemical fertilization resulted in a significant decrease in the bacterial abundance and biodiversity (Zhou et al., 2015). Therefore, opening the black box of soil microbial communities is considered one of the main challenges of modern soil ecology in order to comprehensively understand the patterns and dynamics of soil microbes (Tiedje et al., 1999; Ranjard et al., 2003).

The work described here was conducted at the experimental farm of the Xuzhou Institute of Agricultural Sciences of the Xuhuai District, China, which provides a unique resource to investigate the impact of 38-year long-term fertilization on bacterial and fungal community compositions. This study was aimed to (1) evaluate the effects of fertilization strategies on soil fertility and health, (2) investigate the effects of various 38-year fertilization regimes on the abundance and diversity of bacterial and fungal communities, and (3) assess the mechanisms of fertilization strategies on bacterial and fungal community compositions by linking relationships between environmental factors and taxonomic patterns. We hypothesized that compound organic–inorganic fertilizers would be more favorable than organic or inorganic fertilization alone for enhancing the soil microbial diversity, especially by stimulating eutrophic species.

2. Material and methods

2.1. Site description

The study site is located at the experimental farm of the Xuzhou Institute of Agricultural Sciences of the Xuhuai District, Jiangsu Province, China (117°17′ E and 34°16′ N). The region has a warm and semi-humid continental monsoon climate with 210 frost-free days. The 38-year mean annual temperature and precipitation are 14 °C and 860 mm, respectively. The soil is derived from alluvial sediments of the Yellow River and is classified as a fluvo-aquic soil with a sandy loam texture. The initial properties of the surface soil (0–20 cm) were as follows: soil pH (soil: H_2O ratio 1:2.5) 8.01; total N (TN) content 0.66 g kg^{-1}; soil organic matter (SOM) 10.80 g kg^{-1}; available phosphorus (Olsen P) 12.00 mg kg^{-1}; available potassium (AK) 63.00 mg kg^{-1}; soil cation exchange capacity (CEC) 20.40 cmol kg^{-1}.

2.2. Experimental design

The long-term experiment was initiated in 1980, with each plot measuring 4.8 m × 7.0 m. A 1-m-wide buffer strip was established between every two plots to avoid interactions and to allow for sampling the plots. All plots were maintained with winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) as a two crops per year rotation system from 1980 to 2002. From 2002, the maize was replaced with sweet potato. Crops were manually harvested and the above-ground crop residues were removed from the field.

The fertilization experiment was established usinvg a randomized block design, including eight treatments and three replicates. The treatments included: (1) control treatment without any fertilizer (CK); (2) chemical N fertilizer (N); (3) chemical N and P fertilizer (NP); (4) chemical N, P, and K fertilizer (NPK); (5) manure (M); (6) chemical N fertilizer combined with manure (MN); (7) chemical NP fertilizer combined with manure (MNP); and (8) chemical NPK fertilizer combined with manure (MNPK). The chemical N, P, and K fertilizers used were urea (46 % N), diammonium phosphate (15 % N, 42 % P_2O_5) and potassium sulfate (50 % K_2O), respectively; their application rates were 300 kg N $(ha\ yr)^{-1}$, 150 kg P_2O_5 $(ha\ yr)^{-1}$, and 225 kg K_2O $(ha\ yr)^{-1}$, respectively. The applied manure was horse compost (1981–1984) or cattle compost (1985–present). The application rate of house compost was 75,000 kg $(ha\ yr)^{-1}$, and that of cattle compost was 37,500 kg $(ha\ yr)^{-1}$. The average contents of N, P_2O_5, and K_2O in manure were 6.31 g kg^{-1}, 5.14 g kg^{-1}, and 7.39 g kg^{-1}, respectively, with a C/N ratio of 20.3. The chemical P and K fertilizers, as well as the manure were applied as basal fertilizers. For wheat and maize, N fertilizer (urea) was applied with 50 % as a basal fertilizer and 50 % as a supplementary fertilizer, whereas for sweet potato, N fertilizer (urea) was applied as a basal fertilizer.

2.3. Sampling collection and physico-chemical analysis

Soil samples were collected one week before harvesting the sweet potatoes in October 2018. The samples were collected from the surface layer (0–10 cm) at five random locations per plot using a soil auger (5 cm in diameter). The samples from each plot were combined into one composite sample, wrapped in ice packs, and transported to the laboratory. Each composite soil sample was subjected to physicochemical property analyses. Subsamples were stored at −80 °C for subsequent nucleic acid extraction.

Soil pH was determined for 1:2.5 (weight:volume) soil-distilled water suspensions using a pH meter (Mettler Toledo, USA). Total nitrogen (TN) was measured by Kjeldahl digestion (Bremner and Mulvaney, 1982). Olsen phosphorus (Olsen P) was extracted with 0.5 M $NaHCO_3$ and measured using the molybdenum blue method (Olsen, 1954). Available potassium (AK) was extracted with 1 M ammonium acetate and determined by flame emission spectrophotometry. NO_3^- and NH_4^+ were extracted with 1 M KCl and determined with a flow-injection analyzer (SAN++; Skalar Analytical B.V., Breda, The Netherlands). Soil organic matter (SOM) was measured following the dichromate digestion method (Kalembasa and Jenkinson, 1973).

2.4. Soil DNA extraction, real-time quantitative polymerase chain reaction (qPCR), and high-throughput sequencing analysis

DNA was extracted from 0.5 g fresh soil with the FastDNA Spin Kit for Soil (MP Biomedicals, LLC., Solon, OH, USA), according to the manufacturer's instructions. The DNA integrity and quantity were checked by electrophoresis and a NanoDrop®ND 2000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Real-time quantitative PCR (qPCR) assays targeting the bacterial 16S rRNA and fungal Internal Transcribed Spacer (ITS) rRNA genes were performed on an ABI Q5 real-time PCR system (Applied Biosystems, California, USA). The V4-V5 region of bacterial 16S rRNA

Table 1
Physicochemical properties of the soils used in this study.

Soil properties	CK	N	NP	NPK	M	MN	MNP	MNPK
pH	8.73a	8.38c	8.58b	8.52b	8.51bc	8.11d	8.45bc	8.24d
Moisture	0.240b	0.247ab	0.273a	0.259ab	0.266ab	0.262ab	0.269a	0.241b
TN (g kg^{-1})	0.85e	1.17d	1.15d	1.24c	1.83b	1.89b	2.06a	2.13a
NH_4^+-N (mg kg^{-1})	1.95d	2.20c	2.56c	4.23b	6.83a	6.61a	3.76b	6.63a
NO_3^- -N (mg kg^{-1})	2.81d	2.77d	2.77d	3.24c	3.78b	4.19a	3.71b	3.44bc
Olsen P (mg kg^{-1})	3.91e	3.52e	9.74d	8.32d	143.71a	96.46c	121.55b	105.60c
AK (mg kg^{-1})	64.55e	80.68d	80.68d	121.00ab	112.94b	96.81c	96.81c	129.07a
SOM (g kg^{-1})	34.19d	26.35e	29.09e	29.25e	44.07c	57.70a	47.61b	41.62c

Values are means for triplicate replicates. Different lowercase letters indicate significant differences with a P value < 0.05 based on the analysis of variance. TN: total nitrogen; Olsen P: Olsen phosphorus; AK: available potassium; SOM: soil organic matter.

genes were amplified from genomic DNA using the modified 515 F (5′-GTGYCAGCMGCCGCGGTAA-3′)/806R (5′-GGACTACNVGGGTWT-CTAAT-3′) primer pair (Walters et al., 2016). The fungal ITS rRNA genes were amplified with the ITS 5 F (5′-GGAAGTAAAAGTCGTAACA AGG-3′) and ITS 2R (5′-GCTGCGTTCTTCATCGATGC-3′) primers (White et al., 1990). Each PCR mixture (20 μl) contained 10 μl 2× TransStart® Top Green qPCR SuperMix, 1 mM of each primer, 10 ng of ten-fold diluted DNA template, and 7.0–8.6 μL milli-Q water. The PCR thermal-cycling conditions were as follows: 5 min at 95 °C for the initial denaturation; 40 cycles of 5 s at 95 °C, and 30 s at either 60 °C (for bacteria) or 58 °C (for fungi); and a final step of 40 s at 72 °C. The reactions were followed by melting-curve analysis with temperatures increasing from 50 to 99 °C. Standard curves were generated as previously described by Pan et al. (2018b).

High-throughput sequencing of the bacterial 16S rRNA and fungal ITS rRNA genes was performed using the Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA). Data analysis was performed according to Feng et al. (2019). Only sequences more than 200 bp long with average quality scores of > 25 and no ambiguous base calls, which perfectly matching the forward primers were selected for subsequent analyses. The sequences were clustered using MOTHUR software (Schloss et al., 2009) into operational taxonomic units (OTUs) based on 97 % similarity levels. Sequences were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) under studies PRJNA542408 and PRJNA542409 for the 16S rRNA and ITS rRNA genes, respectively.

2.5. Statistical analysis

Statistical analysis was completed using SPSS software, version 20 (IBM Corp., Armonk, NY, USA). Differences in soil physicochemical properties, functional gene abundances, microbial α-diversity, and the relative abundances of different taxonomic levels of microbe among samples within fertilization patterns were identified by analysis of variance (ANOVA/Duncan). $P <$ 0.05 was considered to reflect a statistically significant difference. Circos graphs for bacterial and fungal community compositions were developed using the online Circos software (Zhang et al., 2018). Principal component analysis (PCA) of bacterial and fungal communities was performed to determine the β-diversities of the bacterial and fungal communities. Partial least-square discriminant analysis (PLS-DA), a supervised method, was performed with the OTU data to identify the effects of fertilization regimes on the bacterial and fungal communities. Analysis of similarity (ANOSIM), based on 999 permutations, was performed on the distance matrix generated above to quantitatively compare the bacterial and fungal community differences resulting from the various fertilization patterns. Redundancy analysis (RDA) with Monte Carlo permutations (999 repetitions) was conducted to determine the relationships between soil properties and microbial communities. PCA and RDA were conducted using the ‘vegan’ package of R software, version 3.5.1. The linear discriminant analysis effect size (LEfSe) method was conducted to identify potential microbial markers (at all taxonomic levels) that reflected the different fertilization treatments. Only taxa meeting an LDA significance threshold of 3 for bacterial communities or 2 for fungal communities are shown. PLS-DA, ANOSIM, and LEfSe analyses were implemented by Visual Genomics. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis was only conducted for the bacterial communities to explore bacterial functional traits from the 16S rRNA gene data (Langille et al., 2013), as the fungal genome data are currently limited (Oh et al., 2016).

3. Results

3.1. Soil properties

The effects of various fertilization strategies on the soil physicochemical properties were summarized in Table 1. Overall, fertilization significantly decreased the soil pH regardless of fertilizer type ($P < 0.05$). The soil pH ranged from 8.11 in MN soil to 8.73 in CK soil. Fertilizer addition significantly increased the soil TN, especially in MNP and MNPK soils ($P < 0.05$). Soil NH_4^+-N contents were significantly increased by all fertilizer additions ($P < 0.05$). NO_3^--N contents were significantly increased under NPK, M, MN, MNP, and MNPK treatments ($P < 0.05$), while changed little under N and NP treatments ($P > 0.05$). Soil Olsen P was less influenced by N treatment, but was significantly increased under other fertilization treatments, especially by amendment of manure treatments ($P < 0.01$). The soil AK was significantly increased by fertilizer addition, especially in MNPK-treated soils ($P < 0.01$). SOM was significantly increased in manure-treated soils (M, MN, MNP, and MNPK), but was obviously decreased by chemical fertilization treatments (N, NP, and NPK) ($P < 0.01$).

3.2. Soil bacterial and fungal abundances

The bacterial and fungal abundances in all soil samples were determined using qPCR targeting the 16S rRNA and ITS rRNA genes (Fig. 1). The abundances of bacteria varied from 4.32×10^8 copies g^{-1} dry soil to 1.42×10^9 copies g^{-1} dry soil, whereas those of fungi varied from 2.14×10^6 copies g^{-1} dry soil to 9.34×10^6 copies g^{-1} dry soil, across all samples. In general, fertilizer addition was followed by immediate increases in bacterial and fungal numbers, but to a less extent under N treatment. The 16S rRNA and ITS rRNA gene-copy numbers in M-treated soils were highest across all samples, up to 1.42×10^9 copies g^{-1} dry soil and 9.34×10^6 copies g^{-1} dry soil, respectively. The ratios of bacteria to fungi (B:F) were calculated using the gene-copy numbers of the 16S rRNA and ITS rRNA genes (Wurzbacher et al., 2014). The B:F ratios ranged from a maximum of 237.65 in CK soils to a minimum of 97.45 in MNP soils. The B:F ratios were significantly decreased by fertilizer addition, irrespective of whether inorganic or organic fertilizer was used.

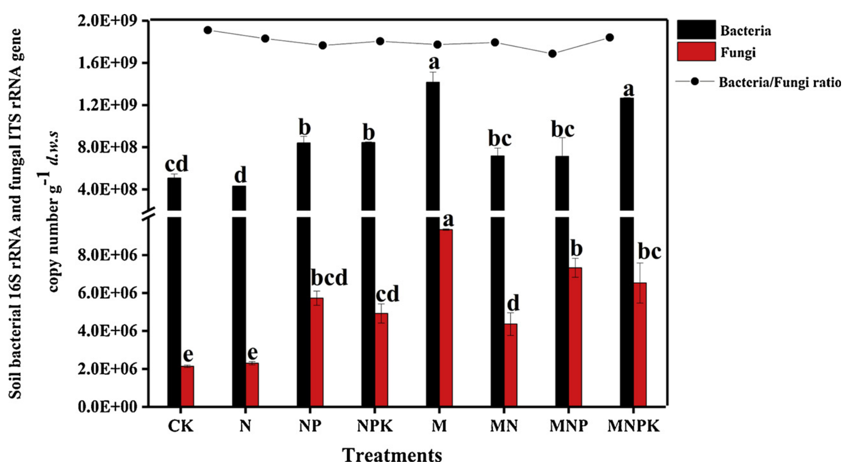

Fig. 1. The quantitative PCR (qPCR) results of bacteria and fungi. The abundance of bacteria as indicated by the number of 16S rRNA and fungi as indicated by the number of ITS rRNA copies measured using qPCR. The error bars represent the standard errors of the mean of triplicate samples. The different letters above the columns indicate a significant difference ($P < 0.05$) based on the analysis of variance.

3.3. Soil bacterial and fungal community structures

Approximately 1,483,151 bacterial reads were generated after quality filtering via Illumina MiSeq sequencing, with the number of reads per sample ranging from 32,219 to 94,494. The sequences derived from all 24 samples were clustered into 99,981 operational taxonomic units (OTUs). With regards to the fungal communities, 1,077,336 fungal sequencing reads were obtained after quality filtering, with the number of reads per sample ranging from 36,433 to 67,159. The sequences were clustered into 9888 OTUs with a 97 % sequence similarity cut-off, after trimming and filtration.

The ten most abundant bacterial phyla in all soil samples included *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes*, *Bacteroidetes, Nitrospirae*, *Firmicutes*, and *Verrucomicrobia* (Fig. 2a and Fig. S1a). Together, these phyla accounted for over 90 % of the bacterial sequences (Fig. S1a). The relative abundance of *Proteobacteria* was significantly decreased from 44.03 % in CK soils to 36.25 % in N soils, but was increased from 44.03 % in CK soils to 54.99 %, 47.29 %, 57.39 %, and 50.38 % in NP, NPK, MN, and MNP soils, respectively. In addition, the relative abundance of *Acidobacteria* was significantly increased by M treatment, but was changed little under other six fertilization regimes.

With regards to the fungal communities, only seven phyla and one unidentified phylum were observed in the soil samples (Fig. 2b and Fig. S1b). The seven determined phyla were *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Neocallimastigomycota*, *Zygomycota*, *Rozellomycota*, and *Glomeromycota*, comprising over 84 % of the fungal sequences (Fig. S1b). The relative abundance of *Ascomycota* was over 40 % among the fungal sequences of each soil sample and was significantly decreased by fertilization, regardless of whether inorganic or organic fertilizer was applied. Conversely, the relative abundances of *Chytridiomycota* and *Neocallimastigomycota* were remarkably increased by all fertilizer treatments. For example, *Chytridiomycota* increased from 0.12 % in CK soils to 0.36 %, 7.61 %, 6.99 %, 16.99 %, 3.24 %, 10.86 %, and 37.17 % in N, NP, NPK, M, MN, MNP, and MNPK soils, respectively; *Neocallimastigomycota* increased from 0.65 % in CK soil to 4.92 %, 13.75 %, 3.5 %, 12.62 %, 9.14 %, 8.47 %, and 2.25 % in N, NP, NPK, M, MN, MNP, and MNPK soils, respectively.

The top 15 bacterial and fungal families were detailed in Fig. 3. *Burkholderiaceae* was the dominant bacterial family across all treatments and was remarkably increased under NP and MN treatments, but was significantly decreased under N, M, and MNPK treatments (Fig. 3a). No significant differences were found in the relative abundance of *Burkholderiaceae* under CK, NPK, and MNP treatments. Regarding the fungal families, fertilizer application led to a significant increase in the relative abundance of *Neocallimastigaceae*, except for with the NP and

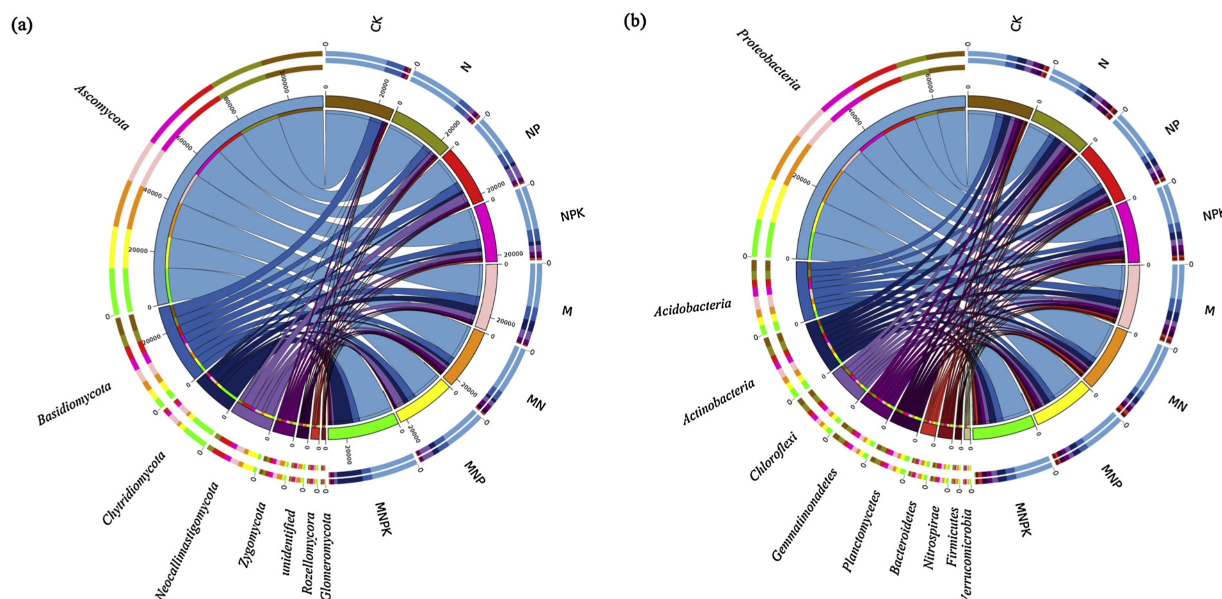

Fig. 2. Distribution of ten most abundant bacterial phyla (a) and eight most abundant fungal phyla in soil. The data was visualized via Circos software (http://circos.ca/). The length of the bars of each sample on the outer-ring represented the percentage of phyla in each sample.

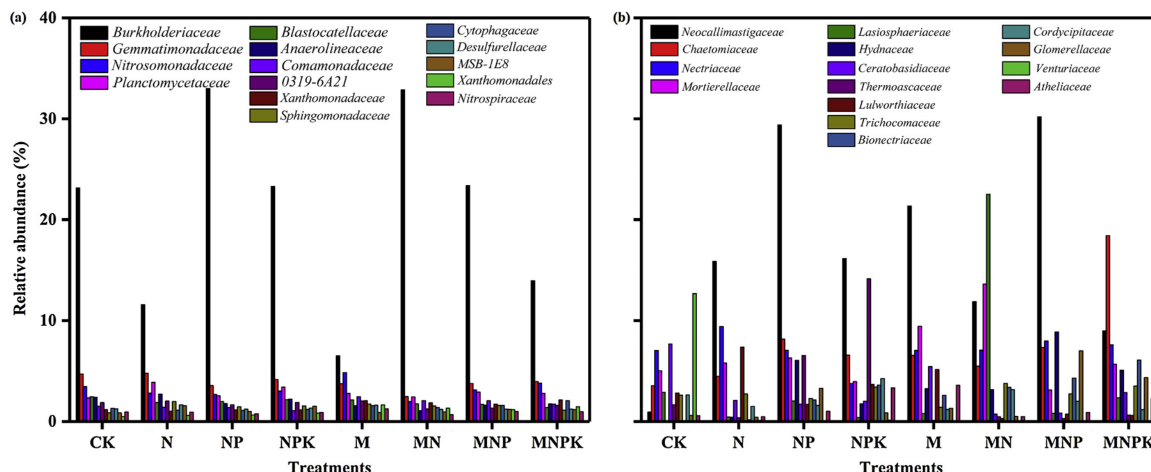

Fig. 3. Top 15 bacterial (a) and fungal (b) families, respectively, in soils under treatments CK (control without any fertilizer), N (chemical N fertilizer), NP (chemical N and P fertilizer), NPK (chemical N, P and K fertilizer), M (manure), MN (chemical N fertilizer combined with manure), MNP (chemical N, P fertilizer combined with manure), MNPK (chemical N, P, K fertilizer combined with manure). Each stripe represents the mean of three replicates.

MNP treatments (Fig. 3b). In contrast, the relative abundance of *Venturiaceae* was remarkably decreased by fertilizer application. The abundances of *Thermoascaceae*, *Lasiosphaeriaceae*, and *Chaetomiaceae* obviously increased in NPK-, MN-, and MNPK-treated soils.

Variations in the bacterial and fungal communities between samples (β-diversities) were evaluated using principal component analysis (PCA) plots (Fig. 4a and b). The first two principle components explained 98.03 % and 49.70 % of the bacterial and fungal community variability, respectively. The PCA plots demonstrated distinct bacterial and fungal community separations between inorganic fertilizer-treated soils (N, NP, and NPK) and organic fertilizer-treated soils (M, MN, MNP, and MNPK), along the first principle coordinates. PLS-DA was performed to validate the results obtained using the unsupervised PCA model (Fig. 4c and d). The PLS-DA analysis showed that both the bacterial and fungal communities in chemical-treated soils (N, NP, and NPK) were separated from those in manure-treated soils (M, MN, MNP, and MNPK) along the first principle coordinate.

ANOSIM was conducted to test the null hypothesis of no compositional differences between the communities of sampling groups. The R statistics from ANOSIM of bacteria and fungi were 0.718 and 0.396, respectively, with $P < 0.01$ observed for all pairwise comparisons, which demonstrated the statistical robustness of our overall analysis of community β-diversity.

3.4. Taxonomic biomarkers of soil microbial communities

LEfSe analysis from the phylum to genus levels was performed to identify high-dimensional biomarker taxa with significantly different abundances among the eight fertilization regimes (Fig. 5). The LDA values of the bacterial and fungal communities are shown in Tables S1

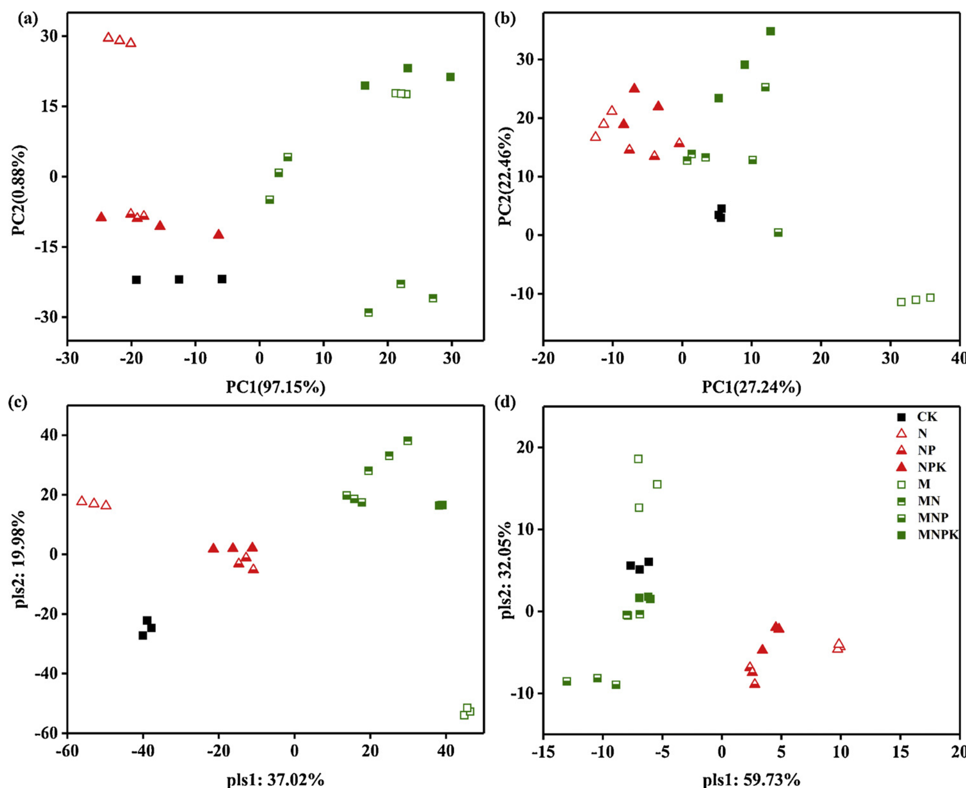

Fig. 4. Principal component analysis (PCA) (a, b) and Partial least-square discriminant analysis (PLS-DA) (c, d) of bacterial (a, c) and fungal (b, d)communities in soils, respectively, under treatments CK (control without any fertilizer), N (chemical N fertilizer), NP (chemical N and P fertilizer), NPK (chemical N, P and K fertilizer), M (manure), MN (chemical N fertilizer combined with manure), MNP (chemical N, P fertilizer combined with manure), MNPK (chemical N, P, K fertilizer combined with manure).

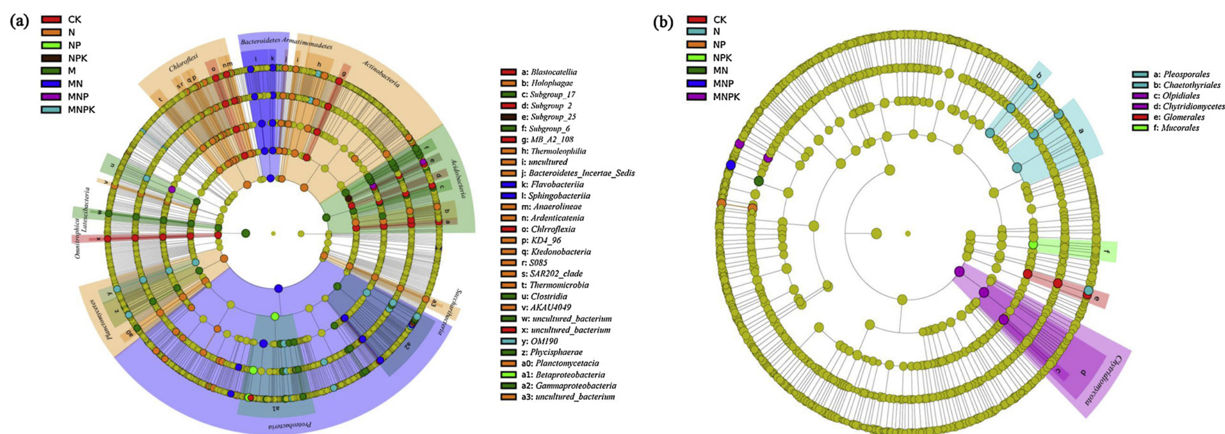

Fig. 5. LEfSe cladograms showing taxa with different abundance values. Taxonomic cladogram obtained from LEfSe analysis of bacterial 16S rRNA and fungal ITS rRNA sequences. Only taxa meeting an LDA significance threshold of 3 for bacterial communities and 2 for fungal communities are shown. Seven rings of the cladogram stand for domain (innermost), phylum, class, order, family, genus, and species (outermost), respectively.

and S2, respectively. By using the LEfSe, we identified 286 and 27 differentially abundant taxa of bacteria and fungi, respectively (Table S1 and S2).

Regarding the bacterial community, the *Omnitrophica* phylum and *Chloroflexia* class were identified as the most abundant biomarkers ($P < 0.01$) present in CK soil (Fig. 5a). The *Planctomycetes*, *Armatimonadetes*, *Saccharibacteria*, *Chloroflexi*, and *Actinobacteria* were the most differentially abundant phyla in N soil. The *Betaproteobacteria* and *Subgroup_25* classes were especially enriched in NP and NPK soils, respectively. In addition, M treatment resulted in two phylum-level markers, *Latescibacteria* and *Acidobacteria*, as well as one class-level marker, *Gammaproteobacteria*. In addition, the *Proteobacteria* and *Bacteroidetes* phyla were significantly enriched under MN treatment. The *OM190* class within the *Planctomycetes* phylum was significantly enriched in MNPK soils.

For the fungal communities, only MNPK soils had a phylum-level marker, namely the *Chytridiomycota* phylum (Fig. 5b). The *Glomerales* order within *Glomeromycota*, the *Pleosporales* and *Chaetothyriales* orders within *Ascomycota*, and the *Mucorales* order within *Zygomycota* were significantly enriched in CK soils, N soils, and NPK soils, respectively. The *Incertaesedis* and *Bionectriaceae* families within *Ascomycota* showed the highest abundance in MN and MNPK soils, respectively, compared with other soils.

3.5. Relationships between microbial community compositions and soil properties

RDA analysis identified distinct soil properties that explained the observed changes in bacterial and fungal community compositions (Fig. 6). For the bacterial communities, the first and second canonical axes explained 57.66 % and 0.81 % of the total variance, respectively (Fig. 6a). The soil pH (R^2 = 0.495, $P < 0.01$), SOM (R^2 = 0.751, $P < 0.01$), NO_3^--N ($R^2 = 0.705$, $P < 0.01$), NH_4^+-N (R^2 = 0.864, $P < 0.01$), TN (R^2 = 0.730, $P < 0.01$), Olsen P ($R^2 = 0.716$, $P < 0.01$), and AK ($R^2 = 0.369$, P = 0.01) all played significant roles in shaping the soil bacterial community structure. For the fungal communities, the first and second canonical axes explained 30.00 % and 24.88 % of the total variance, respectively (Fig. 6b). The soil pH (R^2 = 0.288, $P < 0.05$), NH_4^+-N ($R^2 = 0.314$, $P < 0.01$), TN ($R^2 = 0.376$, $P < 0.01$), Olsen P ($R^2 = 0.344$, $P < 0.01$), and AK ($R^2 = 0.361$, P = 0.01) were the most influential factors in driving the soil fungal community composition.

4. Discussion

The results of this study demonstrated that the 38-year-long application of chemical and organic fertilizers greatly impacted the soil physicochemical properties, as well as the abundances, diversities, and compositions of bacterial and fungal communities.

The soil pH was significantly decreased by fertilization compared to CK treatment, regardless of whether chemical fertilizer was used alone or combined with organic fertilizer (Table 1). It was not surprising that inorganic fertilizer alone decreased the soil pH, as the chemical fertilizer can acidify the soil (van Diepeningen et al., 2006). However, it was intriguing that the application of manure, alone or in combination with a chemical fertilizer, did not stabilize or increase the soil pH, but significantly decreased the soil pH, which disagreed with previous findings in paddy soils (Wang et al., 2017a,b) and winter wheat–summer rice (*Oryza sativa* L.) rotations (Zhao et al., 2014). This discrepancy might be attributed to variance in the soil type, as proposed by Wei et al. (2017), who observed that organic fertilizer could decrease the soil pH in alkaline soils while increasing the soil pH in acidic soils.

Additionally, the amounts of the major soil nutrients (N, P, and K) were significantly increased by amendment with inorganic fertilizer.

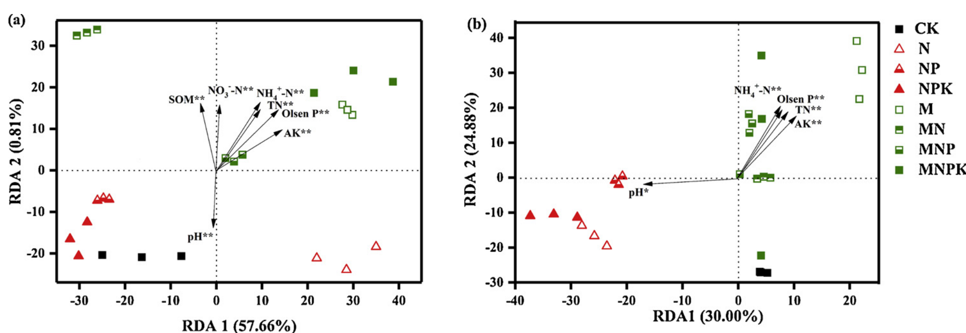

Fig. 6. Redundancy analysis (RDA) between bacterial communities and physiochemical characteristics (a); RDA analysis between the soil properties and fungal communities (b). SOM: soil organic matter; Olsen P: Olsen phosphorus; TN: total nitrogen; AK: Available potassium. The significant differences identified were, ** $P <$ 0.01, and * $P <$ 0.05, based on the mantel test.

Meanwhile, inorganic fertilizer treatments showed significantly lower SOM as compared to control and organic fertilizer treatments, which was in line with findings by Khan et al. (2007), who proposed that N fertilizer application enhanced biomass production while providing only a slight benefit in terms of SOC sequestration. The application of manure, alone or in combination with chemical fertilizer, appreciably stimulated the soil nutrient contents and accelerated the nutrient turnover in soil, which was in agreement with previous studies in red clay soil (Zhong et al., 2010) and in sandy loam soil (Chu et al., 2007a,b). Organic fertilizer was generally perceived to sequester SOM by increasing the input of crop residues (Khan et al., 2007), thus increasing topsoil organic carbon stocks (Zhang et al., 2016). Therefore, these results further suggested that compound organic-inorganic fertilizer would be more favorable for improving the soil quality and agricultural sustainability (Zhong et al., 2010; Zhang et al., 2016; Wei et al., 2017).

QPCR analysis of the 16S rRNA and ITS rRNA genes was performed to characterize the changes of bacterial and fungal abundances after 38 years of different fertilizer-management regimes (Fig. 1). The results showed that the bacterial abundance was significantly increased by organic fertilizations (Fig. 1), in agreement with previous studies (Fracchia et al., 2006; Vivas et al., 2009; Wu et al., 2011; Chaudhry et al., 2012). This was likely attributable to the availability of labile organic substrates and enhanced root exudates in the manure fertilizer (Paul and Beauchamp, 1989; Sørensen, 1998; Bittman et al., 2005). Notably, the bacterial abundance and Olsen P exhibited a strongly positive correlation ($r = 0.626$, $P < 0.01$) in the present soil, as bacteria are considered to be the predominant microorganisms enhancing the available P level (Kucey, 1983; Hu et al., 2009). This could also explain why the Olsen P contents were significantly increased by organic fertilizations relative to inorganic ones (Table 1). Intriguingly, the dynamics of the fungal abundances followed the same trends as those of bacteria under all fertilization regimes (Fig. 1). Specifically, the fungal abundance was little changed by N treatment, but was significantly increased by NP, NPK, M, MN, MNP, and MNPK treatments, especially in M soils. These results further suggested that the fungal community had a wide pH optimum, ranging from a pH of 5–9, without obvious growth inhibition (Rousk et al., 2010a,b). However, in black soils, Zhou et al. (2016) proposed that the fungal population was mildly stimulated by N and P fertilizer after a 34-year fertilization, and Ding et al. (2017) reported that manure decreased fungal gene abundances after a 35-year fertilization, which indicated that the soil type could be a major reason for the disparities. In addition, the fungal abundance in the present study strongly and positively correlated with NH_4^+-N ($r = 0.634$, $P < 0.01$), NO_3^--N ($r = 0.523$, $P < 0.01$), Olsen P ($r = 0.877$, $P < 0.01$), AK ($r = 0.549$, $P < 0.01$), TN ($r = 0.650$, $P < 0.01$), and SOM ($r = 0.451$, $P < 0.05$), which illustrated that the fungal population was mainly driven by soil nutrition, consistent with the saprophytic statuses of most fungi (Ding et al., 2017). Besides, long-term fertilizer applications, irrespective of whether inorganic or organic fertilizers were used, significantly decreased the B:F ratios compared to those in CK soils. This finding could be related to the decreased pH ($r = -0.551$, $P < 0.01$) (Zhou et al., 2016) and increased Olsen P ($r = -0.634$, $P < 0.01$) found in the fluvo-aquic soil under various fertilization patterns. Results from the present study, therefore, further suggested the contributions of soil microorganisms to soil nutrient maintenance and transformation (Zhou and Ding, 2007; Feng et al., 2019), which in turn affected the microbial populations and the self-maintaining capacity of the soil (McGill et al., 1986; Powlson et al., 1987; Wick et al., 1998).

LEfSe results showed that the species of responding bacteria or fungi varied with fertilization patterns. Specifically, 44, 111, and 131 bacteria (Table S1) and 3, 14, and 10 fungi (Table S2) responded to CK, inorganic, and organic fertilizations, respectively. The responding microbes of the bacterial communities in organic fertilizer-treated soils outnumbered those in inorganic-treated soils, whereas those of the fungal communities showed the opposite trend. In a similar vein, PCA and PLS-DA showed that both bacterial and fungal community compositions were separated under inorganic and organic fertilizations in the fluvo-aquic soil (Fig. 4). It is noticeable that the bacterial community compositions under CK and inorganic fertilizer treatments clustered together and were distinct from those found under organic treatments. In contrast, the fungal community distributions under CK and organic fertilizer treatments clustered together, and were separated from those under inorganic fertilization regimes. These results suggested that the bacterial community was more sensitive to organic fertilization than inorganic fertilization. On the contrary, the application of inorganic fertilizer exerted a greater impact on the fungal community than organic fertilizer. These results were further corroborated by the RDA results (Fig. 6), which demonstrated the fundamental importance of SOM in the bacterial community distribution, rather than in the fungal community compositions. Therefore, alterations of the bacterial community distributions were more closely related to organic fertilizer than inorganic fertilizer, whereas changes in the fungal community compositions were mainly driven by inorganic fertilizer rather than organic fertilizer.

Illumina MiSeq sequencing analyses revealed that there were 5276, 5480, 5395, 5709, 5520, 5083, 5563 and 5468 bacterial OTU numbers in CK, N, NP, NPK, M, MN, MNP, MNPK treated soils, respectively, while those of fungal OTU numbers were 1029, 887, 998, 884, 1000, 795, 1033 and 1036, respectively. These results showed that both bacterial and fungal OTU numbers in MNP and MNPK soils were significantly higher than those in CK soils, suggested the microbial community diversity, to some extent, was increased by compound organic-inorganic fertilizers. Furthermore, LEfSe analysis revealed the *Proteobacteria* and *Actinobacteria* phyla were specifically enriched under MN and N treatments, respectively (Fig. 5a; Table S1). This finding was in accordance with the significantly increased SOM and lowest SOM contents in MN and N soils, respectively (Table 1). *Actinobacteria* are thought to participate in the degradation of organic matter, while *Proteobacteria* were shown to have copiotrophic attributes, thriving under conditions of high labile soil organic C concentrations (Meyer, 1994; Tate, 2000; Fierer et al., 2007; Eilers et al., 2010; Nemergut et al., 2010; Pan et al., 2018c). Consequently, soil microbial community differences could be largely due to organic carbon inputs from manure applications, which could differentiate the eutrophic from the oligotrophic microorganisms (Xun et al., 2016). In addition, members of order *Rhodocyclales* (*Betaproteobacteria*), enriched in MNPK-treated soils (Fig. 5a; Table S1), were able to use complex organic sources and perform important steps of soil N cycles, such as denitrification (Pitombo et al., 2016). Compound organic–inorganic fertilizers application, thus, might promote denitrification and lead to N losses from cultivated land, while the confirmation of this point will require much more data. With respect to the fungal communities, the fungal taxonomic diversity involved mainly two phyla, *Ascomycota* and *Basidiomycota*, except for in MNPK soil, whose taxonomic diversity mainly included *Ascomycota* and *Chytridiomycota* (Figs. 2b and S1b). The dominance of *Ascomycota* and *Basidiomycota* was also observed in saline-sodic soil (Feng et al., 2019), silty-clay loam soil (Paungfoo-Lonhienne et al., 2015), and black soil (Zhou et al., 2016). It was worth noting that *Chytridiomycota* species were enriched under MNPK amendment (Fig. 5b), which was ascribed to the highest TN content in MNPK soil (Table 1), in agreement with a study by Pang et al. (2017) revealing a positive correlation between *Chytridiomycota* and TN. It is expected that compound organic–inorganic fertilizers could increase the microbial diversity by making the soil environment more favorable for the proliferation and activity of specific microbial taxa. In this study, significant increase of both bacterial and fungal OTU numbers observed under MNP and MNPK treatments compared to CK treatment, enrichment of *Proteobacteria*, *Rhodocyclales* and *Chytridiomycota* in MN and MNPK soils, respectively, which supported our aforementioned hypothesis.

The RDA and Mantel test results further indicated that the bacterial

community structure was significantly related to the soil properties, such as the pH, SOM, NO_3^--N, NH_4^+-N, TN, Olsen P, and AK (Fig. 6a). It has been demonstrated that both the overall bacterial community patterns (Rousk et al., 2010a,b) or the individual bacterial groups (Jones et al., 2009) were strongly related with soil pH. As most bacterial taxa tend to exhibit relatively and narrowly optimum pH ranges (Rousk et al., 2010a,b). Likewise, the environmental factors (e.g., pH, NH_4^+-N, TN, Olsen P, and AK) were responsible for shaping the fungal community structure (Fig. 6b). Therefore, various fertilization regimes altered the distribution of bacterial and fungal community compositions by affecting the soil characteristics.

5. Conclusion

In conclusion, 38-year long-term inorganic and organic fertilization strategies exerted great effects on the soil properties, soil microbial abundance and distribution. Balanced chemical fertilization (NPK) combined with organic fertilizer application significantly increased the soil fertility, and is recommended as an optimized fertilization pattern. Both inorganic and organic fertilization, especially the addition of organic manure, significantly increased the sizes of bacterial and fungal populations compared to those found with no fertilizer treatment. Both PCA and PLS-DA suggested that organic fertilizer exerted greater impacts on bacterial community distributions than inorganic fertilizer, while the opposite trend was found for fungal community compositions. The number of responding microbes in the bacterial communities of organic fertilizer-treated soils was markedly higher than that in inorganic fertilizer-treated soils. In contrast, the responding fungal species in organic fertilizer-treated soils were dramatically lower than that in inorganic fertilizer-treated soils. RDA further suggested the primary importance of organic matter in shaping community distributions of bacteria, rather than fungal communities. Therefore, organic fertilizer and inorganic fertilizer dominated in shaping bacterial and fungal community distributions, respectively, by affecting the physicochemical properties of soil.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.still.2019.104540.

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