

Microbial community diversity and enzyme activity varies in response to long-term fertilisation in a continuous potato (*Solanum tuberosum* L.) cropping system

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ABSTRACT

Context. The misuse of chemical fertilisers is still prevalent in potato cultivation. However, the large-scale application of manure is increasingly being accepted by growers to improve soil health. **Aim.** To clarify what effect manure and chemical fertilisers have on the development of soil microbes in potato fields. **Methods.** The status of soil microbial community abundance and structure were determined by analysing soil metagenomes, which were assessed by applying high through-put sequencing technology. The potato field received one of the following treatments for 9 years: manure (M); manure plus nitrogenous (N), phosphatic (P), and potassic (K) fertiliser (MNPK); NPK fertiliser; NP fertiliser; NK fertiliser; PK fertiliser; and no fertiliser. **Key results.** The application of manure significantly increased soil organic matter, and this increase was one of the main factors in reducing bacterial Shannon and Heip indices and increasing the Simpson index. The MNPK treatment significantly reduced the relative abundance of α -Proteobacteria and β -Proteobacteria in the soil, which are essential microorganisms involved in nitrogen cycling. The M treatment significantly increased the abundance of Actinobacteria. **Conclusions.** In summary, applying manure increases Actinobacteria's abundance, and using MNPK fertilisers decreases the abundance of Proteobacteria, whereas chemical fertilisers are detrimental to soil fungal diversity. **Implications.** Applying MNPK fertilisers has a complex effect on soil microorganisms. It is not simply a combination of the effects of manure and chemical fertilisers on microbes; the interaction mechanism of microbial succession needs to be further explored.

Keywords: bacteria, community diversity, enzyme activity, fertilisation, fungi, microbial abundance, potato, soil microbes.

Introduction

Soil is the natural medium in which crops are grown, and the microorganisms within the soil play an important role in promoting the cycling of nutrients and energy in farmland ecosystems. Improving the ability of crops to obtain nutrients by exploiting the role of soil microorganisms has been the focus of global sustainable agricultural development in recent decades (Freitas *et al.* 2007). The Food and Agriculture Organization (FAO) of the United Nations promotes sustainable soil management using means that increase soil organic matter (OM) content in the surface layer of farmland soil and create a suitable environment for the survival and reproduction of beneficial microorganisms, which in turn promote the better utilisation of soil nutrients by crops (FAO 2016). In resource-poor developing countries, low soil fertility remains the most important factor limiting increase in crop yield (Mohammadi and Sohrabi 2012). Thus, growers often use large amounts of nitrogen and phosphate fertilisers to promote yield, but this can lead to soil acidification and consequently reduce crop yield and quality over time (Liang *et al.* 2013).

Physical, chemical, and biological characteristics of fertile soil and the chemical fertility of soil are affected by biological processes (Abbott and Murphy 2007). Soil biological fertility involves direct or indirect activities of microorganisms, such as organic compound mineralisation and mineral dissolution facilitated by microorganisms. Soil biological fertility can be quantified by determining the abundance, diversity, and level of activities of microbial communities (Lemanceau *et al.* 2015). Studies have shown that the microbes that promote plant growth are members of common bacterial and fungal genera found in soils. *Bacillus*, *Enterobacter*, *Rhizobium*, *Bradyrhizobium*, *Pantoea*, *Erwinia*, and *Pseudomonas* are the common bacterial genera, and *Aspergillus*, *Trichoderma*, and *Penicillium* are the common fungal representatives found in soil (Marschner 2008; Saharan and Nehra 2011; Ahemad and Kibret 2014; Yadav 2017). Maintaining the diversity and activity of beneficial microorganisms in soil promotes the continuous absorption of nitrogen and phosphorus by the crops present (Barea *et al.* 2007; Richardson *et al.* 2009; Lugtenberg *et al.* 2013; De Bruijn 2015).

The application of nitrogen and phosphate fertilisers can greatly affect soil microorganisms in farmland, and thus microbial activity in farmland ecosystems is often limited by unsuitable fertilisation management (Fan *et al.* 2020). Fungi often play a more important role in the decomposition and transformation of soil nutrients when fertiliser supplies are scarce (Bardgett *et al.* 1999). Changes in soil-available nitrogen and phosphorus contents can alter the structure and function of the microbial community (Cruz *et al.* 2009; Das *et al.* 2017). Mainly, available nitrogen content strongly influences changes in the microbial community (Liu *et al.* 2020a). Ammonium-nitrogen fertiliser had a more significant negative influence on microbial community structure than manure application; the abundance ratio of fungi to bacteria in manure treatment was significantly higher than that in chemical fertiliser treatment, and the ratio in the no fertiliser treatment was similar to that in manure treatment (Suzuki *et al.* 2005). Liu *et al.* (2020b) and Zhang *et al.* (2019) found that soil fertility was positively correlated with the α -diversity of fungi in agricultural soils. Compared with abundances in abandoned farmland, long-term chemical fertiliser application significantly reduced the abundance of bacteria and fungi (Li *et al.* 2005).

As one of the most important food crops, potato (*Solanum tuberosum* L.) is widely cultivated and produced worldwide. Continuous progress in potato production requires sustained soil health and high microbial activity. Rational application of fertilisers can improve soil microbial diversity and community structure (Gu *et al.* 2019), and potato's growing environment. However, the effects of long-term application of different fertilisers on soil microbial diversity and community structure in potato fields have rarely been reported. Hence, this study aims to explore the changes in soil microbial species abundance and community structure in a continuous potato cropping system exposed to

long-term fertilisation with different organic and chemical sources of nutrients.

Materials and methods

Experimental design and soil sampling

The long-term fertilisation experiment was conducted in a potato field from 2010 to 2018 in the northern foothills of Yinshan Mountains (41°10'57"N, 111°36'17"E, 1606 m above sea level), which is one of the main potato-producing areas in China (Fig. 1). The area has a mid-temperate continental climate with abundant sunshine and large temperature differences during the day and night. The average annual temperature is 2.4°C; the active accumulated temperature is about 2000°C from mid-May to mid-September. The annual average precipitation is about 350 mm, mostly concentrated in summer, and the annual evaporation is 2068 mm, which is almost six times the annual average precipitation. The annual frost-free period varies in the range of 110–120 days. Soils here belong to the group Calcic Kastanozem.

The cropping system was continuous summer potato cultivation (variety Kexin 1) followed by winter fallow. We established seven fertiliser treatments: (1) manure (M), (2) inorganic nitrogen, phosphorus, and potassium fertilisers (NPK), (3) manure combined with NPK (MNPK), (4) inorganic nitrogen and phosphorus fertilisers (NP), (5) inorganic nitrogen and potassium fertilisers (NK), (6) inorganic phosphorus and potassium fertilisers (PK), and (7) a no-fertiliser control (CK). Annual NPK fertilisation comprised 150 kg N hm⁻² as urea, 75 kg P₂O₅ hm⁻² as calcium superphosphate, and 270 kg K₂O hm⁻² as potassium sulfate. Annual sheep manure (0.6%N, 0.5%P₂O₅, 0.2%K₂O) application rate was 22 500 kg hm⁻². The treatments, each with four replicate plots, were set up using a randomised block design (the design was unchanged throughout the entire period). The potato seed tubers with a diameter of about 2 cm were sown in a ditch at a depth of 8 cm, the proposed fertiliser was applied all at once. The experimental fields were manually weeded and watered by trickle irrigation. Field management practices were the same in all treatments throughout the experimental period.

On 24 August 2018, three intact soil samples were randomly collected at the tillage horizon (0–20 cm) in each plot using a stainless-steel soil sampler with a diameter of 5 cm. All samples from each plot were carefully mixed to form a composite sample and transported to the laboratory in a constant temperature box containing ice. Each sample was divided into two parts: one part was air-dried for determination of the physicochemical properties and the other part was sealed in a plastic bag with Drikold® (–78.5°C) for determination of the metagenome of soil microorganisms.

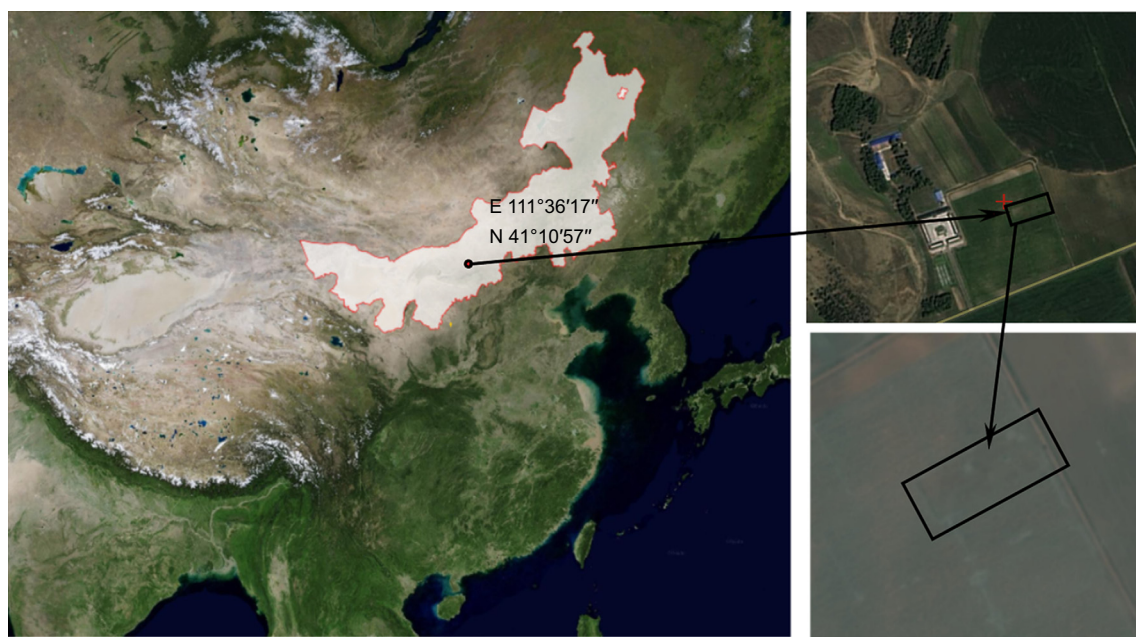


Fig. 1. The geographical location of the research area.

Soil properties analysis

Soil pH was measured by using a glass electrode (FE20-FiveEasy™ pH; Mettler Toledo, Weilheim, Baden-Württemberg, Germany) with a 1:1 soil-to-water ratio. Soil humidity was measured by using a soil water content meter (Diviner2000; Sentek, Adelaide, Australia). Soil available potassium (AK) was extracted using 1 M ammonium acetate and measured using flame photometry (FP640 Flame Photometer; INESA, Shanghai, China). Soil available nitrogen (AN) was calculated as ammonia-nitrogen using the methods described in detail in [Bremner and Shaw \(1955\)](#). Soil available phosphorus (AP) was extracted using 0.5 M NaHCO₃, and its concentration determined using the molybdenum blue method with colorimetry (660 nm) ([Holman 1943](#)). Soil OM was determined using dilution heat method with a 1:0.2 K₂Cr₂O₇-to-H₂SO₄ ratio, and direct titration with 0.1 M FeSO₄ ([Bisutti et al. 2004](#)).

DNA extraction and metagenome sequencing analysis

Bacterial and fungal community genomic DNA was extracted from each soil sample using the DNeasy® PowerSoil® DNA Kit (QIAGEN, Redwood City, CA, USA) according to the manufacturer's instructions. The quality, purity, and concentration of the extracted DNA were determined by 1% agarose gel electrophoresis and NanoDrop™ 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 341F (5'-CCTACGGGNG GCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAAT CC-3'). The internal transcribed spacer (ITS) region of rRNA

gene was amplified with primer pairs V43NDF (5'-GGCAA GTCTGGTGCCAG-3') and EukV4R (5'-ACGGTATCTRATC RTCTTCG-3'). All rRNA genes were amplified using an ABI GeneAmp® 9700 PCR thermocycler (ABI, Carlsbad, CA, USA). The purifying and quantifying of the extracted PCR product were performed with 2% agarose gel, AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and a Quantus™ Fluorometer (Promega, Madison, WI, USA).

Library construction was performed using the NEXTFLEX® Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on a MiSeq PE300 platform platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw rRNA gene sequencing reads were deposited into the Nucleotide Sequence Database. The raw reads were demultiplexed, quality-filtered by fastp ver. 0.20.0 ([Chen et al. 2018](#)) and merged by FLASH ver. 1.2.7 ([Magoč and Salzberg 2011](#)). Using UPARSE ver. 7.1 ([Edgar 2013](#)), operational taxonomic units (OTUs) with 97% similarity cut-off were clustered and chimeric sequences were identified and removed. Representative sequences were annotated by RDP Classifier ver. 2.2 ([Wang et al. 2007](#)) against the Silva v138 (<http://www.arb-silva.de>).

Soil enzymatic activity analysis

Enzyme activities were determined using the following methods. The soil urease (EC 3.5.1.5) activity was determined using 10% urea solution as the substrate and citrate buffer (pH 6.7); the ammonium released from urea hydrolysis was quantified using absorbance at 578 nm (Evolution™

One Thermo Scientific™, Waltham, MA, USA). The activity level was expressed as NH_4^+ per gram of dry weight of soil per 24 h (Guan 1986; Nannipieri *et al.* 2012). The activity of soil invertase (EC 3.2.1.26) was determined, using 8% sucrose solution as the substrate and phosphate buffer (pH 5.5), calorimetrically according to the reaction of glucose with 3,5-dinitrosalicylic acid, taking absorbance at 508 nm (Evolution™ One Thermo Scientific™, Waltham, MA, USA). The value was expressed as glucose per gram of dry weight of soil per 24 h (Guan 1986). Soil alkaline phosphatase (EC 3.1.3.1) activity was measured with disodium phenyl phosphate solution as a substrate and borate buffer (pH 9.6), and absorbance was measured at 660 nm (Evolution™ One Thermo Scientific™, Waltham, MA, USA). The value was expressed as phenol per gram of dry weight of soil per 2 h (Guan 1986). Soil catalase (EC 1.11.1.6) activity was measured with H_2O_2 (0.3%) as a substrate, the mixture was shaken for 30 min, and then 5 mL of H_2SO_4 at 1.5 mol L^{-1} was added. Its filtrate was titrated with $0.002 \text{ mol L}^{-1} \text{ KMnO}_4$. The reacted amount of KMnO_4 was calculated per gram of the sample to represent catalase activity (Roberge 1978).

Statistical analysis

The SPSS Statistics package for Windows (SPSS, ver. 25.0, Chicago, IL, USA) was used for one-way ANOVA of abundances of bacterial and fungal communities and soil enzyme activities. Multiple comparisons were performed using the S–N–K method (Hilton and Armstrong 2006) and a confidence interval of 95%. Because the sequencing depth varied across samples, we used a sub-sampling procedure to normalise the number of reads to the minimum observed across all samples (19 461 reads in bacterial data and 4245 reads in fungal data). The α -diversity indices of microbes were calculated using Mothur (ver. 1.30.2) (Xu *et al.* 2018). The community abundances of microbes and the calculation of β -diversity were performed by QIIME (ver. 1.9.1) (Caporaso *et al.* 2010). Using the vegan package of the R language

(Dixon 2003), clustering was carried out according to the similarity of species abundance. The results are displayed on the community heatmap map, which clusters high- and low-abundance species in blocks through colour change and similarity degree to reflect the similarities and differences in community composition of different treatments at the genus level. Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa 2002), the PICRUST software package was used for functional prediction analysis on the 16S and ITS sequencing data. Through the GreenGene ID corresponding to the measured OTU, the Clusters of Orthologous Groups (COG) information and KEGG Orthology (KO) information of the OTU were obtained, and its abundance was calculated. Information about enzymes and enzymatic reactions could be annotated on each OTU. By analysing the OTU quantity data annotated by the target enzyme in each fertiliser treatment, the activity of the target enzyme-catalysed reaction in the corresponding soil was judged.

Results

Soil properties

Compared with CK, soil pH was lower in the MNPK soil, but was similar to the soils with the other treatments. The highest OM (33.36 g kg^{-1}) and AN contents (82.12 mg kg^{-1}) were in the M soil, and were similar to the MNPK soil. Application of MNPK also significantly increased AP (64.39 mg kg^{-1}) and AK contents ($359.83 \text{ mg kg}^{-1}$) compared to the other treatments (Table 1).

Microbial diversity

When analysing the diversity of bacterial and fungal communities, the corresponding α -diversity index value of each sample was used as a Y-axis, and the extracted data volume as a X-axis to draw the distribution rarefaction

Table 1. Variations of soil properties under different fertiliser treatments.

Treatments	Soil properties					
	Soil pH	Soil humidity (%)	Organic matter (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
MNPK	8.15 ± 0.05^b	22.80 ± 1.76^a	27.71 ± 1.71^{ab}	82.07 ± 3.96^a	64.39 ± 0.52^a	359.83 ± 14.37^a
M	8.21 ± 0.04^{ab}	20.59 ± 1.45^a	33.36 ± 6.61^a	82.12 ± 3.19^a	42.73 ± 2.33^b	210.75 ± 6.17^c
NPK	8.18 ± 0.06^{ab}	21.84 ± 1.45^a	16.30 ± 6.03^{bc}	66.02 ± 2.64^b	32.84 ± 3.47^c	256.06 ± 18.61^b
NP	8.20 ± 0.07^{ab}	20.55 ± 0.46^a	15.39 ± 7.60^c	69.54 ± 3.90^b	30.56 ± 4.45^c	136.79 ± 3.12^d
NK	8.22 ± 0.06^{ab}	22.41 ± 1.52^a	16.30 ± 5.07^{bc}	71.30 ± 3.01^b	17.01 ± 0.29^d	262.93 ± 4.91^b
PK	8.27 ± 0.02^{ab}	21.31 ± 0.99^a	15.22 ± 2.63^c	63.55 ± 1.98^b	41.70 ± 4.74^b	237.72 ± 26.43^{bc}
CK	8.32 ± 0.03^a	20.85 ± 1.23^a	14.64 ± 3.42^c	63.52 ± 1.44^b	15.85 ± 0.70^d	137.77 ± 13.07^d

Superscript letters a–d indicate significant difference at $P < 0.05$ in a given column for fertiliser treatments. MNPK, manure combined with NPK; M, manure; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers; CK, no-fertiliser control.

curves. All curves reached a smooth state, indicating that the sequencing data volume was sufficient to accurately represent the communities in the field soil.

We analysed the diversity of microbial communities from the perspectives of richness and evenness. Fig. 2 shows the α -diversity boxplots of the bacterial community. The Chao1 values for the different treatments showed no significant differences, but the medians of Chao1 values of M and MNPK were lower than those of the lower quartiles of the other treatments, indicating reductions in the richness of the bacterial communities in M and MNPK (Fig. 2a). Analysis of the Shannon index values showed that the CK, NP, and NPK treatments had values higher than the other treatments (Fig. 2c). The range of Heip values for NP was wider than that of the other treatments (Fig. 2b). The Shannon values of M were narrower in range than those of the other

treatments, and the medians of Heip and Shannon values of M were lower than those of the other treatments. Compared with M, the medians of Heip and Shannon values for MNPK were higher and wider ranging. The Simpson index represents species richness and evenness, where a greater numerical value indicates that some microbes are more represented than others in samples. The median Simpson's index value of M was significantly higher than those of NPK and PK. Additionally, MNPK's upper quartile was also higher than those of the chemical fertiliser treatments (Fig. 2d). All four index values indicated that the application of manure substantially promoted the dominance of particular soil bacterial populations, and reduced evenness of the bacterial community.

Fig. 3 shows the α -diversity indices of fungal communities. Compared with the CK, only the upper quartile of M was

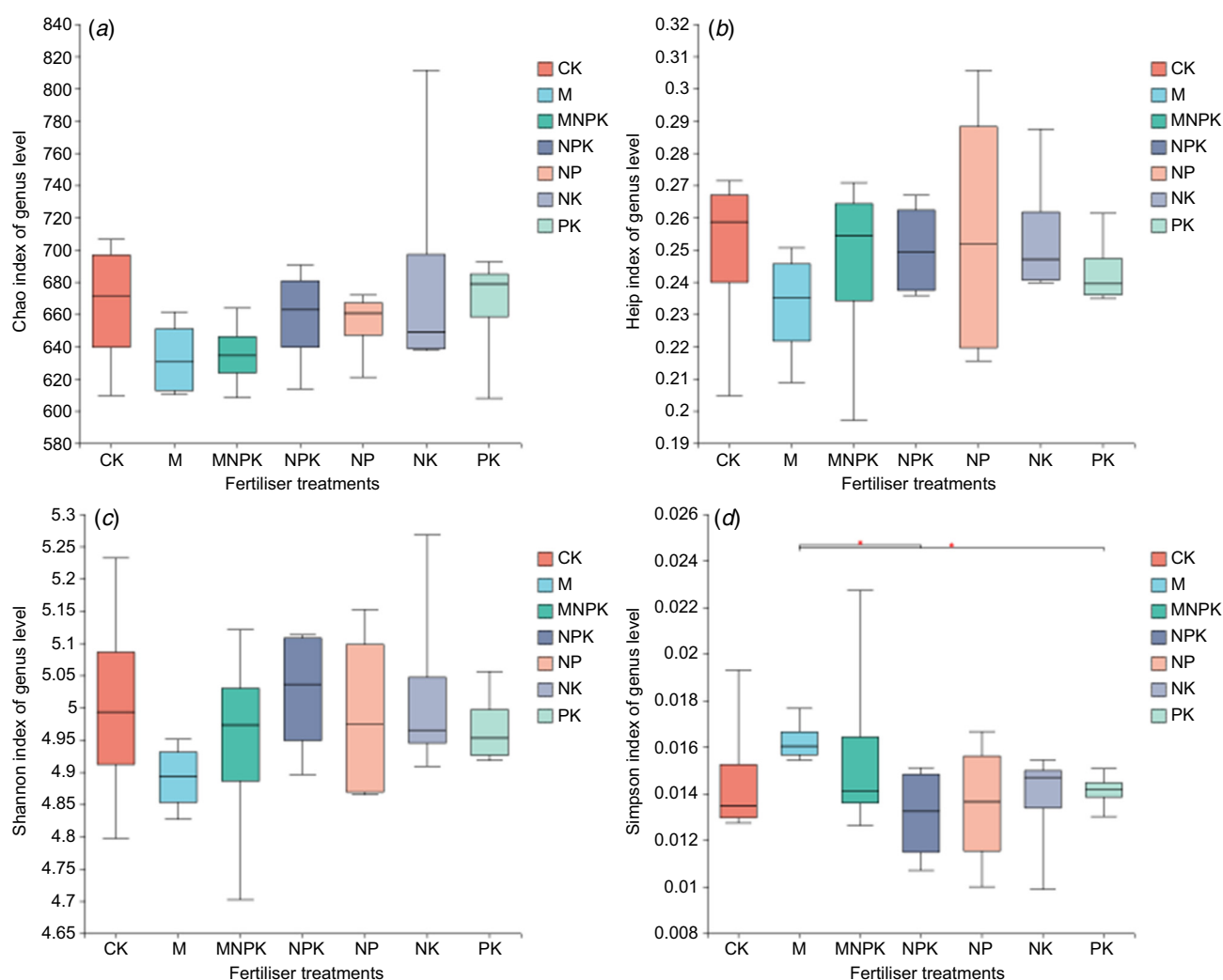


Fig. 2. (a) Chao index, (b) Heip index, (c) Shannon index and (d) Simpson index of soil bacterial communities in different fertiliser treatments. Boxplots with same single asterisk significantly differ at $P < 0.05$. CK, no-fertiliser control; M, manure; MNPK, manure combined with NPK; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers.

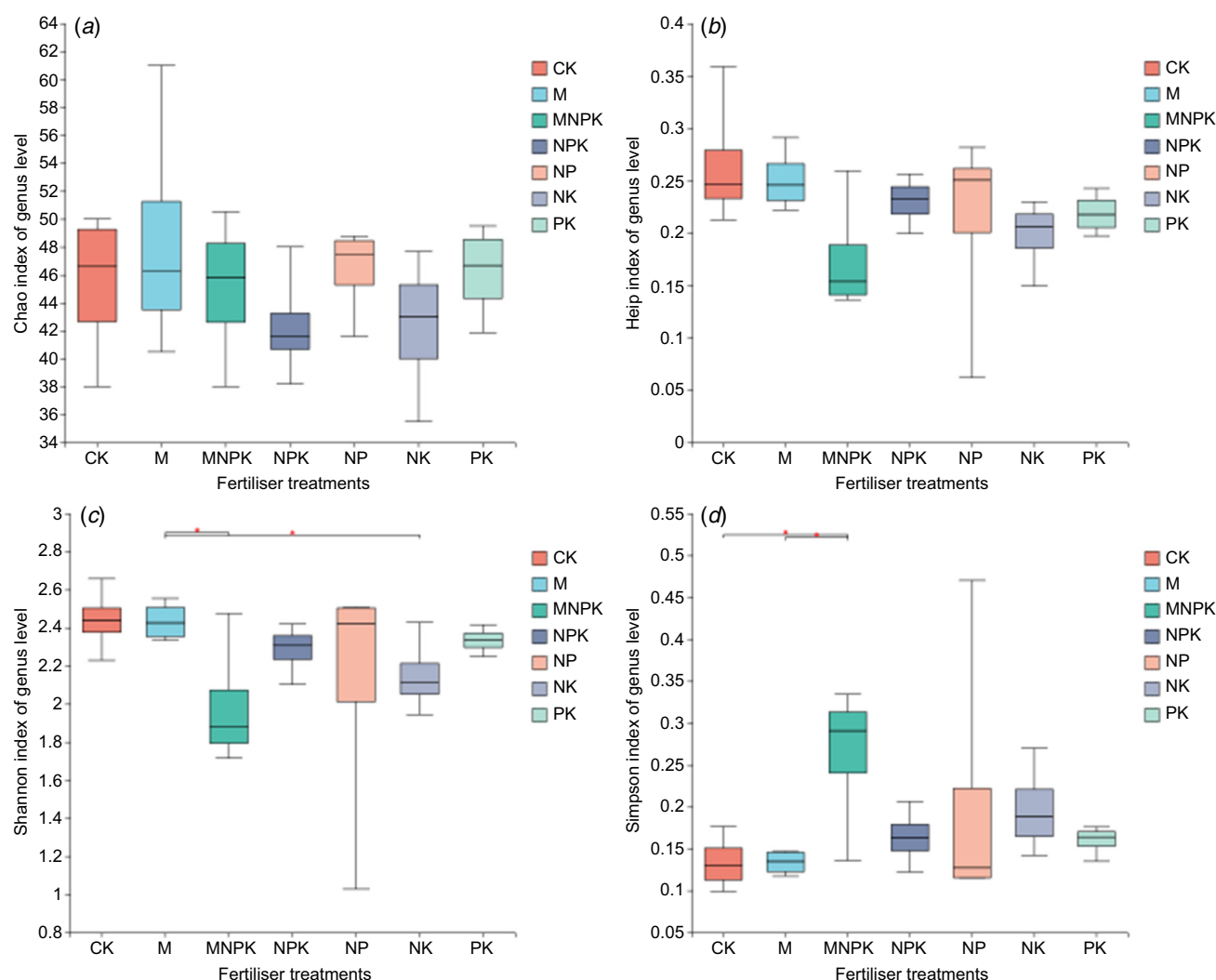


Fig. 3. (a) Chao index, (b) Heip index, (c) Shannon index and (d) Simpson index of soil fungal communities in different fertiliser treatments. Boxplots with the same single asterisk significantly differ at $P < 0.05$. CK, no-fertiliser control; M, manure; MNPK, manure combined with NPK; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers.

higher for the Chao1 index values of the fungal communities, while the upper quartiles of other treatments were lower. The medians and upper quartiles of NPK and NK were much lower than the respective values of the CK (Fig. 3a). These data suggest that manure helped to maintain the richness of the soil fungal community, while NPK fertiliser had the opposite effect. Results of the Heip and Shannon indices suggested that fungal evenness of MNPK and NK was substantially lower compared to those of CK, and the Heip index value of M was closer to that of CK (Fig. 3b). Moreover, the Shannon index value of M was significantly higher than those of MNPK and NK (Fig. 3c). The Simpson index value of MNPK was significantly larger than those of M and CK. The Simpson index values of all fertiliser treatments were higher than that of CK to different degrees, except for that of M (Fig. 3d). Overall, the results showed that soil fungal communities were

more sensitive to inorganic fertilisers, while manure had little to no effect on altering the diversity of the fungal communities in potato field soil.

Further analysis of the Pearson correlations between the α -diversity index and soil properties showed that bacterial Shannon index was significantly negatively correlated with OM content ($P < 0.01$), bacterial Simpson index was significantly positively correlated with OM content ($P < 0.05$), and the Heip index of bacteria was significantly negatively correlated with OM content ($P < 0.05$) (Table 2).

Microbial community composition and abundance

Seven phyla (with relative abundance $>5\%$) made up a core community of bacteria, comprising Proteobacteria,

Table 2. Pearson correlation coefficients between α -diversity index and soil characteristics.

Item	Moisture	OM	AN	AP	AK	pH
Shannon (B)	0.321	−0.505**	−0.284	−0.283	−0.031	0.038
Sig. (two-tailed)	0.096	0.006	0.143	0.145	0.877	0.847
Simpson (B)	−0.146	0.433*	0.129	0.160	−0.078	0.077
Sig. (two-tailed)	0.460	0.021	0.512	0.415	0.693	0.696
Chao1 (B)	0.168	−0.060	−0.151	−0.056	0.095	−0.034
Sig. (two-tailed)	0.391	0.761	0.444	0.778	0.632	0.862
Heip (B)	0.290	−0.463*	−0.127	−0.229	−0.080	0.057
Sig. (two-tailed)	0.135	0.013	0.519	0.242	0.684	0.775
Shannon (F)	0.032	−0.307	−0.140	−0.172	−0.168	−0.164
Sig. (two-tailed)	0.871	0.112	0.479	0.380	0.393	0.404
Simpson (F)	−0.151	0.276	0.148	0.220	0.308	0.142
Sig. (two-tailed)	0.443	0.156	0.452	0.261	0.111	0.472
Chao1 (F)	−0.016	−0.041	0.052	0.110	0.267	−0.294
Sig. (two-tailed)	0.936	0.834	0.794	0.576	0.169	0.129
Heip (F)	−0.059	−0.338	−0.221	−0.319	−0.265	−0.033
Sig. (two-tailed)	0.764	0.078	0.258	0.097	0.173	0.869

*Correlation significant at $P < 0.05$; **Correlation significant at $P < 0.01$.

B, bacterium; F, fungus; OM, organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium.

Actinobacteria, Planctomycetes, Acidobacteria, Chloroflexi, Bacteroidetes, and Gemmatimonadetes. Among the different fertilisation treatments, Proteobacteria and Actinomycetes were the dominant groups (Table 3). The relative abundance of Proteobacteria in NPK was the highest (34.96%), followed by that of NK; both were significantly higher than those of CK and MNPK. The Proteobacteria abundance in CK was the lowest (28.50%). All treatments with only chemical fertilisers were higher in Proteobacteria abundance than M. The relative abundance of Actinobacteria was the highest in M (32.69%) and lowest in NK (19.88%). The relative abundance of Actinobacteria was significantly higher in M than in NK, NP, and PK, while those for NPK and CK

were significantly higher than in NK. Different fertilisation treatments also produced significant differences in relative abundances of Planctomycetes and Acidobacteria. The MNPK had the highest relative abundance of Planctomycetes (13.25%), which was significantly higher than in NPK. The NK had the highest relative abundance of Acidobacteria (9.29%), significantly higher than in the other treatments.

We analysed the relative abundance data of the top 50 OTUs in total that were each at least 0.5% of the total abundance. Among the top 50 bacterial communities in the sum of relative abundances of all treatments, there were 26 communities with significant differences in relative abundance between treatments (Table 4). Firstly, there were

Table 3. The relative abundance of dominant bacterial phyla after different fertiliser treatments in a potato field (%).

Phylum	Treatments						
	MNPK	M	NPK	NP	NK	PK	CK
Proteobacteria	29.47 ^b	31.26 ^{ab}	34.96 ^a	32.95 ^{ab}	34.81 ^a	32.93 ^{ab}	28.50 ^b
Actinobacteria	25.94 ^{abc}	32.69 ^a	29.11 ^{ab}	24.97 ^{bc}	19.88 ^c	25.68 ^{bc}	27.16 ^{ab}
Planctomycetes	13.25 ^a	6.39 ^{ab}	6.18 ^b	7.23 ^{ab}	7.12 ^{ab}	8.38 ^{ab}	8.63 ^{ab}
Acidobacteria	6.87 ^b	6.79 ^b	6.69 ^b	7.30 ^b	9.29 ^a	7.33 ^b	6.75 ^b
Chloroflexi	5.94 ^a	5.57 ^a	5.06 ^a	5.26 ^a	5.94 ^a	5.91 ^a	6.39 ^a
Bacteroidetes	4.14 ^a	4.40 ^a	4.50 ^a	6.81 ^a	6.94 ^a	5.25 ^a	6.61 ^a
Gemmatimonadetes	4.20 ^a	5.87 ^a	4.99 ^a	4.83 ^a	5.52 ^a	5.40 ^a	4.76 ^a

Superscript letters a–c indicate significant difference at $P < 0.05$ in a given row for fertiliser treatments. MNPK, manure combined with NPK; M, manure; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers; CK, no-fertiliser control.

Table 4. The relative abundance of bacterial genera after different fertiliser treatments in a potato field (%).

Genus	Treatments						
	MNPK	M	NPK	NP	NK	PK	CK
<i>Pseudarthrobacter</i>	3.09 ^{Ab}	5.79 ^{Aa}	3.80 ^{Aab}	3.86 ^{Aab}	3.51 ^{Aab}	4.44 ^{Aab}	4.74 ^{Aab}
<i>Sphingomonas</i>	2.44 ^{Ab}	4.66 ^{Aa}	4.64 ^{Aa}	3.68 ^{Aab}	4.67 ^{Aa}	4.54 ^{Aa}	3.36 ^{Aab}
c_Actinobacteria	1.54 ^{Bb}	2.94 ^{Aa}	2.21 ^{ABab}	2.16 ^{ABab}	1.84 ^{ABb}	2.33 ^{ABab}	2.31 ^{ABab}
<i>Rubrobacter</i>	1.30 ^{Ab}	2.28 ^{Aab}	1.41 ^{Aab}	2.15 ^{Aab}	1.91 ^{Aab}	2.44 ^{Aa}	1.76 ^{Aab}
<i>Blastococcus</i>	2.25 ^{Aa}	2.03 ^{Aab}	1.95 ^{Aab}	0.93 ^{Aab}	0.73 ^{Ab}	1.27 ^{Aab}	1.85 ^{Aab}
f_Nitrosomonadaceae	1.04 ^{Bc}	1.44 ^{ABabc}	1.84 ^{Aa}	1.43 ^{ABabc}	1.7 ^{ABab}	1.62 ^{ABab}	1.23 ^{ABbc}
f_Cytophagaceae	0.92 ^{Cc}	1.38 ^{ABCbc}	1.30 ^{ABCbc}	2.24 ^{Aa}	1.97 ^{ABab}	1.28 ^{ABCbc}	1.07 ^{BCc}
f_Planctomycetaceae	2.31 ^{Aa}	1.01 ^{Ab}	1.00 ^{Ab}	1.02 ^{Ab}	1.08 ^{Ab}	1.38 ^{Aab}	1.36 ^{Aab}
f_JG34-KF-161	0.66 ^{Bb}	1.33 ^{ABa}	1.44 ^{Aa}	1.06 ^{ABab}	1.53 ^{Aa}	1.55 ^{Aa}	1.06 ^{ABab}
<i>Gaiella</i>	1.07 ^{ABab}	1.59 ^{Aa}	1.57 ^{Aa}	1.04 ^{ABab}	0.77 ^{Bb}	1.21 ^{ABab}	1.16 ^{ABab}
<i>Nocardioides</i>	1.60 ^{ABa}	1.46 ^{ABCa}	1.71 ^{Aa}	0.70 ^{Cb}	0.69 ^{Cb}	0.82 ^{BCb}	1.29 ^{ABCab}
RB41	0.70 ^{Bb}	1.08 ^{ABb}	1.02 ^{ABb}	1.28 ^{ABab}	1.83 ^{Aa}	1.31 ^{ABab}	1.02 ^{ABb}
<i>Streptomyces</i>	1.23 ^{Aab}	1.44 ^{Aa}	1.28 ^{Aab}	1.07 ^{Aab}	0.73 ^{Ab}	1.10 ^{Aab}	1.24 ^{Aab}
o_Gaiellales	0.72 ^{Bb}	1.38 ^{Aa}	1.32 ^{ABa}	0.92 ^{ABab}	0.80 ^{ABb}	1.10 ^{ABab}	1.08 ^{ABab}
<i>Steroidobacter</i>	0.63 ^{Ab}	0.70 ^{Aab}	0.85 ^{Aab}	0.95 ^{Aab}	1.02 ^{Aa}	0.84 ^{Aab}	0.72 ^{Aab}
c_Gemmatimonadetes	0.58 ^{Ab}	0.84 ^{Aab}	0.68 ^{Aab}	0.70 ^{Aab}	0.94 ^{Aa}	0.78 ^{Aab}	0.77 ^{Aab}
f_OM1_clade	0.46 ^{Bb}	0.84 ^{ABa}	0.69 ^{ABab}	0.91 ^{Aa}	0.73 ^{ABab}	0.84 ^{ABa}	0.72 ^{ABab}
<i>Bryobacter</i>	0.51 ^{Bb}	0.71 ^{ABab}	0.66 ^{ABab}	0.73 ^{ABab}	0.89 ^{Aa}	0.90 ^{Aa}	0.61 ^{ABb}
<i>Pirellula</i>	1.00 ^{Aa}	0.50 ^{Ab}	0.58 ^{Aab}	0.59 ^{Aab}	0.64 ^{Aab}	0.80 ^{Aab}	0.81 ^{Aab}
o_TRA3-20	0.46 ^{Bb}	0.74 ^{ABa}	0.73 ^{ABa}	0.67 ^{ABab}	0.81 ^{Aa}	0.72 ^{ABa}	0.68 ^{ABab}
f_JG34-KF-361	0.44 ^{Bb}	0.80 ^{Aa}	0.73 ^{ABa}	0.66 ^{ABab}	0.63 ^{ABab}	0.82 ^{Aa}	0.67 ^{ABab}
<i>Devosia</i>	0.64 ^{Aab}	0.43 ^{Ab}	0.50 ^{Ab}	1.17 ^{Aa}	0.53 ^{Ab}	0.46 ^{Ab}	0.50 ^{Ab}
<i>Iamia</i>	0.64 ^{Aab}	0.57 ^{Aab}	0.71 ^{Aa}	0.57 ^{Aab}	0.32 ^{Ab}	0.47 ^{Aab}	0.61 ^{Aab}
<i>Variibacter</i>	0.40 ^{Bb}	0.58 ^{ABab}	0.75 ^{Aa}	0.59 ^{ABab}	0.46 ^{Bb}	0.57 ^{ABab}	0.48 ^{ABb}
<i>Mesorhizobium</i>	0.71 ^{Aa}	0.45 ^{Aabc}	0.51 ^{Aabc}	0.68 ^{Aab}	0.39 ^{Abc}	0.35 ^{Ac}	0.44 ^{Aabc}
<i>Nordella</i>	0.28 ^{Ab}	0.50 ^{Aa}	0.51 ^{Aa}	0.51 ^{Aa}	0.44 ^{Aab}	0.57 ^{Aa}	0.40 ^{Aab}

Superscript letters A–C indicate significant difference at $P < 0.01$ in a given row for fertiliser treatments. Superscript letters a–c indicate significant difference at $P < 0.05$ in a given row for fertiliser treatments. 'c', 'o', and 'f' in genus list represent class, order, and family, respectively. MNPK, manure combined with NPK; M, manure; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers; CK, no-fertiliser control.

11 communities with significant changes in the relative abundance of bacteria under MNPK treatment. Seven bacterial genera were from Proteobacteria, and the relative abundance of *Sphingomonas* in MNPK was 2.44%, which was significantly lower than for M, NPK, NK, and PK. The relative abundance of Nitrosomonadaceae (Family) in MNPK was 1.04%, significantly lower than those of NPK, NK, and PK. The relative abundance of JG34-KF-161 (Family) in MNPK was 0.66%, significantly lower than those of PK, NK, NPK, and M; the relative abundance of JG34-KF-361 (Family) in MNPK was 0.44%, significantly lower than that of PK, M, and NPK. The relative abundance of TRA3-20 (Order) of MNPK was 0.46%, significantly lower than those of NK, M, and NPK. The relative abundance of *Nordella* in MNPK was 0.28%, significantly lower than those of PK, NPK, NP, and M.

The relative abundance of *Mesorhizobium* in MNPK was 0.71%, significantly higher than those of PK and NK. Two bacterial genera were derived from Actinobacteria. The relative abundance of *Blastococcus* in MNPK was 2.25%, which was significantly higher than that of NK; the relative abundance of OM1-clade (Family) in MNPK was 0.46%, significantly lower than those of NP, M, and PK. Two bacterial genera were derived from Planctomycetes. The relative abundance of Planctomycetaceae (Family) in MNPK was 2.31%, which was significantly higher than those of M, NPK, NP, and NK; and the relative abundance of *Pirellula* in MNPK was 1.00%, significantly higher than that of M.

Secondly, there were nine communities with significant changes in the relative abundance under partial chemical fertiliser treatment. Two bacterial genera were from

Proteobacteria, and the relative abundance of *Steroidobacter* in NK was 1.02%, significantly higher than that of MNPK; the relative abundance of *Devosia* in NP was 1.17%, significantly higher than for M, PK, CK, NPK, and NK. The three bacterial genera were from Actinobacteria. The relative abundance of *Rubrobacter* in PK was 2.44%, significantly higher than that of MNPK. The relative abundance of *Gaiella* in NK was 0.77%, significantly lower than those of M and NPK; the relative abundance of *Nocardioideis* in NK, NP, and PK was 0.69–0.82%, significantly lower than for NPK, MNPK, and M. One bacterial genus was derived from Bacteroidetes. The relative abundances of Cytophagaceae (Family) in NP and NK were 2.24% and 1.97%, respectively, significantly higher than those in MNPK and CK. Two bacterial genera were from Acidobacteria. The relative abundance of RB41 in NK was 1.83%, significantly higher than those in MNPK, NPK, CK, and M; the relative abundance of *Bryobacter* in PK and NK was 0.90% and 0.89%, respectively, significantly higher than those in MNPK and CK. One bacterial genus was derived from Gemmatimonadetes, and the relative abundance of Gemmatimonadetes (Class) in NK was 0.94%, significantly higher than that in MNPK.

Thirdly, the relative abundance of four bacteria from Actinobacteria increased significantly under M treatment. The relative abundance of *Pseudarthrobacter* in M was 5.79%, significantly higher than that of MNPK. The relative abundance of Actinobacteria (Class) in M was 2.94%, significantly higher than those of MNPK and NK. The relative abundance of *Streptomyces* in M was 1.44%, significantly higher than that of NK. The relative abundance of Gaiellales (Order) in M was 1.38%, significantly higher than those of MNPK and NK. The relative abundances of two bacteria belonging to Proteobacteria and Actinobacteria increased significantly under NPK treatment. The relative abundance of *Variibacter* was 0.75%, which was significantly higher than those of MNPK and NK. The relative abundance of *Iamia* was 0.71%, which was significantly higher than that of NK.

Three fungal phyla (Ascomycota, Chytridiomycota, and Basidiomycota) had relative abundances >1%. Ascomycota clearly dominated in abundance across all treatments, with relative abundances with range of 88–92%, and the relative abundances of other fungal phyla were <3% with the exception of the unclassified fungi. The relative abundance of Chytridiomycota in NPK reached 2.58%, which was

significantly higher than in the other treatments. Furthermore, the Chytridiomycota abundance was 0.72% in M, the lowest among all treatments. The abundance of Basidiomycota was highest in CK (1.15%) followed by that in M (1.59%), which were significantly higher than abundances in the other treatments. Among the treatments with chemical fertilisers, the range of relative abundances of Basidiomycota was 0.09–0.64%, suggesting an inhibitory effect of chemical fertilisers on Basidiomycota (Table 5). In Fig. 4, the abundance data of all 63 genus-level communities of Ascomycota are shown along with the cluster analysis by treatments. The M, PK, and CK treatments were more similar than the others, while MNPK, NK, and NPK could be classified as one lineage. Overall, the data indicate that fungal community changes of Ascomycota were greatly affected by nitrogen fertiliser.

Microbial community structure affected by different fertilisers

Based on an unweighted UniFrac distance algorithm, species evolutionary relationships and relative abundances of microbes were examined using principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) analysis. The amounts of variance explained by axes one and two in the PCoA of bacteria were 37.75% and 19.45%, respectively (Fig. 5a). The amounts of variance explained by axes one and two of the PCoA of fungi were 71.44% and 7.07%, respectively (Fig. 5c). The NMDS analysis (Fig. 5b, d) showed that the stress values of bacteria and fungi were 0.111 and 0.041, respectively. The bacterial community results (OTU level) for the samples from NPK, NP, NK, and PK were clustered around the coordinate origin (Fig. 5a). The samples from NPK and PK were most similar, clustering closer to the origin than samples of other treatments. The MNPK and M samples clustered beyond the origin and were located on both sides of the origin along axis one. The CK samples were also located far from the origin, as well as from samples of the other treatments. The fungal community results (OTU level) for the NPK and M samples clustered nearer to the coordinate origin than samples from the other treatments (Fig. 5c). The NK and MNPK samples were also clustered close to the origin, but the positions of the two

Table 5. Effect of different fertilisers on Chytridiomycota and Basidiomycota community abundance (%).

Phylum	Treatments						
	MNPK	M	NPK	NP	NK	PK	CK
Chytridiomycota	1.09 ^b	0.72 ^b	2.58 ^a	0.93 ^b	1.16 ^b	0.98 ^b	0.88 ^b
Basidiomycota	0.09 ^b	1.59 ^a	0.16 ^b	0.53 ^b	0.31 ^b	0.64 ^b	1.15 ^{ab}

Superscript letters a and b indicate significant difference at $P < 0.05$ in a given row for fertiliser treatments. MNPK, manure combined with NPK; M, manure; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers; CK, no-fertiliser control.

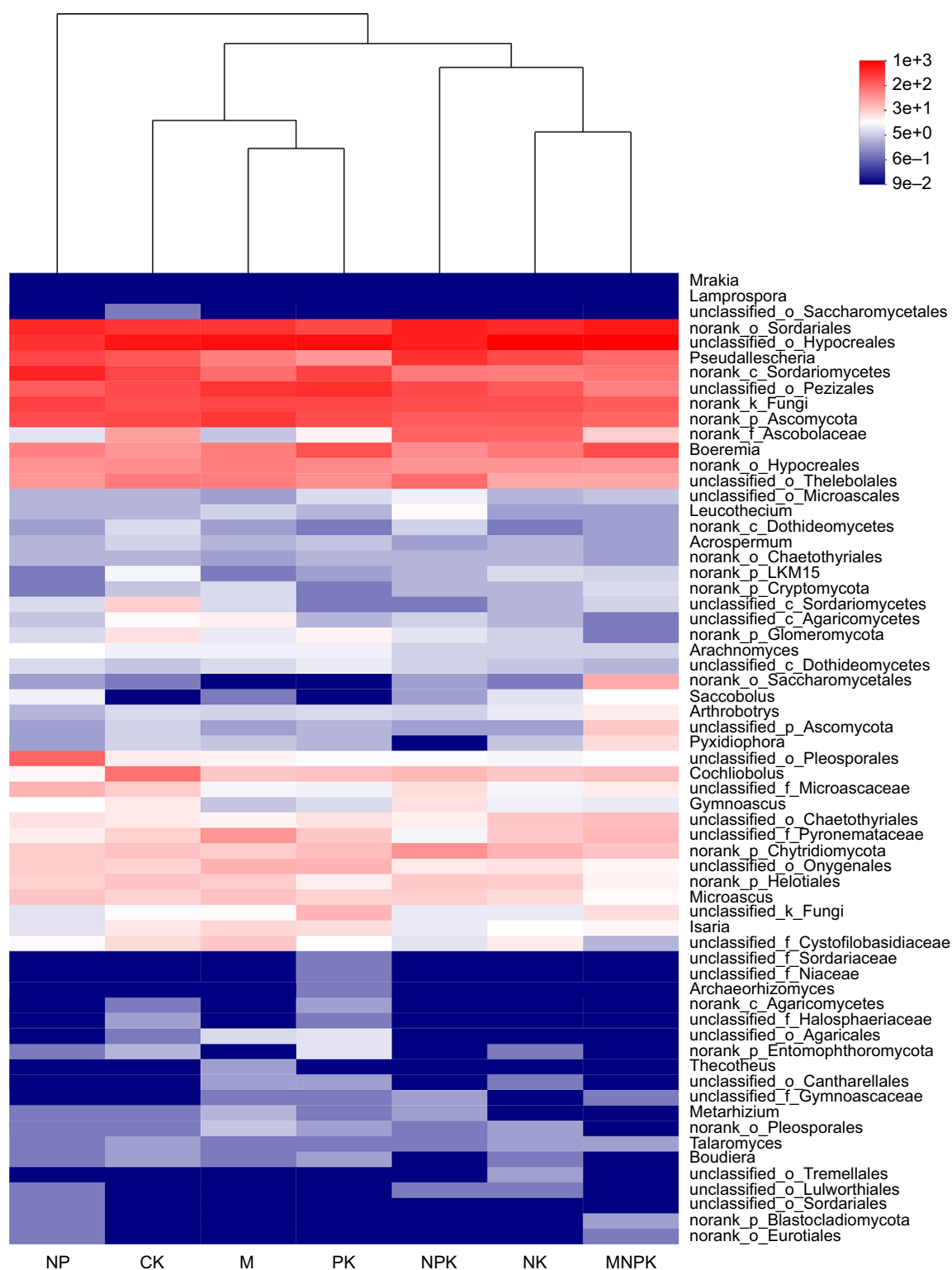


Fig. 4. The relative abundance of Ascomycota at genus level after long-term different fertiliser treatments. 'k', 'p', 'c', 'o', and 'f' represent kingdom, phylum, class, order, and family, respectively. NP, inorganic nitrogen and phosphorus fertilisers; CK, no-fertiliser control; M, manure; PK, inorganic phosphorus and potassium fertilisers; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NK, inorganic nitrogen and potassium fertilisers; MNPK, manure combined with NPK.

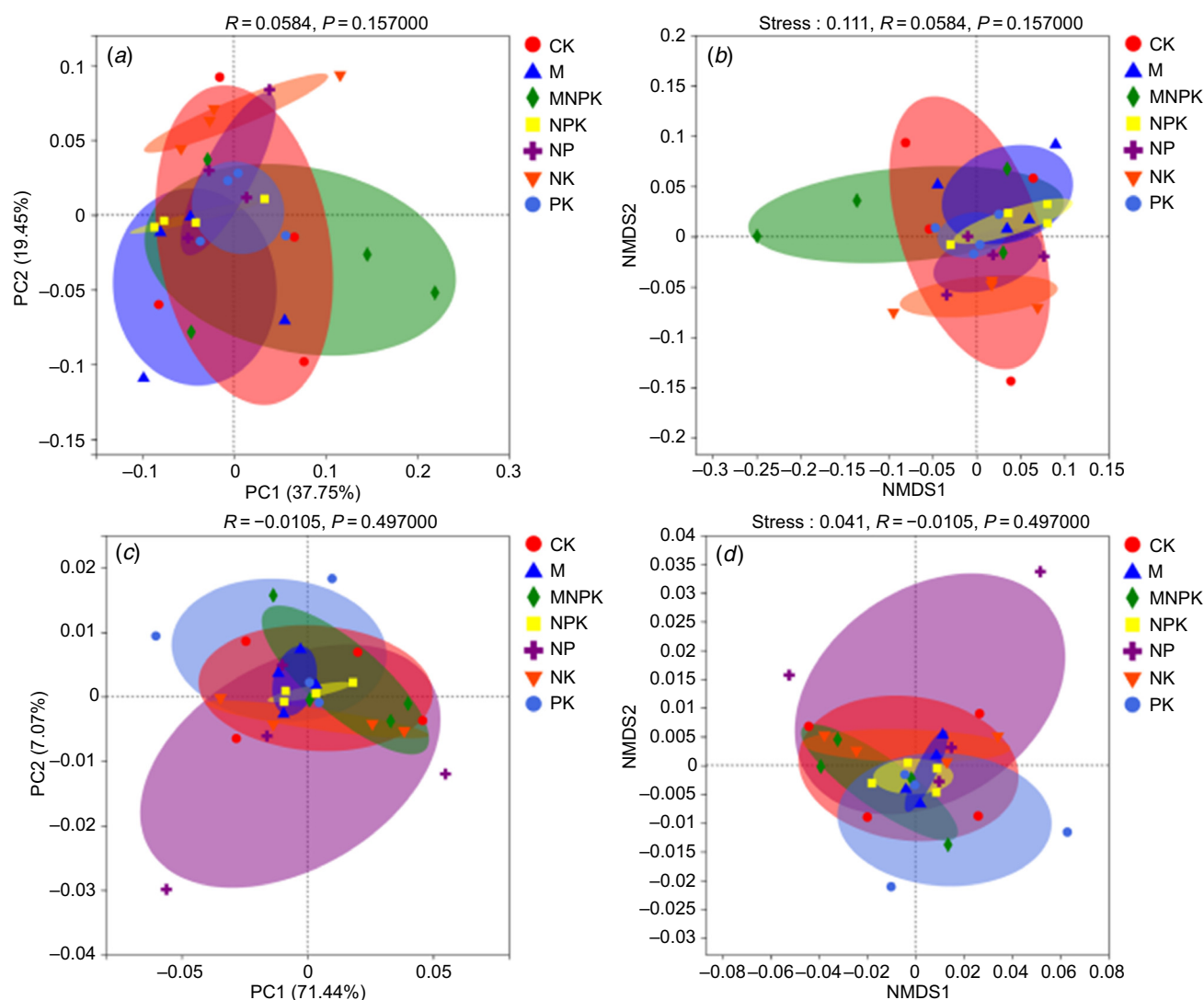


Fig. 5. Community structure of bacteria and fungi after long-term fertiliser treatments. The bacterial community structure was presented by (a) PCoA results and (b) NMDS results. The fungal community structure was presented by (c) PCoA results and (d) NMDS results. CK, no-fertiliser control; M, manure; MNPK, manure combined with NPK; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers.

groups of samples were not similar. Samples of NP and PK clustered far from the origin and had obvious differences.

Microbial abundance under soil enzyme annotation and soil enzyme activity

Five soil enzymes, urease, invertase, alkaline phosphatase, catalase, and alcohol dehydrogenase, are considered to be the key enzymes associated with plant nutrient absorption, and so their activities were measured (Table 6). Facilitated by the information on microbial genes and their associated five enzymes annotated in the KEGG database, the abundances of these soil enzyme-associated microbes were obtained (Table 7). By analysing these OTU quantity

data annotated by the five soil enzymes, we appraised the activity of the target enzyme-catalysed reaction in the corresponding soil. The bacterial and fungal communities associated with alcohol dehydrogenase had the highest abundances. Among the bacterial communities, the group associated with invertase had the lowest abundance, and the group associated with urease had a higher abundance of 4–5 times that of invertase-associated bacteria. The fungal data exhibited the lowest abundance in the community associated with urease, which was significantly lower than that associated with invertase.

Among the seven fertiliser treatments, the differences in abundances of the bacteria and fungi associated with each of these five enzymes were similar. The bacterial abundance

Table 6. Effect of different fertiliser treatments on soil enzyme activities in a potato field.

Treatments	Urease (mg NH ₃ -N g ⁻¹ 24 h ⁻¹)	Invertase (mg glucose g ⁻¹ 24 h ⁻¹)	Alkaline phosphatase (mg phenol g ⁻¹ 2 h ⁻¹)	Catalase (mol KMnO ₄ g ⁻¹ 30 min ⁻¹)
MNPK	0.4595 ^{ab}	28.52 ^a	2.407 ^{ab}	35.54 ^a
M	0.4796 ^a	34.12 ^a	3.232 ^a	35.66 ^a
NPK	0.3268 ^c	33.87 ^a	1.376 ^b	35.07 ^a
NP	0.3644 ^{abc}	32.17 ^a	1.814 ^b	35.29 ^a
NK	0.3730 ^{abc}	33.08 ^a	1.995 ^b	35.33 ^a
PK	0.3043 ^c	31.94 ^a	1.376 ^b	35.10 ^a
CK	0.3410 ^b	34.86 ^a	1.840 ^b	35.24 ^a

Superscript letters a–c indicate significant difference at $P < 0.05$ in a given column for fertiliser treatments. MNPK, manure combined with NPK; M, manure; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers; CK, no-fertiliser control.

Table 7. Effect of different fertiliser treatments on soil enzyme-associated microbial abundance (OTUs).

Kingdoms	Treatments	Enzymes				
		Urease	Invertase	Alkaline phosphatase	Catalase	Alcohol dehydrogenase
Bacteria	CK	25 569 ± 1067 ^{bc}	6363 ± 1239 ^{ab}	28 925 ± 1228 ^{bc}	19 878 ± 935 ^{bc}	73 754 ± 1457 ^{ab}
	M	33 612 ± 4287 ^a	7323 ± 1444 ^a	39 713 ± 2324 ^a	25 904 ± 3035 ^a	93 461 ± 9290 ^a
	MNPK	22 980 ± 2678 ^{bc}	4543 ± 953 ^{bc}	28 875 ± 3158 ^{bc}	20 888 ± 3757 ^{bc}	60 118 ± 4962 ^{ab}
	NPK	27 259 ± 342 ^{ab}	4849 ± 23 ^{abc}	31 340 ± 493 ^{bc}	22 545 ± 486 ^{abc}	76 960 ± 1623 ^{ab}
	NP	20 711 ± 3591 ^{bc}	3551 ± 822 ^c	35 964 ± 4578 ^{ab}	16 454 ± 2729 ^{ab}	79 359 ± 11 607 ^b
	NK	19 933 ± 1829 ^c	3325 ± 199 ^c	25 548 ± 2484 ^c	17 022 ± 1249 ^c	58 616 ± 4555 ^b
	PK	24 769 ± 2213 ^{bc}	4141 ± 796 ^{bc}	30 410 ± 2496 ^{bc}	20 351 ± 1427 ^{bc}	70 133 ± 4753 ^{ab}
Fungi	CK	1775 ± 77 ^{ab}	2761 ± 128 ^a	3813 ± 146 ^{ab}	8295 ± 466 ^{ab}	23 385 ± 899 ^{ab}
	M	1833 ± 76 ^{ab}	2740 ± 103 ^a	3680 ± 99 ^{ab}	8713 ± 549 ^a	22 993 ± 788 ^{ab}
	MNPK	1577 ± 187 ^b	2259 ± 182 ^b	3268 ± 439 ^b	6510 ± 571 ^c	20 703 ± 1480 ^b
	NPK	1980 ± 53 ^a	2863 ± 90 ^a	4315 ± 257 ^a	8931 ± 251 ^a	23 376 ± 882 ^{ab}
	NP	1786 ± 48 ^{ab}	2722 ± 101 ^a	4232 ± 350 ^a	8089 ± 411 ^{abc}	24 672 ± 1872 ^a
	NK	1587 ± 48 ^b	2253 ± 78 ^b	3093 ± 122 ^b	6807 ± 178 ^{bc}	19 793 ± 1041 ^b
	PK	1812 ± 203 ^{ab}	2616 ± 140 ^{ab}	3856 ± 358 ^{ab}	8370 ± 1083 ^{ab}	21 893 ± 641 ^{ab}

Superscript letters a–c indicate significant difference at $P < 0.05$ in a given column for fertiliser treatments. OTUs, operational taxonomic units; CK, no-fertiliser control; M, manure; MNPK, manure combined with NPK; NPK, inorganic nitrogen, phosphorus and potassium fertilisers; NP, inorganic nitrogen and phosphorus fertilisers; NK, inorganic nitrogen and potassium fertilisers; PK, inorganic phosphorus and potassium fertilisers.

associated with each enzyme in the M treatment was the highest among all treatments, and the bacterial community abundances associated with urease, invertase, and alkaline phosphatase were significantly higher in M than in NK and PK. In the soil treated with MNPK, except for the bacterial abundance associated with alcohol dehydrogenase, the abundances of bacterial communities associated with other enzymes were significantly lower than those of the M treatment. The bacterial community abundance associated with alkaline phosphatase under the NPK treatment was significantly lower than that of the M treatment, while the differences in abundances between the NPK and M treatments associated with the other enzymes were not significant.

The abundances of bacterial communities associated with these five enzymes under the NK treatment were lower than those in the other treatments.

Regarding the fungal communities associated with each of the five enzymes (Table 7), except for that associated with alcohol dehydrogenase, the highest values of fungal community abundance associated with the other enzymes were all obtained from the NPK treatment. The differences in abundance between the M treatment and the treatments with chemical fertiliser application were lower than the same comparisons of the bacterial community. Moreover, the differences in abundances of fungal communities associated with each of the five soil enzymes among the CK, M, and

NPK treatments were not significant. Among the abundances associated with urease, those in the MNPK and NK treatments were significantly lower than those in the NPK treatment. The abundances associated with invertase under the MNPK and NK treatments were significantly lower than those under the M, NPK, NP, and CK treatments. The abundances of fungal communities associated with alkaline phosphatase under the MNPK and NK treatments were significantly lower than those under the NPK and NP treatments. The abundances of communities associated with catalase under the MNPK treatment were significantly lower than those under the CK, M, NPK, and PK treatments. Among the fungal community abundances associated with alcohol dehydrogenase, MNPK and NK treatments had significantly lower abundance than the NP treatment. The abundances of fungal communities associated with all five enzymes under the NK and MNPK treatments were significantly lower than those under the other treatments.

Soil enzyme activities determined by a microbial inactivation treatment and substrate culture are presented in Table 6. Urease activity in the M treatment was 50.1% higher than that in NPK, 62.0% higher than PK, and 43.4% higher than CK. Urease activity of the MNPK treatment was also 43.5% higher than that of the NPK and 54.9% higher than PK. The M and MNPK treatments had relatively higher alkaline phosphatase activity, and that of the M treatment was significantly higher than those of the NPK, NP, NK, PK, and CK treatments. Activity levels in the NPK and PK treatments were the lowest. There were no significant differences in the activities of soil invertase and catalase among all fertilisation treatments.

Discussion

Effect of different fertilisers on microbial community diversity

The diversity of microbial communities is affected by many factors, such as different land-use types and farmland soil management methods (Zhong and Cai 2004). The type of applied fertiliser also has significant effects on soil microbial diversity. Research by Kamaa *et al.* (2011) suggests that the application of chemical fertilisers negatively influences the diversity of soil bacterial communities. Moreover, the effects of manure versus chemical fertiliser treatment on the diversity of fungal communities differ greatly. A study in Chernozem soils of northeastern China showed that the α -diversity of bacterial communities in soils treated with the combined application of manure and chemical fertiliser was higher than that of communities in soils treated with NPK fertiliser (Ding *et al.* 2016). In our study, the Chao1 and Shannon indices of soil bacterial communities were higher in the NPK than the MNPK treatment. Nie *et al.* (2018) found in paddy soils that the Chao1 and ACE indices

of bacterial communities in soils supplied with N, P, and K fertilisers were lower than those without fertilisers, and there were no significant differences in other α -diversity indices between the two fertiliser treatments. These inconsistencies with our results may be due to differences in soil types in the experimental areas.

Zhou *et al.* (2016) showed that the diversity of soil fungi somewhat depends on pH of the soil. They reported that with the greater amount of chemical fertiliser applied, there was greater decline in soil pH and greater decrease in fungal community diversity. However, the adverse effects of chemical fertilisers on soil fungal communities can be alleviated by addition of manure (Ding *et al.* 2017). The PCoA results of this study showed that the composition of the soil fungal community in the plots applied with a combination of manure and chemical fertiliser was similar to that of plots that did not receive fertiliser. Murphy *et al.* (2007) showed that this phenomenon is due to the slow decomposition of manure and release of nutrients in the soil, which helps maintain populations of all kinds of soil microorganisms.

This study showed that the diversity of soil bacterial and fungal communities varied in response to the different long-term fertilisation treatments. Chao1 and Shannon indices of soil bacteria decreased under the MNPK treatment compared to the CK. However, this is contrary to the results of Lu *et al.* (2015), who found that the Shannon and richness indices of soil bacteria increased significantly under the MNPK treatment compared to the CK, while there were no changes in the two indices of fungi. The Pearson correlations between bacterial α -diversity index and soil properties showed a significant negative correlation between OM content and bacterial Shannon index ($P < 0.01$); however, the correlation between OM content and the bacterial Chao1 index was not significant. This result indicated that the increased OM of manure plus chemical fertilisers provided more assimilable carbon sources for fungi, significantly reducing soil bacterial richness. In addition, the soil pH range in the experimental area of Lu *et al.* (2015) was 4.46–4.67, and the dominant population of soil bacteria clearly differed from that in our study, which may be one reason for the inconsistent results.

Consistent with the results of Xia *et al.* (2015), the α -diversity indices of bacterial and fungal communities in treatment with no fertilisation and application of NPK fertilisers were relatively similar. Based on the Pearson correlation results, the increase of soil OM content significantly decreased the bacterial Shannon index (richness) and Heip index (evenness), while the increase of soil OM content significantly increased the bacterial Simpson index (dominance of the main community). These indices for fungi also had similar correlations with OM content, but were less significant than for bacteria. In addition, the AN, AP, and AK contents had low correlations with the α -diversity index of

the above bacteria and fungi, indicating that only chemical fertilisers or no fertilisers had little effect on soil bacterial and fungal communities. Hence, the community structure of bacteria and fungi under the CK treatment was more similar to that of NPK than MNPK. This result is also consistent with PCoA and NMDS results.

Fungal richness was significantly greater with a single application of manure than without any fertiliser, while fungal richness was significantly lower with applications of NPK and NK fertilisers than without fertiliser. Together, the Shannon and Simpson index data also showed that the single application of manure increased the quantity and diversity of fungi in Calcic Kastanozem, while the single application of chemical fertilisers or combined application of manure and chemical fertilisers had the opposite effect. This effect may be because the fungal abundance of Basidiomycota decreased significantly under the combined action of manure and chemical fertilisers, which in turn reduced the fungal richness (Shannon index). The ecological niche left by Basidiomycota was occupied by other dominant fungi, which increased the Simpson index of fungi. The Heip index results indicated that manure alone did not affect the evenness of the fungal community in Calcic Kastanozem, while the application of NK and combined application of manure and NPK fertilisers significantly reduced the evenness of fungi. This is basically consistent with the results of Nie *et al.* (2018). Xu *et al.* (2015) found that the Shannon indices of the soil fungal community subjected to a treatment of manure in combination with NPK fertilisers and a treatment of only NK fertilisers were higher than those of N, NP, PK, and NPK treatments. In addition, they reported that a single application of NPK fertilisers or combined application of manure and NPK fertilisers could improve diversity of the soil fungal community in grey desert soil, which is inconsistent with our results. Their results may be related to the low content of AN and AP in the grey desert soil. A large amount of nitrogen and phosphorus provided by NPK fertiliser or manure plus NPK fertiliser increased the abundance of various microorganisms, thus enhancing the diversity of fungi. Other results from red paddy soils showed that the Shannon and Chao1 indices of the fungal community of soil that did not receive fertiliser did not differ from those of fertilisation treatments (Lu *et al.* 2015). Altogether, the results of all these studies differed from our results to varying degrees, possibly due to the different soil types, crops, and soil pH in the experimental areas. In this study, the community diversities of soil bacteria and fungi varied among treatments with no clear pattern, regardless of whether the addition of nutrients was withheld in the CK or the addition of a sufficient amount of nutrients was present in the MNPK treatment. The variability in our results is consistent with the results of the long-term fertilisation experiment of Kamaa *et al.* (2011).

Effects of different fertilisers on microbial community abundances

Under different fertilisation treatments, the relative abundances of Proteobacteria and Actinobacteria changed the most, followed by Acidobacteria, Planctomycetes, Bacteroidetes, and Gemmatimonadetes. In the soil with the combined application of manure and NPK fertiliser, the relative abundance of *Sphingomonas* bacteria decreased significantly, which can produce catalase and have a solid ability to decompose aromatic hydrocarbons (Janbandhu and Fulekar 2011). Declining abundance is not conducive to purifying contaminated farmland soils. The relative abundance of Nitrosomonadaceae and various Rhizobiales decreased significantly, and these microorganisms can promote the soil nitrogen cycle (Kowalchuk and Stephen 2001) and decomposition of AP and AK (Zai *et al.* 2021). The decrease in their abundance may be related to the plentiful nitrogen in the soil. However, abundance of *Mesorhizobium* significantly increased, and it was more suitable for MNPK treatment than other Rhizobia bacteria. In addition, the abundance of Planctomycetaceae, which are aquatic bacteria and are obligately aerobic, increased significantly, indicating a significant improvement in soil water content and aeration. Due to the presence of a large number of easily metabolised carbon sources in the soil with a single manure application, the relative abundance of Actinobacteria increased significantly (Chessa *et al.* 2016), which have a better ability to decompose lignin (Wang *et al.* 2014) and can promote soil carbon and nitrogen cycles. The relative abundance of *Iamia* (Acidimicrobiales) was the highest in the NPK-fertilised soil. This was significantly higher than in the NK treatment, indicating that the lack of phosphorus fertiliser was not conducive to its community development. This may be because phosphates lower the soil pH appropriately to a range of 7.0–8.0, which is optimum for *Iamia* (Kurahashi *et al.* 2009). In the soil with unbalanced fertilisation, the relative abundance of soil bacteria in the NK treatment changed the most, and the relative abundances of Cytophagaceae and *Bryobacter* were significantly higher than those in the MNPK and CK treatments, which may be beneficial to the development of cellulose-degrading microorganisms.

The dominant phyla of fungi were Ascomycota, Chytridiomycota, and Basidiomycota. The fertilisers differentially affected the relative abundances of the different fungi in the soil. Depending on the fertiliser, different phyla may dominate the fungal community as demonstrated by research on changes in dominant communities of fungi in tea field soil in response to various fertilisers (Ji *et al.* 2018). In this study, Ascomycota was the dominant group in the Calcic Kastanozem fungal community, followed by Chytridiomycota and Basidiomycota. The single application of manure reduced the abundance of Chytridiomycota and increased that of Basidiomycota. The combined

application of NPK fertilisers significantly increased the abundance of Chytridiomycota and decreased the abundance of Basidiomycota. This is consistent with results from tobacco field soils (Chen *et al.* 2014) and continuous-cropping experiments with cucumber (Zhang 2016). Milkereit *et al.* (2021) found that soils with more complex organic compounds present can provide soil nutrients for fungal growth and reproduction, while the soil bacteria are limited in using these complex organic compounds. Therefore, long-term application of manure not only helps to maintain and stabilise development of the soil fungal community, it also alters composition of the soil bacterial community. This facilitates the utilisation of nutrients by crops during the growth process.

Effects of different fertilisers on soil enzyme activities

Soil enzyme activity is composed of the activity of the enzymes that have accumulated in soil from cells that collapse after the death of some organism and the enzymes released into the soil by microorganisms. Our long-term fertilisation treatments significantly affected soil enzyme activity. The activities of the accumulated urease and alkaline phosphatase in the soil treated with manure were significantly higher than those of the chemical fertiliser and no-fertilisation treatments, consistent with the microbial abundances associated with the corresponding soil enzymes. Long-term application of manure significantly increased the microbial abundance associated with the major soil enzymes: urease, invertase, alkaline phosphatase, catalase, and alcohol dehydrogenase. Only the NPK application had positive effects on the fungal abundances associated with the major soil enzymes, while the other chemical fertiliser treatments reduced the abundances of bacteria and fungi associated with the major soil enzymes. This result is similar to those of studies by Tan *et al.* (2007), Jin *et al.* (2012), Li *et al.* (2015), and Zhang *et al.* (2018).

Conclusions

This study revealed the response of microorganisms to sheep manure and nitrogen, phosphorus, and potassium fertilisers in terms of community structure and relative abundance in Calcic Kastanozem. The application of manure significantly increased soil OM, and the increase of OM content was one of the main factors in reducing soil bacterial richness and evenness and increasing bacterial dominance. The combined application of manure and chemical fertiliser significantly reduced the relative abundance of Proteobacteria in the soil, mainly due to α -Proteobacteria and β -Proteobacteria, which are essential microorganisms involved in nitrogen cycling. The single application of manure significantly increased the abundance of Actinobacteria and effectively

enhanced the soil's ability to decompose OM. In North China, we conducted a 9-year continuous positioning fertilisation experiment in potato fields for the first time. We found that the combined application of manure and NPK fertilisers had a complex effect on soil microorganisms and was not simply a combination of the effects of manure and chemical fertilisers on microbes. The interaction mechanism between the two on microbial succession changes needs further exploration.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare no conflicts of interest.

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