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# Linkages between nutrient ratio and the microbial community in rhizosphere soil following fertilizer management

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ABSTRACT

To unravel the linkages between ecological ratios (C:N:P) and the microbial community in rhizosphere soil in response to fertilizer management, soil samples were collected from a proso millet (*Panicum miliaceum* L.) field under different fertilizer management systems, including nitrogen fertilizer (NF), phosphorus fertilizer (PF), combined N and P (NP) fertilizer, and organic fertilizer (OF); no fertilizer (CK) was used as a control. Furthermore, 16S rRNA and ITS gene sequencing were applied to represent the bacterial and fungal diversity in the soil. Moreover, the elemental properties, including the carbon (C), nitrogen (N), and phosphorus (P) contents, in the microbial biomass and rhizosphere soil were evaluated. The results showed that the C, N, and P contents and microbial biomass (MBC, MBN and MBP, respectively) in the rhizosphere soil were augmented following fertilizer management. Increases in the alpha diversity indices (Shannon and Chao 1) of soil bacteria and fungi were observed in response to the fertilizers, and the responses were more closely related to the soil C:N and N:P ratios than to the C:P ratio. Additionally, with high relative abundances (> 1%) across all soil samples, the composition of soil microbial phyla levels revealed different trends following fertilizer management. The abundances of Actinobacteria and Gemmatimonadetes increased, while the abundances of Acidobacteria and Nitrospirae decreased ($P < 0.05$) following fertilizer management. Among the fungal taxa, the abundances of *Ascomycota* and *Mortierellomycota* responded positively to fertilizer. These results were largely influenced by changes in the C:N and N:P ratios in both the soil and microbial biomass. Overall, significantly increased C:N and decreased N:P ratios in the soil reflected the N deficiency that would limit increased microbial biomass and diversity. Together, all of these results indicated that interactions between ecological ratios (C:N:P) and microbial community composition play vital roles in resource imbalance in dynamic environments. Thus, N status should be an important factor for sustainable agricultural management. Moreover, the synergistic effects were better with the combination of C, N, and P or with organic fertilizer than with C, N and P separately.

## 1. Introduction

Farmland ecosystems are experiencing unprecedented increases in nutrient inputs due to the use of large amounts of chemical fertilizers (Gao et al., 2014). These unbalanced fertilizer inputs could strongly influence farmland ecological ratios and have further impacts on the elemental ratios of plants and soil and ecosystem diversity (Finzi et al., 2011). Nutrient availability in soil can be affected by fertilizers (organic or inorganic) and can further regulate soil biogeochemical cycles (Gong et al., 2009). Carbon (C), nitrogen (N), and phosphorus (P) are three dominant macroelements in soil, and inappropriate C, N, and P cycling in ecosystems is a common problem that has been explored globally (Zhang et al., 2019b). C:N:P ratios, as a criterion for evaluating soil nutritional conditions, provides a link between aboveground plant productivity and the states of belowground soil nutrients (Mooshammer et al., 2012); furthermore, this indicator has been widely used to gain an in–depth understanding of natural nutrient limitations in farmland ecosystems to a certain extent. Many studies have shown that element

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enrichment accelerates cycling by altering plant nutrient ratios (Marklein and Houlton, 2012); these factors may have reciprocal effects with plant interactions and plant productivity (Brooker, 2006) and modify organic matter decomposition (Wild et al., 2014). Consequently, a better understanding of the influence of fertilizer management on ecological ratios (C:N:P) is vital to appraise soil nutrients and regulate the sustainable development of agricultural ecosystems.

Generally, fertilizers increase plant production and inevitably alter the elemental nutrient status in plants, thus changing soil nutrient pools and directly influencing soil microorganisms (Acosta–Martínez et al., 2008). In addition, as indicated by the theory of consumer–driven nutrient recycling, C, N and P cycling are also widely limited by the elemental requirements of microbes and the elemental content in rhizosphere soil (Zechmeister-Boltenstern et al., 2015). Microorganisms in soil are thought to maintain their elemental ratios to (a) obtain and fix required nutrients, (b) regulate element transfer times by retaining limited elements in their biomass for a long time or (c) discharge elements that are present in excess according to microbial needs. Evidence has indicated that soil microbes can establish symbiotic or pathogenic relationships with plants as well as animals and can also facilitate the decomposition of organic matter (Mooshammer et al., 2014) by degrading unstable and nutrient–enriched organic matter to satisfy the elemental demands of rapidly growing bacteria with low C:P or N:P ratios (Ganie et al., 2016); in contrast, fungi, including those in the orders Thelephorales and Cantharellales, are the main microbes restricting N and P mobilization rates in soil (Verbruggen et al., 2015). A recent meta–analysis demonstrated that fertilizers (e.g., N) decreased both soil microbial diversity and the relative abundances of Actinobacteria and Nitrospirae; however, the relative abundances of the primary fungal groups, Ascomycota and Basidiomycota, did not significantly change (Wang et al., 2018). Soil microbial diversity and composition also respond differently to the application of fertilizers, such as P and organic fertilizers. The underlying mechanisms for these responses are still unknown, and feedback between microbial activity changes and C:N:P homeostasis may in turn modify the environment. Relevant research can reveal microbial activity changes in response to the external environment, and this knowledge can provide new insights regarding the connection between microbial adaptation and evolution and the balanced states of soil nutrients.

Numerous studies related to C:N:P ratios and the microbial community in soil have primarily focused on the plant population or ecosystem level in forests and grasslands (Jenerette and Chatterjee, 2012; Yang et al., 2018). Information on ecological ratios in farmland soils is extremely scarce, especially regarding fertilizer management. Proso millet (*Panicum miliaceum* L.), one of the earliest cultivated crops in China, which has a short growing season, is a predominant food and feed source along the Great Wall of China; this crop has excellent drought resistance and is tolerant to poor–quality soil that is highly saline or alkaline (Zhang et al., 2017; Gong et al., 2019). In recent years, with the modification of China's agricultural structure, people have paid increasing attention to proso millet. Consequently, knowledge concerning the relationship between C:N:P ratios and the microbial diversity in rhizosphere soil is necessary to promote the advancement of proso millet. Therefore, we hypothesized that the C:N:P ratios and microbial biomass in rhizosphere soil may be simultaneously altered following fertilizer management and that this ratio may predict bacterial and fungal diversity changes in the soil. Additionally, we anticipated that variations in specific bacterial and fungal taxa may illustrate the changes in C:N:P levels. Thus, the key goals in this study were as follows: to (i) determine the influence of fertilizer management on C, N, and P levels and microbial biomass in rhizosphere soil; (ii) investigate the linkages between the C:N:P ratios and microbial biomass in rhizosphere soil following fertilizer management; and (iii) analyze the feedback between microbial diversity and community composition and variations in C:N:P ratios. This study provides basic information for subsequent fertilizer management and sustainable development.

## 2. Materials and methods

### 2.1. Experimental sites

Field studies were performed at the experimental site of Northwest A&F University (37°56′26″N, 109°21′46″E), Yulin city, Shaanxi Province, China, which has a semi–arid continental monsoon climate. The local average annual temperature and precipitation are 8.3 °C and 400 mm, respectively. Before sowing, the soil was characterized by a loess–like loam texture in the 0–20 cm soil layer with a pH of 8.5, organic matter content of 7.34 g $kg^{-1}$, total N content of 0.36 g $kg^{-1}$, total P content of 0.75 g $kg^{-1}$ and total K content of 18.47 g $kg^{-1}$.

### 2.2. Experimental design and treatments

A randomized block experimental design with four replicates was used. There were five treatments: 1) a control (CK) without any fertilizer; 2) nitrogen fertilizer (NF), 150 kg $ha^{-1}$ N; 3) phosphorus fertilizer (PF), 100 kg $ha^{-1}$ $P_2O_5$; 4) combined N and P fertilizer (NP), 150 kg $ha^{-1}$ N and 100 kg $ha^{-1}$ $P_2O_5$; and 5) organic fertilizer (OF), 15,000 kg $ha^{-1}$ of organic fertilizer that consisted of 40.0% organic matter and 50 million effective microorganisms. Compared with the other treatments, the OF treatment equally balanced the actual N and P inputs. The proso millet cultivar 'Shanmi–1' was planted on June 10 and harvested on September 30, 2018. Each experimental plot was 6 m long and 5 m wide; there were 12 rows, and the row spacing was 42 cm. When the proso millet was at the 4- to 5-leaf stage, 400,000 plants per hectare were planted in all the plots. Border rows were not used for sampling. No other fertilizers were used during the proso millet growth period. Weeds were managed by hand weeding, particularly in the early growing season.

### 2.3. Soil sampling

At the flowering stage (August 2018, 60 days after sowing the proso millet), rhizosphere soil was taken from each fertilizer plot. Plants that had an 'S'–shaped pattern were selected and uprooted with a spade. Attached soil was carefully removed from the roots. Rhizosphere soils were collected with a brush and sieved with a 2 mm mesh to filter out other impurities. Soil samples from five sampling points in one treatment were merged to produce mixed samples for subsequent analyses. A portion of every soil sample was rapidly stored at −80 °C in the laboratory for molecular analysis. One portion of the soil samples was preserved at 4 °C for microbial biomass analysis. Another portion of the soil samples was dried and then preserved at room temperature for chemical component analysis.

### 2.4. Measurement of the soil physical and chemical properties

Water was added to the soil samples (1:5 *w/v*), and the samples were shaken for 30 min. The pH of the soil was then measured with a pH meter. The soil water content (SWC) was measured by oven drying at 105 °C until reaching a constant mass. The soil temperature (ST) was measured by five temperature probes (Hongxing Instruments Factory, Hebei, China) at a 10 cm depth close to the proso millet. The soil bulk density (BD) was determined from the volume of each individual core by gravimetric oven drying at 105 °C for 24 h. The soil total carbon (TC) and total nitrogen (TN) contents were measured with an elemental analyzer (Vario MACRO cube CN, Germany); the total phosphorus (TP) content was measured by wet digestion with $HClO_4$–$H_2SO_4$ (UV spectrophotometer) as reported by Ren et al. (2018). The soil MBC, MBN, and MBP were analyzed in fresh soil samples by the chloroform fumigation–extraction method (Ren et al., 2016).

**Table 1**
Properties of the rhizosphere soil under fertilizers managements. Different letters indicate significant differences (ANOVA, $P < 0.05$) among different fertilizers managements. CK, NF, PF, NP, and PF represent no fertilizer, nitrogen fertilizer, phosphorus fertilizer, combined N and P fertilizer, and organic fertilizer, respectively.

| Fertilizers managements | CK | NF | PF | NP | OF | F | *P* |
|---|---|---|---|---|---|---|---|
| pH | 8.72 ± 0.15a | 8.94 ± 0.04a | 8.90 ± 0.06a | 8.88 ± 0.12a | 8.95 ± 0.09a | 5.992 | 0.004 |
| Soil bulk density (BD, g·cm$^{-3}$) | 1.38 ± 0.03a | 1.35 ± 0.05a | 1.35 ± 0.04a | 1.33 ± 0.02a | 1.23 ± 0.02b | 10.582 | < 0.001 |
| Soil temperature (ST, °C) | 27.98 ± 1.06c | 28.40 ± 0.73bc | 28.15 ± 0.87c | 29.63 ± 0.43a | 29.43 ± 0.52 ab | 4.021 | 0.021 |
| Soil water content (SWC, %) | 8.94 ± 0.33a | 7.48 ± 1.01bc | 8.32 ± 1.22 ab | 6.58 ± 1.03c | 6.75 ± 0.70c | 4.886 | 0.010 |

### 2.5. Soil DNA extraction, PCR amplification and illumina sequencing analysis

Fresh rhizosphere soil DNA (0.5 g) was extracted four times with a MoBio Laboratories (USA) Power Soil DNA Isolation Kit. The genomic DNA quality and concentration were detected using agarose gels (1.0%). The genomic DNA purity and quality were verified by 0.8% sepharose. The bacterial 16S rRNA genes in the V3–V4 hypervariable region were amplified with primers (Jiang et al., 2013). A 10-digit barcode sequence was added at the 5′ ends of both the forward (F) and reverse (R) primers (provided by Auwigene Company, Beijing). PCR was conducted on a 50 μL reaction volume that consisted of 10 × Ex Taq buffer ($Mg^{2+}$ plus) (5 μL), 12.5 mM dNTP mix (4 μL), Ex Taq DNA polymerase (1.25 U), template DNA (2 μL), 200 nm barcoded F and R primers and dd$H_2O$ (36.75 μL) in a sterilized centrifuge tube. The PCR procedure was as follows: first, 94 °C (2 min); then, 30 cycles of 94 °C (30 s), 57 °C (30 s) and 72 °C (30 s); and a final extension at 72 °C (10 min). The fungal ITS region was amplified with the primers ITS1 and ITS2 in a sterilized centrifuge tube (Jing et al., 2019). The 5′ ends of the two primers were tagged. The PCR procedure was as follows: 5 × FastPfu buffer (4 μl), 5 μM primer (1 μl), 2.5 mM dNTP mixture (2 μl), template DNA (2 μl) and $H_2O$ (10 μl). Thermocycling consisted of initial denaturation at 95 °C (2 min); 30 cycles of 95 °C (30 s), 55 °C (30 s) and 72 °C (30 s); and a final extraction at 72 °C (5 min).

### 2.6. Calculation of the 16S rRNA and ITS gene data

First, the original data were filtered. Sequences shorter than 200 bp with a weak quality score (≤20) that included undefined bases or that did not precisely suit the primer sequences and barcode tags were excluded.

Eligible reads were partitioned by the sample-specific barcode sequences and repaired with Illumina Analysis Pipeline Version 2.6. The data were processed by QIIME. Based on a level of approximately 97%, the sequences were classified into operational taxonomic units (OTUs) to determine the diversity and richness indices (Edgar, 2013). All sequences were categorized into different taxonomic groups by the Ribosomal Database Project (RDP) tool (Cole et al., 2009).

According to the OTU information, clustering analyses and principal component analysis (PCA) were applied to investigate the similarity between different samples by R language (Wang et al., 2007). This analysis was used to form a Newick–formatted tree file. The membership and structure of communities were represented by heat maps including the top 20 OTUs in the different samples (Jami et al., 2013).

### 2.7. Statistical analyses

The taxonomic alpha diversity (Shannon and Chao1 indices, representing community diversity and richness, respectively) was calculated with Mothur software (v.1.30.1). The taxonomic beta diversity (weighted UniFrac distance) was calculated to illuminate the clustering of different soil samples. Nonmetric multidimensional scaling (NMDS) was used to reflect the microbial community structures. Redundancy analysis (RDA) was performed by the CANOCO 4.5 software package to calculate the correlations among the soil physicochemical properties and soil microbial compositions. The relationships among the C:N:P ratios, soil properties, and microbial diversity in the soil were determined via Spearman's correlation analysis (SPSS Institute, Chicago, USA). One–way analysis of variation (ANOVA) was used to analyze all data for the different fertilizer management schemes ($P < 0.05$). The least significant difference (LSD) test ($P < 0.05$) was used to represent differences between mean values, as indicated by different letters. Origin Pro 2018 was used to draw all figures.

## 3. Results

### 3.1. Effect of fertilizer management on rhizosphere soil properties

Table 1 lists the rhizosphere soil properties. The soil BD changed from 1.23 to 1.35 g cm$^{-3}$, but the different fertilizer treatments had no effect on the soil BD. Compared with the values in the CK treatment, the soil pH and temperature in NF, PF, NP, OF treatments were 2.6%, 2.0%, 1.8%, 2.6% higher, respectively, and 1.5%, 0.6%, 5.9%, 5.2% higher, respectively. Compared with that in the CK treatment, the SWC in the soil fertilizer management treatments (NF, PF, NP, and OF) significantly decreased by 16.4%, 6.9%, 28.3% and 24.5%, respectively.

### 3.2. Effect of fertilizer management on C, N, and P contents and microbial biomass in rhizosphere soil

Compared with the values in the CK treatment, the C, N, and P contents in the fertilizer management treatments were higher by 26.7%–122.1%, 3.8%–15.4%, and 6.8%–23.7%, respectively (Table 2).

**Table 2**
The C, N, P in rhizosphere soil and microbial biomass as affected by fertilizers managements. Different letters indicate significant differences (ANOVA, $P < 0.05$) among different fertilizers managements. CK, NF, PF, NP, and PF represent no fertilizer, nitrogen fertilizer, phosphorus fertilizer, combined N and P fertilizer, and organic fertilizer, respectively.

| Fertilizers managements | CK | NF | PF | NP | OF | F | *P* |
|---|---|---|---|---|---|---|---|
| Total carbon (TC, g kg$^{-1}$) | 3.89 ± 0.14e | 5.64 ± 0.27c | 4.93 ± 0.12d | 6.51 ± 0.31b | 8.64 ± 0.12a | 297.508 | < 0.001 |
| Total nitrogen (TN, g kg$^{-1}$) | 0.26 ± 0.03b | 0.28 ± 0.01 ab | 0.27 ± 0.02 ab | 0.30 ± 0.02a | 0.30 ± 0.01a | 3.56 | 0.31 |
| Total phosphorus (TP, g kg$^{-1}$) | 0.44 ± 0.01e | 0.47 ± 0.01d | 0.53 ± 0.01c | 0.61 ± 0.01a | 0.56 ± 0.001 | 357.835 | < 0.001 |
| Microbial biomass carbon (MBC, mg kg$^{-1}$) | 43.73 ± 2.24c | 53.12 ± 4.67b | 47.30 ± 1.81bc | 71.77 ± 6.07a | 77.03 ± 3.38a | 56.847 | < 0.001 |
| Microbial biomass nitrogen (MBN, mg kg$^{-1}$) | 4.90 ± 0.42c | 5.78 ± 0.41b | 4.99 ± 0.19c | 6.27 ± 0.09a | 6.60 ± 0.15a | 27.664 | < 0.001 |
| Microbial biomass phosphors (MBP, mg kg$^{-1}$) | 0.96 ± 0.06d | 1.10 ± 0.09d | 2.58 ± 0.06b | 3.58 ± 0.19a | 1.43 ± 0.23c | 240.083 | < 0.001 |

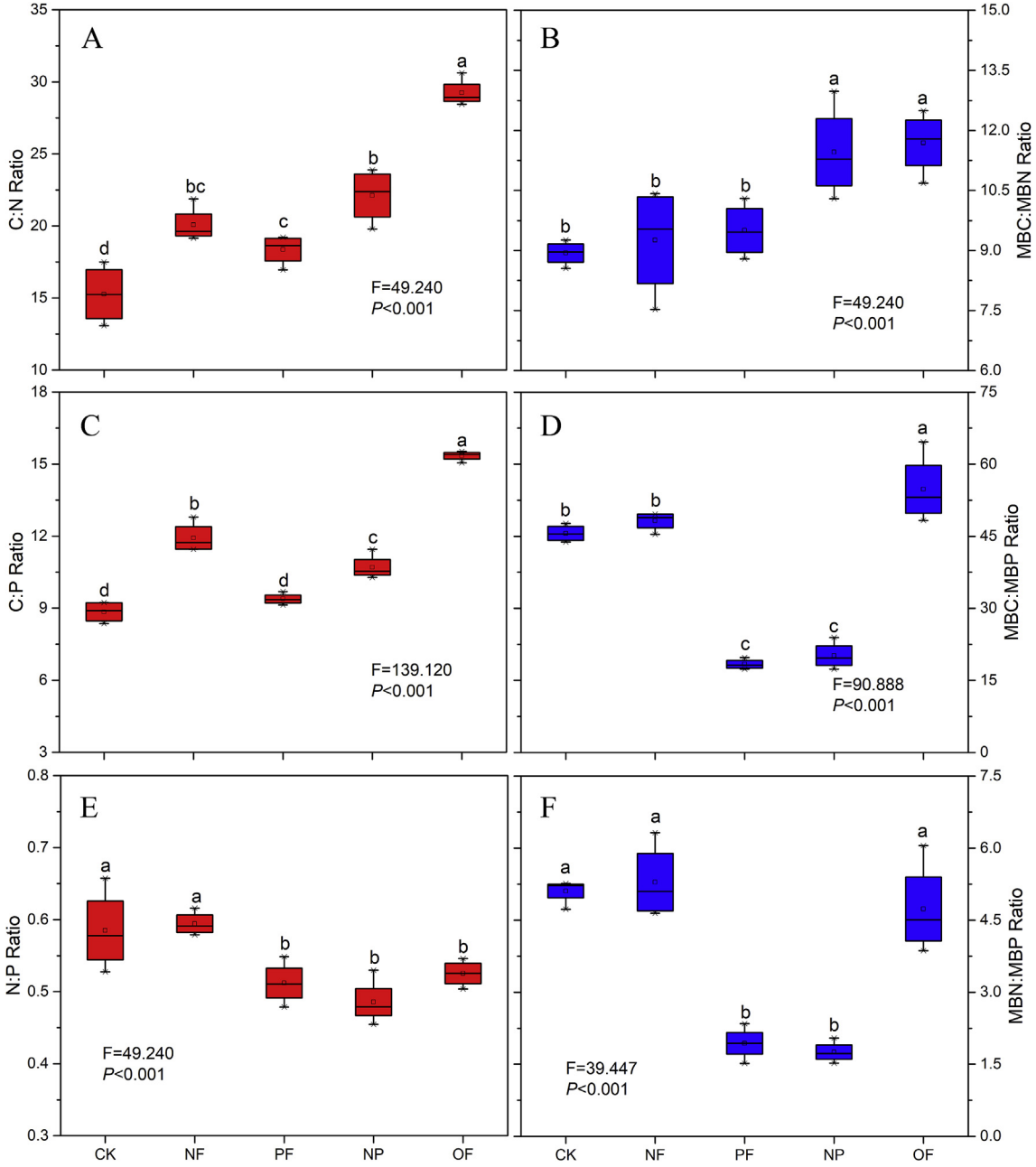


**Fig. 1.** Changes in C:N ratio (A), MBC:MBN ratio (B), C:P ratio (C), MBC:MBP ratio (D), N:P ratio (E), and MBN:MBP ratio (F) in rhizosphere soil following fertilizers managements. Different letters indicate significant differences among different fertilizers managements ($P < 0.05$). CK, NF, PF, NP, and PF represent no fertilizer, nitrogen fertilizer, phosphorus fertilizer, combined N and P fertilizer, and organic fertilizer, respectively.

MBC, MBN, and MBP increased by 8.2%–76.1%, 1.8%–34.7%, and 14.6%–272.9%, respectively, following fertilizer management. Furthermore, the C ratios were higher in the rhizosphere soil than in the microbial biomass, while the opposite trend was obtained for the P ratios, being higher in the microbial biomass than in the rhizosphere soil.

### *3.3. Effect of fertilizer management on C:N:P ratios and microbial biomass in rhizosphere soil*

The C:N, C:P, and N:P ratios in the microbial biomass and rhizosphere soil are displayed in Fig. 1. The C:N and C:P ratios in rhizosphere soil increased by 20.3%–91.5% (Figs. 1A) and 6.0%–73.4% (Fig. 1C), respectively, compared to the levels in the CK treatment. Similarly, the MBC:MBN ratio increased by 3.6%–30.8% following the fertilizer treatments (Fig. 1B). Moreover, the N:P ratios in the PF, NP, and OF treatments were lower than that in the CK treatment by 10.2%–17.0% (Fig. 1E), and similarly, the MBN:MBP ratio decreased by 7.3%–65.7% following the fertilizer treatments (Fig. 1F). In other words, the C:N:P ratios of the soil was similar to the C:N:P ratios of the microbial biomass, and both parameters were substantially affected by fertilizer management.

### *3.4. Effect of fertilizer management on the rhizosphere soil bacterial and fungal community abundance and diversity*

After quality sequencing, the bacterial communities in all soil samples (982,958 sequences) were determined by the 338F/806R primers, and the fungal communities (912,880 paired–end sequences) were determined by the ITS1/ITS2 primers. The number of bacterial

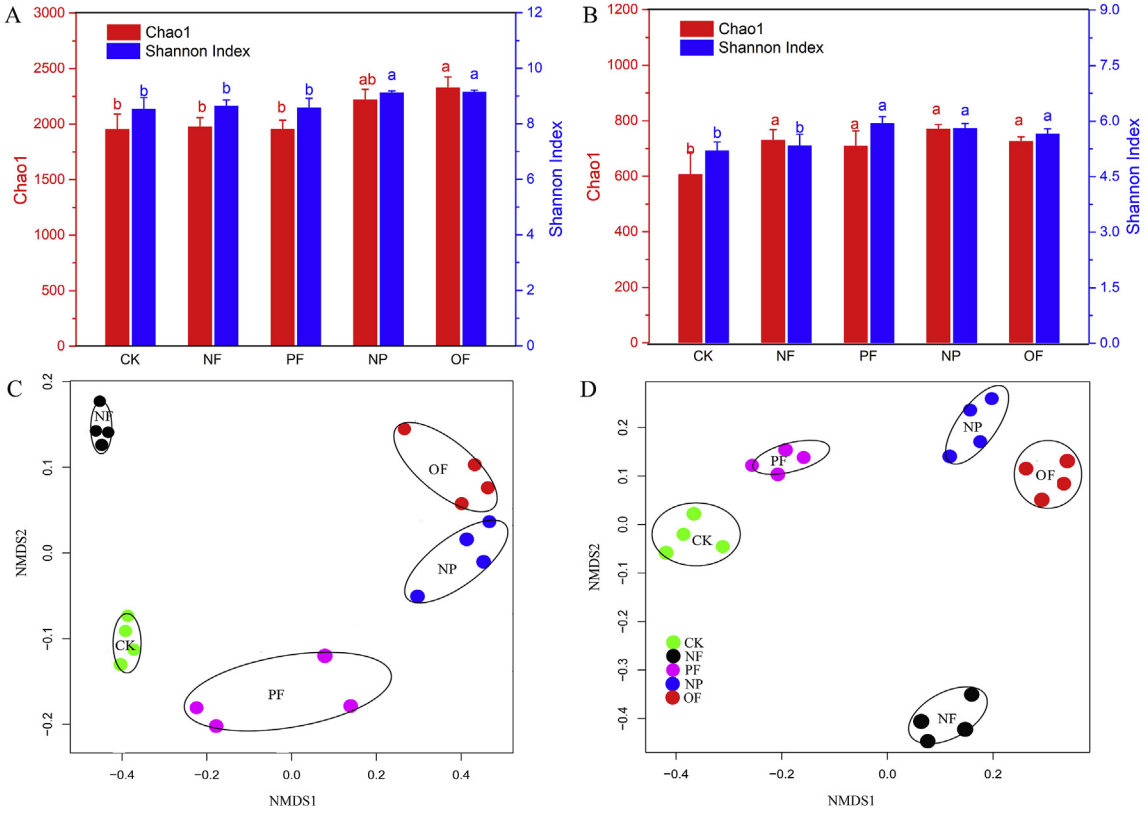


**Fig. 2.** Changes in soil bacterial (alpha, A; beta, C) and fungal (alpha, B; beta, D) diversity in rhizosphere soil following fertilizers managements. Different letters indicate significant differences among different fertilizers managements ($P < 0.05$). CK, NF, PF, NP, and PF represent no fertilizer, nitrogen fertilizer, phosphorus fertilizer, combined N and P fertilizer, and organic fertilizer, respectively.

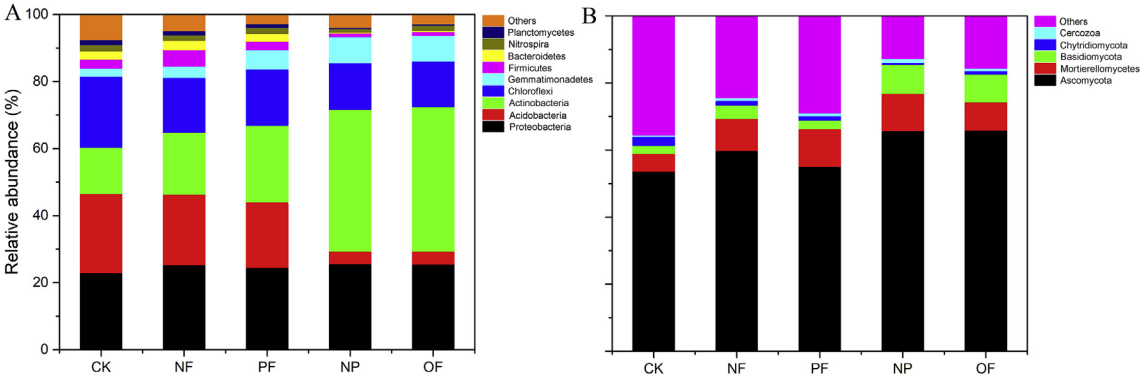


**Fig. 3.** Distribution of 16S rRNA and ITS2 sequences across bacterial (A) and fungal (B) phyla communities following fertilizers managements. CK, NF, PF, NP, and PF represent no fertilizer, nitrogen fertilizer, phosphorus fertilizer, combined N and P fertilizer, and organic fertilizer, respectively.

sequences varied from 22,272 to 148,996 per sample (mean = 49,148), while the number of fungal sequences varied from 29,564 to 62,107 per sample (mean = 45,644). The datasets were purified to 22,000 sequences to analyze the bacterial downstream sequences and to 29,500 sequences to analyze the fungal downstream sequences.

The Shannon and Chao 1 indices were used to indicate the OTU-level alpha diversity and richness, respectively, of the soil bacteria and fungi (Fig. 2). For bacterial diversity, the Shannon index values of the NF, PF, NP, and OF treatments were 8.66 ( ± 0.20, SE), 8.60 ( ± 0.32, SE), 9.14 ( ± 0.05, SE), and 9.17 ( ± 0.04, SE), respectively, which were significantly higher than the CK value (8.56 ± 0.38, SE) (Fig. 2A). Moreover, the Chao 1 index results showed the same tendency, increasing from 1958 ( ± 134, SE) in the CK treatment to 1981 ( ± 77, SE), 1960 ( ± 75, SE), 2227 ( ± 86, SE), and 2336 ( ± 89, SE) in the NF, PF, NP, and OF treatments, respectively (Fig. 2A). Regarding fungal diversity, the Shannon and Chao 1 indices varied from 5.22 to 5.96 and from 610 to 772, respectively (Fig. 2B). Both the diversity and richness were higher in the fertilizer management treatments, although the differences were not significant. NMDS was used to determine the microbial beta diversity among the sample points (four replicates each within the five different fertilizer management treatments) (Fig. 2). For bacteria, compared with that in the CK treatment, the microbial structure at adjacent sampling sites in the fertilizer treatments was similar and was greatly affected by fertilizer management (Fig. 2C); for fungi, the degree aggregation of the sample points from each other was relatively low, and the communities were greatly affected by fertilizer management (Fig. 2D).

### 3.5. Effect of fertilizer management on the rhizosphere soil bacterial and fungal community compositions

The principal bacterial phyla with relative abundances greater than 1% were Acidobacteria (28.1%), Proteobacteria (24.8%), Chloroflexi (16.4%), Actinobacteria (14.3%), Gemmatimonadetes (5.4%), Firmicutes (2.4%), Bacteroidetes (1.6%), Nitrospirae (1.6%), and Planctomycetes (0.9%) in all sequences (Fig. 3A and Table S1). Markedly, Proteobacteria, Actinobacteria and Gemmatimonadetes were more abundant in the different fertilizer treatments (NF, PF, NP, and OF) than in the CK treatment, and the abundances of other phyla, including Acidobacteria, Chloroflexi, Nitrospirae, and Planctomycetes, were as follows: CK > NF or PF > NP or OF (Fig. 3A and Table S1). Additionally, the class-level classification showed that Alphaproteobacteria and Actinobacteria were the dominant classes and were more abundant in the fertilizer plots (NF, PF, NP, and OF) than in the CK plots (Fig. S1A). Within Alphaproteobacteria, the orders Rhizobiales and Sphingomonadales were the most abundant. The relative abundance of Rhizobiales was markedly higher in the fertilizer treatments than in the CK treatment, while the opposite trend was found for the relative abundance of Sphingomonadales in soil following fertilizer management (Fig. S2A and Table S1).

Based on the distribution of fungal community compositions in the different fertilizer management treatments, across all samples, the predominant phyla were Ascomycota, Mortierellomycota, Basidiomycota, Chytridiomycota, and Cercozoa, with average contributions of 60.1%, 9.1%, 5.2%, 1.4%, and 0.8%, respectively (Fig. 3B and Table S2). In particular, the relative abundances of Ascomycota, Mortierellomycota, Basidiomycota, and Cercozoa were significantly higher under the fertilizer treatments than under the CK treatment. Chytridiomycota exhibited the opposite trend, with a dramatically lower relative abundance under fertilizer management. Further taxonomical classification showed that the relative abundances of Sordariomycetes and Eurotiomycetes, the predominant classes, were 31.1% and 15.0%, respectively (Fig. S1B). Compared with the CK treatment, the abundance of Sordariomycetes was markedly higher following fertilizer management, but the relative abundance of Eurotiomycetes was irregular. Additionally, at the order level, Onygenales, Hypocreales, Mortierellales, and Sordariales had relative abundances greater than 40% and were significantly higher in the soils with fertilizer application (NF, PF, NP, and OF) than in the CK soil.

### 3.6. Correlations of rhizosphere soil bacterial and fungal communities with soil properties and C:N:P ratios

The Spearman coefficients showed marked correlations between the rhizosphere soil bacterial and fungal diversity, soil properties, the C:N:P ratios and the microbial biomass in the rhizosphere soil (Fig. 4). The bacterial diversity changes were closely linked to the C:N ratio and C:P ratio in the microbial biomass and rhizosphere soil and unrelated to the pH and N:P ratio. For fungi, the MBN:MBP ratio was most closely related to the soil diversity and richness, and other factors played less prominent roles in rhizosphere soil.

RDA and Spearman correlation analysis were used to analyze the relationship between changes in environmental variables (i.e., pH, BD, ST, SWC, C:N ratio, C:P ratio, N:P ratio, MBC:MBN ratio, MBC:MBP ratio, and MBN:MBP ratio) and the relative abundances of the predominant bacterial and fungal taxa (Fig. 5 and Table S3). The BD, C:N ratio, N:P ratio, and MBC:MBP ratio accounted for 98.6% and 97.1% of the total variation in the bacterial and fungal taxa, respectively. Furthermore, the C:N ratio and MBC:MBN ratio were dramatically and positively related to the abundances of Actinobacteria and Gemmatimonadetes and negatively related to the abundances of Acidobacteria, Chloroflexi, Nitrospirae, and Planctomycetes (Fig. 5A and Table S3). Moreover, Fig. 5B shows that the MBC:MBP ratio and MBN:MBP ratio affected the abundances of Ascomycota and Mortierellomycota and the abundance of Cercozoa was also slightly affected by variations in these two ratios.

## 4. Discussion

### 4.1. Linkage between C:N:P ratios and microbial biomass in rhizosphere soil following fertilizer management

The total C, N, and P contents and microbial biomass in rhizosphere soil are simultaneously enhanced following fertilizer applications, suggesting that there is a strong interaction between the soil and microbes. Moreover, the maximum C, N, and P contents and microbial biomass were recorded in the NP and OF treatments (Table 2). To meet the elemental nutrient inputs needs of poorly fertilized soils, fertilizer management systems have been changed from the use of no fertilizer to the use of chemical fertilizers and OFs (Zhan et al., 2017). C, N, and P fertilizers increase agricultural productivity and reduce soil disturbance; therefore, they are beneficial for increasing C, N, and P levels and microbial biomass in rhizosphere soil (Ball et al., 2018; Chen et al., 2019). In this study, we found that the C:N:P ratios in the soil varies widely that the fertilizers had significant effect on the soil C:N:P ratios (Fig. 1), both of which are in agreement with the results of previous studies (Flynn, 2010; Borja et al., 2013; Liang et al., 2019). Compared with that under the CK treatment, the soil C:N ratio under the NF, PF, NP and OF treatments increased (15.3, 20.1, 18.4, 22.11 and 29.2, respectively), and the levels were higher than the average levels in China (from 10.1 to 12.1) and the global average (13.33) (McGroddy et al., 2004; Tian et al., 2010). The high C:N ratios indicated that organic matter may be accumulating faster than it is decomposing. However, under the CK, NF, PF, NP and OF treatments, the soil C:P (8.85, 11.92, 9.38, 10.70, and 15.35, respectively) and N:P ratios (0.59, 0.59, 0.51, 0.49, and 0.53) were lower than the average ratios in China (C:P ~ 61.0, N:P ~ 5.1), suggesting that the soil C and N contents were relatively low in this region, which indirectly proved the status of soil aridity in the arid regions of Northwest China. Consequently, these fertilizer inputs altered the environmental (soil and plant) nutrient status, and such resultant changes also correspond to variations in soil properties in unstable farmland ecosystems. Zhang et al. (2019a) noted that fertilizers largely altered the C:N and N:P ratios. Therefore, the C:N and N:P ratios could offer more valuable information than the C:P ratio in terms of demonstrating the relationship between soil nutrient conditions and soil microbial activities. In addition, lower BD and higher

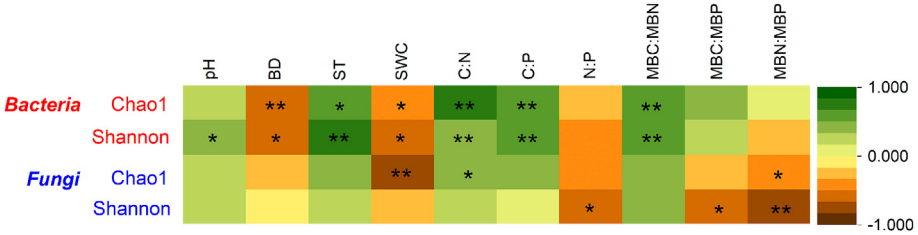


**Fig. 4.** Spearman's rank correlation coefficients between the bacterial and fungal alpha diversity (Shannon index) and richness (Chao1), and the soil properties and C:N:P ratios. CK, NF, PF, NP, and PF represent no fertilizer, nitrogen fertilizer, phosphorus fertilizer, combined N and P fertilizer, and organic fertilizer, respectively.

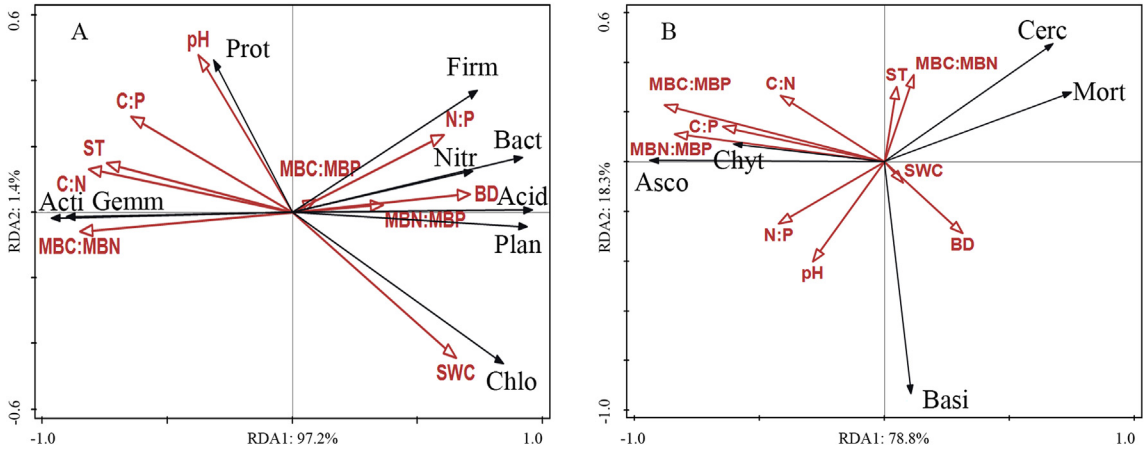


**Fig. 5.** Ordination plots of the redundancy analysis (RDA) to identify the relationship between the abundance of microbial phyla taxa (black arrows) and soil properties (red arrows). A. The relationship between soil bacterial taxa and soil properties; these are the abbreviations of bacterial populations: Proteobacteria (Prot), Acidobacteria (Acid), Actinobacteria (Acti), Chloroflexi (Chlo), Gemmatimonadetes (Gemm), Firmicutes (Firm), Bacteroidetes (Bact), Nitrospirae (Nitr), Planctomycetes (Plan). B. The relationship between soil fungal taxa and soil properties; these are the abbreviations of fungal populations: Ascomycota (Asco), Mortierellomycota (Mort), Basidiomycota (Basi), Chytridiomycota (Chyt), Cercozoa (Cerc). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ST values induced soil nutrient element increases (Table 1) in the fertilized plots compared with the contents in the CK. Many studies have also reported that the accumulation of nutrients and microbial biomass in rhizosphere soil under fertilizer management systems accounts for higher net prime productivity rates due to inputs attributed to soil microorganisms (Schleuss et al., 2019).

Ecological ratios not only help to understand the elemental balance in farmland ecological interactions and processes (Borja et al., 2013; Liang et al., 2019) but also provides an integrative nutrient guide connecting biogeochemical models on a global scale (Flynn, 2010). Wu et al. (2017) studied nutrient limitation by analyzing the ecological ratios of C, N and P and suggested conspicuous effects on the soil nutrient balance in farmland ecosystems. In turn, with increased organic matter inputs to the soil, it is expected that soil microbes would respond rapidly to the increased C availability. In our study, the C:N:P ratios of the rhizosphere soil and microbial biomass were markedly altered because of disproportionate C, N, and P improvements under the different fertilizer management treatments. C:N:P increased in the microbial biomass in the fertilized plots, revealing that enhancement of MBC content was contingent on a sufficient abundance of soil N and P to maintain the required microbial element ratio. Moreover, increases in MBP were more than twice as frequent as increases in MBC and MBN in the fertilizer management treatments compared to the CK treatment. Previous studies have shown that P availability is a crucial factor regulating symbiotic N fixation (Pourhassan et al., 2016) and asymbiotic N fixation (Augusto et al., 2013). In soil with limited P, $N_2$–fixing plants cannot acquire adequate $PO_4^+$ for their symbiotic $N_2$–fixing root nodules (Vance, 2001). Thus, rational fertilizer management can improve the C and N contents of these plants (because of the increased photosynthetic translocation of C and N to the roots and more organic C and N uptake by heterotrophic microbes) and use more P, altering the C:N, C:P and N:P ratios of the microbial biomass and rhizosphere soil.

### *4.2. Effect of C:N:P ratios on bacterial and fungal diversity and their composition in rhizosphere soil*

Despite increasing numbers of studies on reducing the use of fertilizers, guaranteeing the balance of soil fertility strongly affects the microbial community in the soil. Compared with those of the no fertilizer control, our results of combined N and P fertilizer and OF showed that each caused significant increases in bacterial alpha diversity (both the richness index and Shannon index) and fungal alpha diversity, (Fig. 2A and B), suggesting that shifts in rhizosphere soil nutrients directly drove differences in microbial diversity and that high bacterial diversity was sensitive to the soil environment (Zhang et al., 2016). These findings were in accordance with the increases in soil C, N, and P levels and the positive effects on microbial habitats in the soil. Some studies have reported inorganic fertilizer dramatically differentially affects the abundance of microbes involved in N cycling (Sun et al., 2015). Concordantly, organic fertilizers can provide abundant and balanced nutrients (such as high organic C and some inorganic salts) that are beneficial for the growth of microorganisms involved in the N cycle, because organic C from applications of organic fertilizers stimulates N denitrification. In addition, soil microorganisms are key regulators of soil P mobilization and transformation because they can enhance soil P availability through inorganic P solubilization and organic P mineralization, which are controlled by the regulation of P-cycling genes and the expression of phosphatases. Taken together, our data indicate that the combination of N and P fertilizers and organic fertilizer were more conducive than were only applications of individual fertilizers to increased diversity of soil microbial communities. Moreover, the improvements in microbial diversity were closely related to variations in ST (Fig. 4). It has been well established that fertilizer management under a high ST could offer a satisfactory environment for soil microbial growth by stimulating microbial activities (Yuste et al., 2007).

Correspondingly, changes in soil microbial diversity were induced by fertilizer management, which regulated microbial C:N:P ratios. The bacterial diversity was strongly correlated with C:N ratio of both the soil and microbial biomass but weakly correlated with the C:P and N:P ratios (Fig. 4). These findings indicated that C:N ratio gave more useful information than did other two ratios (the C:P and N:P ratios) in terms of changes in the bacterial community. Schleuss et al. (2019) noted that increased N input might predominantly lead to soil C and N cycling to keep the biomass ratio constant. The reason for this result is that soil organic matter, as the predominant substrate, is the basis for soil microorganism growth, and its C:N ratio determines whether N is limited or adequate based on microbial demands (Deng et al., 2016). Both a relatively high C:N ratio resulting from increased organic inputs and soil inorganic N are beneficial for increasing microbial diversity, and similar results have been reported in other studies (Ren et al., 2016). In addition, Marklein and Houlton (2012) indicated that microorganisms are nearly homeostatic in response to C:P and N:P ratios under P-deficient conditions. Similar characteristic changes in rhizosphere soil were observed in our study in that the fungal diversity was strongly

correlated with N:P ratio of both the soil and microbial biomass, and the N:P ratios in the fertilizer management treatments were lower than those in the CK treatment (Fig. 4), suggesting that P enrichment occurred and indicating that sufficient P levels are beneficial for the production of ATP. Moreover, P may affect some processes in the N cycle, promoting heterotrophic nitrification in soils, which may stimulate microbial growth. These results are based on applications of only P fertilizer increasing the fungal diversity (Fig. 2). Additionally, previous studies have shown that the N:P ratio is generally balanced in most farmland and grassland ecosystems (Davidson and Howarth, 2007). Hence, during the process of fertilizer management, P improvement because of C:N:P imbalances and higher nutrient additions may induce changes in the relationship between soil microbial diversity and the N:P ratio.

The microbial community composition varies in response to the soil environment under field conditions, especially changes in nutrient conditions (Mouhamadou et al., 2013). Allison and Martiny (2008) supported this conclusion in that 84% of 38 studies reported that the microbial community composition is sensitive to N, P and other fertilizers. In the present study, changes in the bacterial and fungal composition in the rhizosphere soil in response to fertilizer management were detected and were closely related to both the C:N ratios and N:P ratios of the microbial biomass and rhizosphere soil (Fig. 5). Moreover, the bacterial diversity in response to fertilizer management was mainly due to changes in the relative abundance of specific bacterial taxa belonging to the Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Firmicutes, Bacteroidetes, Nitrospirae and Planctomycetes, which accounted for more than 95% of the total bacterial phyla. Similar results have been reported, and these bacterial phyla have been shown to be directly related to the soil C and N cycles (Fierer et al., 2007). Regarding the soil bacterial community compositions, we discovered that the phyla Acidobacteria, Actinobacteria, Gemmatimonadetes and Nitrospirae responded differently to the different fertilizers and were closely related to the C:N ratio and the N:P ratio (Fig. 5 and Table S3). In particular, members of the Actinobacteria, a highly abundant bacterial phylum (Fig. 3), grow rapidly and use cellulose, hemicellulose, protein, lignin and other C and N compounds; thus, these bacteria require more C and N than do slow-growing bacteria (Ventura et al., 2007). Accordingly, the C:N ratio may be affected by the abundance of Actinobacteria (Fig. 5). The abundance of Actinobacteria could also be interpreted in terms of Micrococcales, a suborder of Actinobacteria (Table S1) regarded as a branch of plant-promoting bacteria in rhizosphere soil that can fix atmospheric $N_2$ in symbiosis with plants; therefore, increases in Actinobacteria abundance can result in N accumulation, impacting the C:N ratio (Van der Heijden et al., 2008). The percentage of Acidobacteria and Nitrospirae decreased in all the fertilized plots, which might indicate a reduction in N cycling (Zhang et al., 2013). Acidobacteria may be able to reduce nitrates and nitrites, are widespread in the soil, and thrive in soils with a low availability of resources. In contrast, the abundances of both Acidobacteria and Nitrospirae were negatively correlated with the C:N ratio, illustrating that these microbes could inhibit N accumulation. Acidobacteria are likely adapted to low-pH conditions and stoichiometric imbalances. Therefore, the relative abundance of Acidobacteria and Nitrospirae may influence the growth of these phyla by competing for resources in the fertilized plots, thus increasing their N fixation capability, which, in turn, is beneficial for increasing the soil C:N and C:P ratios under different fertilizer treatments. Furthermore, fertilizer management markedly increased the relative abundance of Gemmatimonadetes. Previous studies have also reported that Gemmatimonadetes respond inconsistently to N fertilization (Nemergut et al., 2008; Cederlund et al., 2014). In our study, Gemmatimonadetes were significantly positively correlated with the C:N and C:P ratios, which contrasts with the results for the N:P ratio. In line with hypothesis regarding the growth rate, an increase in bacterial species requires a large amount of P due to the synthesis of ribosomal RNA (Elser et al., 1996). This could be attributed to the added N and P effecting changes in the chemical properties of the soil. Therefore, the relative abundances of the dominant bacterial phyla were extraordinarily hypersensitive to the N:P ratio changes in rhizosphere soil. Thus, these results revealed that changes in the bacterial community compositions, such as Actinobacteria, Acidobacteria, Gemmatimonadetes, and Nitrospirae, accounted for changes in the C:N ratio and N:P ratio following fertilizer management.

Ascomycota and Mortierellomycota were found in sixty percent of the soil fungal sequences. The abundances of both phyla had an interaction with the N:P ratio of the soil and the MBN:MBP ratio (Fig. 5 and Table S3), and these changes resulted in a strong correlation between the soil fungal community compositions and the N:P ratio. Ascomycota and Mortierellomycota rapidly metabolize rhizodeposited organic matter in rhizosphere soil, so their abundances are stimulated by nutrient substances (Bastida et al., 2013). The relative abundance of Ascomycota and Mortierellomycota markedly increased in response to the fertilizer treatments. Hence, fertilizer management may result in suitable circumstances for phyla that obtain sufficient levels of C, N and P from the top soil (Šťursová et al., 2012). Moreover, the combination of N and P fertilizer caused the greatest relative abundance of Ascomycota and Mortierellomycota, indicating that microbes may be highly sensitive to a N and P imbalance. However, although the use of these elements results in major changes in the environment, why could the microbial community maintain its biomass ratio? We ascertained that the community N and P cycling rates could be modulated in terms of the microbes' stoichiometric demands. Moreover, fungi are thought to contribute more than bacteria in terms of influencing N and P availability for microbial biomass, with considerable changes observed for the N:P ratio and for the distribution of Ascomycota and Mortierellomycota (Hu et al., 2017).

## 5. Conclusion

Compared with those following no fertilizer application (CK), the C:N ratios and microbial biomass in rhizosphere soil following fertilizer applications were greater in this study, whereas lower N:P ratios were detected in the latter. Furthermore, fertilizers dramatically shaped the soil bacterial and fungal diversity and community composition. These alterations were affected more by changes in the C:N ratios and N:P ratios than by changes in the C:P ratios of the microbial biomass and rhizosphere soil. The compositions of certain microbial taxa, such as the bacteria Acidobacteria, Actinobacteria, Gemmatimonadetes, and Nitrospirae and the fungi Ascomycota and Mortierellomycota, in agricultural ecosystems can influence changes in C:N:P ratios. Therefore, with respect to fertilizer applications, researchers may use these ratios as reference values when evaluating the nutrient status of rhizosphere soil and microbial activity. On the other hand, the synergistic effects were better with the combination of C, N, and P or with organic fertilizer than with C, N and P separately.

## CRediT authorship contribution statement

**Chunjuan Liu:** Writing - original draft, Investigation, Formal analysis. **Xiangwei Gong:** Formal analysis, Writing - original draft, Investigation. **Ke Dang:** Investigation. **Jing Li:** Investigation. **Pu Yang:** Formal analysis. **Xiaoli Gao:** Formal analysis. **Xiping Deng:** Methodology, Project administration. **Baili Feng:** Methodology, Project administration.

## Declaration of competing interest

No conflict of interest exists in the submission of this manuscript, and the manuscript has been approved by all authors for publication.

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## Appendix A. Supplementary data

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