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RESEARCH ARTICLE

Combined organic-inorganic fertilization builds higher stability of soil and root microbial networks than exclusive mineral or organic fertilization

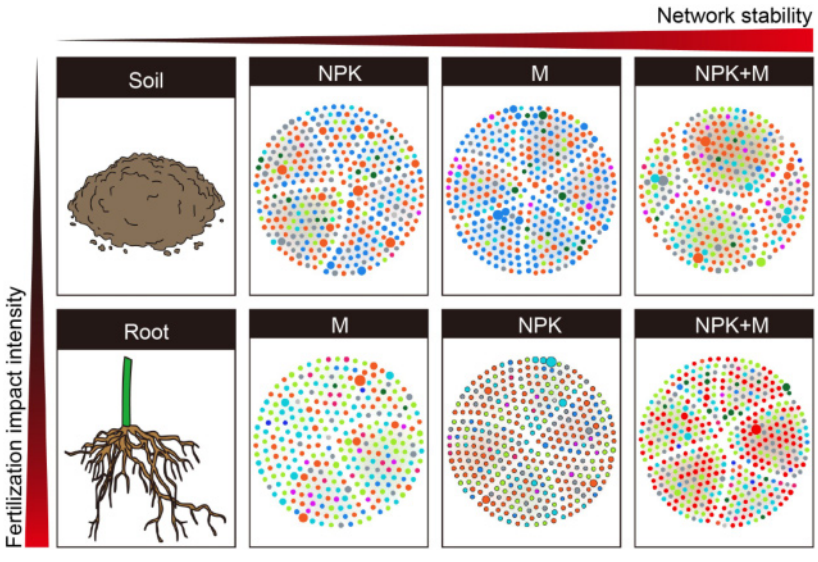
Luhua Yang[1,2,3,†], Renhua Sun[1,4,†], Jungai Li[1], Limei Zhai[1], Huiling Cui[5,6], Bingqian Fan[1], Hongyuan Wang[1,*], Hongbin Liu[1]

1 Key Laboratory of Nonpoint Source Pollution Control, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China
2 Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
3 Ningbo Environment Observation and Research Station, Chinese Academy of Sciences, Ningbo 315800, China
4 Rural Energy & Environment Agency, Ministry of Agriculture and Rural Affairs, Beijing 100125, China
5 Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
6 University of Chinese Academy of Sciences, Beijing 100049, China

HIGHLIGHTS

- Fertilization had stronger impact on the root microbiome than on the soil microbiome.
- Organic-inorganic fertilization led to higher microbial network stability than exclusive mineral or organic fertilization.
- The variances of the soil and root microbiome were attributed to the soil organic matter and the total nitrogen respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Plant health and performance are highly dependent on the root microbiome. The impact of agricultural management on the soil microbiome has been studied extensively. However, a comprehensive understanding of how soil types and fertilization regimes affect both soil and root microbiome is still lacking, such as how fertilization regimes affect the root microbiome's stability, and whether it follows the same patterns as the soil microbiome. In this study, we carried out a long-term experiment to see how different soil types, plant varieties, and fertilizer regimens affected the soil and root bacterial communities. Our results revealed higher stability of microbial networks under

* Corresponding author
E-mail address: wanghongyuan@caas.cn (H. Wang)
† These authors contributed equally to this work.

combined organic-inorganic fertilization than those relied solely on inorganic or organic fertilization. The root microbiome variation was predominantly caused by total nitrogen, while the soil microbiome variation was primarily caused by pH and soil organic matter. Bacteroidetes and Firmicutes were major drivers when the soil was amended with organic fertilizer, but Actinobacteria was found to be enriched in the soil when the soil was treated with inorganic fertilizer. Our findings demonstrate how the soil and root microbiome respond to diverse fertilizing regimes, and hence contribute to a better understanding of smart fertilizer as a strategy for sustainable agriculture.

1 Introduction

The root microbiota, sometimes known as the "second genome" of plants (Berendsen et al., 2012), have important impact on the plant health and productivity (de Vries and Wallenstein, 2017). By secreting root exudates, plants selectively attract microbes in the rhizosphere, forming hotspots different from the bulk soil (Reinhold-Hurek et al., 2015; Schlaeppi and Bulgarelli, 2015; Zhalnina et al., 2018). Many prior studies focused solely on the soil microbiome (Hartmann et al., 2015; Ling et al., 2016), with only a few taking into account both the soil and root microbiomes. It is yet still unclear how the root microbiome is affected by agricultural practices, and whether to the same extent as the soil microbiome. More integrative studies of the soil and root microbiome in response to agricultural practices are still missing.

While changes in the microbiome of bulk soil are linked to environmental effects, the microbial communities in the rhizosphere are more directly linked to yield results. The plant-microbe interactions in the rhizosphere could promote both plant productivity and agroecosystem sustainability (Schmidt et al., 2019). Understanding how the soil and root microbiome respond to various fertilization regimes is critical for establishing improved fertilization strategies and enhancing soil fertility and function. It was reported that fertilization changed the functioning of the soil microbiome, however, with a soil-specific manner. One example was that fertilization reduced ammonia-oxidizing bacteria (AOB) and predatory/exoparasitic bacteria in acidic soils (Zhao et al., 2020). In addition, bioorganic fertilizer was found to maintain a more stable soil microbiome than chemical fertilizer for monocropping (Cai et al., 2017). The phyllosphere and root endosphere were less affected by fertilization than the soil microbiome (Sun et al., 2021). However, relatively less is known about the response of the rhizosphere microbiota.

To disentangle the complexities of the soil and root microbiome, a system-level understanding of the community function and structure is needed (Fierer, 2017). In the past decades, network-based approaches have been found useful in unravelling microbe-microbe associations in complex ecosystems such as human intestines, oceans, and soils (Cram et al., 2015; Sung et al., 2017; de Vries et al., 2018; Lurgi et al., 2019). By investigating co-occurrence patterns, the network analysis could offer new insights into potential interactions of community members (Barberán et al., 2012; Berry and Widder, 2014). However, previous studies of agricultural management mainly focused on the network complexity (Banerjee et al., 2019), yet few have considered the network stability, which is ecologically important.

Communities that make less adaptations in response to environmental disturbances are thought to be more stable, and they are more likely to return to their previous state after the disruption (Shade et al., 2012; Griffiths and Philippot, 2013; Coyte et al., 2015). The gut microbiome, for instance, is known for its stability, which is important for the health and well-being of the host (Faith et al., 2013). In natural systems, the stability of microbial networks decreased with increasing environmental stress (Hernandez et al., 2021). Although theoretically related to community functions, there is a scarcity of data on network stability in agro-ecosystems. The impact of agricultural management on microbial networks, particularly fertilization regimes, is poorly known.

With these ideas in mind, we conducted a large-scale experiment using amplicon sequencing and network analysis to assess the impact of soil types, plant types and fertilization regimes on the soil and root (rhizosphere) microbiomes. We addressed the following questions: (i) Are there differences in the responses of soil and root microbial populations to fertilization practices? (ii) Which bacteria serve as indicators for different fertilization regimes? (iii) What effect does fertilizer have on the stability of soil and root microbial networks? Our work will contribute to a better understanding of smart fertilizer as a strategy for sustainable agriculture.

2 Materials and methods

2.1 Site description

The samples were collected from 9 sites in China (Additional file 1). All sites are long-term fertilization research fields and have been under consistent treatment since establishment. The sampling sites spanned from the North-east Semi-humid Plain to the Huanghuaihai Semi-humid Plain, representing 3 typical soil types in China, namely black soil, cinnamon soil, and fluvo-aquic soil. The sampling sites were distributed in 3 provinces, i.e., Jilin (black soil), Beijing (cinnamon soil), and Henan (fluvo-aquic soil).

In black soil (North-east Semi-humid Plain), two plant types, spring maize (in open fields) and eggplants (in the greenhouse), were selected. The sites were treated with inorganic N, P and K fertilizers (NPK) for 4 years. In fluvo-aquic soil (Huanghuaihai Semi-humid Plain), the fields were

cropped with winter wheat from October to June and summer maize from June to September (i.e., wheat maize rotation). All fluvo-aquic soil sites were fertilized with mineral NPK for 11 years. In cinnamon soil (Huanghuaihai Semi-humid Plain), spring maize, wheat maize rotation, as well as fallow fields were also sampled. For spring maize, five different kinds of fertilization were applied, including inorganic P and K fertilizers without N (PK), inorganic N, P, and K fertilizers (NPK), half substitution of the inorganic fertilizer by manure (1/2NPK + 1/2M), and equal substitution of the inorganic fertilizer with manure (M), respectively. For wheat maize rotation, five fertilization regimes were applied, namely no fertilization (CK), NPK, inorganic fertilization plus straw (NPK + S), inorganic fertilization plus 22.5 t ha^{-1} manure (NPK + M), and inorganic fertilization plus 33.75 t ha^{-1} manure (NPK + 1.5M). The sites were under consistent long-term treatment up to 28 years (Additional file 1). The fertilization details were recorded in Additional file 1.

2.2 Sample collection

The soil and root samples were collected two days after plants were harvested. Fields cropped with spring maize were sampled in the early September of 2018. Fields cropped with summer maize or eggplants, as well as the fallow fields were sampled in the later September of 2018. Each treatment in each site has three replicates. In total, 95 samples were collected (one sample was missing), including 51 soil samples and 44 rhizosphere (hereafter as root) samples (2 agro-climatic area, 3 soil types, 4 plant types, 8 fertilization treatments) (Additional file 2).

In each plot between plant rows, five soil cores (at 0−20 cm depth) were collected and pooled as one bulk soil replication. Each replicate contained around 80 g of bulk soil. About 10 g of each well-mixed bulk soil replicate were put into sterile falcons on ice, transferred to the laboratory immediately and stored at −80 °C until DNA extraction. The rest bulk soil was used for physiochemical analysis.

In each sampled subplot, five plants were selected. Plant roots were taken out from the soil and shacked to remove the loosely-attached bulk soil. The remaining soils attached to plant roots including that need to be brushed off from plant rhizoplane were sampled and mixed as the rhizosphere. The rhizosphere samples were treated in the same way as the bulk soil described above and were stored at −80 °C. The rest rhizosphere was used for physiochemical analysis.

2.3 Physiochemical analysis

The samples were sieved through 2 mm and kept at 4 °C. Samples were analyzed for water content, pH, total nitrogen (TN), NO_3^--N, NH_4^+-N and soil organic matter (SOM). The properties were determined according to the methods described in previous studies (Gai et al., 2018).

2.4 DNA extraction, PCR, library preparation, and sequencing

The DNA was extraction using Griffiths' protocol (Griffiths et al., 2000). The primer pair 515F and 806R were used for DNA amplification (Caporaso et al., 2011). PCR reactions were carried out in 30 μL reactions containing 15 μL Phusion® High-Fidelity PCR Master Mix (New England Biolabs, UK), 0.2 μM primers, 10 ng template DNA and sterile distilled water. The PCR cycles were set as 98 °C for 1 min, followed by 30 cycles of 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 5 min. Triplicate PCR products were then purified with GeneJET TM Gel Extraction Kit (Thermo Scientific, US) according to the manufacturer's instructions.

Following the manufacturer's instructions, libraries were created using the Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific, US). After quality test and quantification on the Qubit@ 2.0 Fluorometer (Thermo Scientific, US), the libraries were pooled into equal concentrations. The library was sequenced via the Ion S5 TM XL platform (Thermo Scientific, US). The clean reads were deposited under the accession number CRA003656 in the GSA database.

2.5 Bioinformatics

The raw sequences were quality-filtered and de-multiplexed using Cutadapt (v1.9.1) (Martin, 2011). Barcode and primer sequences were thus truncated. Chimeric sequences were screened using UCHIME (Edgar et al., 2011) against the Silva database (v138) (Quast et al., 2013) and removed. The clean reads were denoised using the unoise3 algorithm (Edgar, 2016a) implemented in usearch (v11). The denoised sequences, which are the correct biological sequences in the reads, are called "zOTUs" (zero-radius OTU) (here after OTUs). Taxonomy assignment was performed using the RDP training set v16 (Maidak et al., 2001) with the SINTAX taxonomy prediction algorithm (Edgar, 2016b) implemented in usearch (v11). Reads which were assigned to chloroplast, mitochondria and archaea were filtered out.

2.6 Statistical analysis

All statistical analyses were conducted in R (v4.0.2). Figure S1 depicts the process of the analysis procedures discussed below, as well as the figures created by each phase (Additional file 3).

2.6.1 α-diversity

The α-diversity was calculated at each rarefaction level in usearch (v11). We tested the effects of compartments, soil types, plant types and fertilization in overall samples and in subset samples respectively. The normality of the data set

was checked using Shapiro-Wilk test and the homogeneity of variance across groups was computed using Levene's test. For the two-group comparison, the differences were tested using Student's *t*-test if the data set is normally distributed, or Wilcoxon test otherwise. For comparison of more than two groups, the differences were tested using one-way ANOVA if the samples have equal variance, or Kruskal–Wallis test otherwise. Tukey's Honest Significant Differences test was carried out for pair-wise comparison using the R package *TukeyC* (Faria et al., 2018) if applicable.

2.6.2 β-diversity

With all of the samples combined, we ran a general study of β-diversity on the bacterial populations, followed by more specific hypothesis testing. We then carried out unconstrained principle coordinates analysis (PCoA) using Bray-Curtis dissimilarities. Constrained analysis of principal coordinates (CAP) was employed for in-depth investigation. The R package "*phyloseq*" was used for all ordination studies (McMurdie and Holmes, 2013). The community dissimilarity in PCoA and CAP were tested using *adonis* and *permutest* in R package "*vegan"* respectively (Oksanen et al., 2013). Pairwise comparisons, where applicable, were performed with the R package *RVAideMemoire* (Hervé, 2018).

2.6.3 The microbiome network construction and analysis

OTUs with a relative abundance no less than 0.05% in at least one third samples were selected for co-occurrence networks using Spearman correlations with R package "*psych"* (Revelle, 2017). Significant correlations ($r > 0.7$ and FDR adjusted $p < 0.001$) were visualized by Gephi (Bastian et al., 2009) with the Fruchterman-Reingold layout.

We then calculated cohesion (Herren and McMahon, 2017) and modularity (Hernandez et al., 2021) as properties of network stability. Modules were identified with the Clauset-Newman-Moore algorithm (greedy_modularity_communities) from the Python package *networkx* (Clauset et al., 2004). Cohesion was calculated using 'taxa shuffle' null model with the provided R code (Herren and McMahon, 2017).

2.6.4 Identification of fertilization sensitive OTUs (fsOTUs)

We first carried out correlation based indicator species analysis with the R package *indicspecies* (De Caceres et al., 2010). Furthermore, OTUs differed in abundance among fertilization regimes (false discovery rate (FDR) corrected *p* value < 0.05) were identified with the R package *edgeR* (Hobbs et al., 2008). The fertilization sensitive OTUs (fsOTUs) were then classified as OTUs that were confirmed by both methods. Bipartite networks were used to illustrate the fsOTUs with the R package "*igraph*" (Csardi and Nepusz, 2006).

2.6.5 Identification of key drivers in networks

The soil and root communities under each fertilization regime were combined to construct meta-networks. Four meta-networks were constructed consequently, in accordance with the fertilization regime NPK, M, NPK + M, and NPK + 1.5M.

To define the topological properties of individual nodes (OTUs), we employed a set of parameters (i.e., within-module connectivity (Zi) and connectivity among modules (Pi)) (Guimerà and Amaral, 2005). The distribution of nodes in the networks was visualized using R package *ggplot2*. Based on the values of Zi and Pi, nodes might be divided into four subcategories (Olesen et al., 2007): (i) highly linked connector nodes ($Zi \leqslant 2.5$, $Pi > 0.62$), (ii) module hubs ($Zi > 2.5$, $Pi \leqslant 0.62$), (iii) network hubs ($Zi > 2.5$, $Pi > 0.62$), and (iv) peripheral nodes ($Zi \leqslant 2.5$, $Pi \leqslant 0.62$).

We also used the method NetShift (Kuntal et al., 2019) to find key microbial taxa that act as "drivers" between two networks.

3 Results

3.1 The main drivers of the soil and root microbiota

We conducted bacterial community profiling of 51 soil and 44 root samples from 9 sites with 3 different soil types, 4 plant types and 8 fertilization regimes. A total of 2 758 622 high-quality sequences was yielded (range 8165−57 939; median 28 701; Additional file 2). In sum, we identified 18 097 bacterial zOTUs (zero radius OTU) across all samples.

The major phyla of the bacterial community were Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, and Firmicutes (Fig. S2). Soil and root, as different microbial habitats, were found inhabited by specific sets of microbes (Fig. 1A). Principal coordinate analysis (PCoA) indicated that microbial communities were clearly separated by soil types, when taking all some samples into consideration (Fig. 1B; Table S1). The discrete outlier in the bacterial communities was consistent with TN (Fig. S3b) and SOM (Fig. S3c). Soils exhibited a higher diversity than rhizosphere (Fig. S4 and Table S2).

For the in-depth analysis, we employed the canonical analysis of principal coordinates (CAP). Soil types could explain 13% of the variance in the soil microbiome and 15% of the root microbiome, both of which were confirmed by pairwise PERMANOVA tests (Fig. S5; Table S3). A higher diversity of the soil and root microbiome was found in cinnamon soil than in fluvo-aquic soil by the comparison of Shannon index. Unexpectedly, no differences were observed in the α-diversity between black and cinnamon soil, though black soil is considered more fertile in general (Fig. S5; Table S4).

We further analyzed samples under the sample soil type and the same fertilization regime. Both the soil microbiome

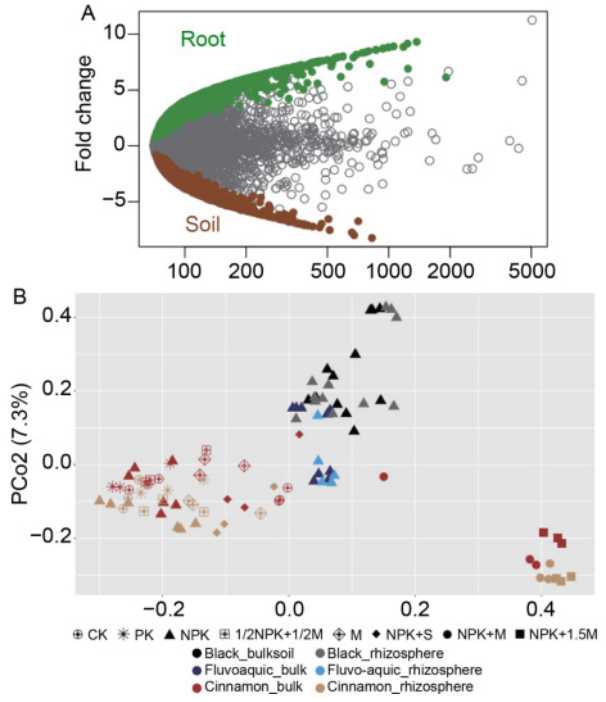

Fig. 1 The main drivers of soil and root microbiome. (A) The plot displays the abundance patterns of bacteria in soil and root microbiomes. *X*-axis reports average OTU abundance (as counts per million, CPM), and *Y*-axis $\log_2$-fold change (root relative to soil). Root and soil-specific OTUs are colored in green and brown, respectively. Non-differentially abundant OTUs are colored in gray (likelihood ratio test, $p < 0.05$, FDR corrected). (B) Unconstrained PCoA ordinations of bacteria. Percentage of variation given on each axis refers to the explained fraction of total variation in the community. Symbols refer to the different fertilization treatments. Figure (B) are colored by soil types and compartments.

and the root microbiome were clearly separated by plant types, confirmed by both PCoA plots and PERMOVA tests (Fig. S6; Table S5), indicating that plant type is a driving factor in shaping both soil and root microbiome. However, no statistical differences of α-diversity were found between different plant types (Fig. S7; Table S6).

We further investigated the fertilization impacts on soil and root bacterial communities. Clear differences of beta diversity among fertilization regimes were indicated by CAP and PERMANOVA tests (Fig. 2; Fig. S8).

The impact of fertilization on α-diversity was only observed in the root microbiome but not in the soil microbiome, regardless with crop types (Fig. S8; Table S7). The lowest α-diversity of the root microbiome was observed under fertilization regime PK, where N was missing. Unexpectedly, the root microbiome without fertilization and fertilized with NPK did not differ in the α-diversity. Interestingly, the addition of organic materials (straw and manure) significantly lowered the α-diversity. In particular, the addition of the straw (NPK + S) showed the lowest α-diversity, much lower than the addition of the manure (NPK + M and NPK + 1.5M).

3.2 Fertilizatiton sensitive OTUs

We identified OTUs varied in abundance among different fertilization regimes confirmed by both indicator species analysis and likelihood ratio tests. The resulted fertilization sensitive OTUs (hereafter: fsOTUs) were summarized in bipartite networks (Fig. 2; Fig. S9). The patterns resembled those observed in the beta diversity analysis. A specific subset of soil and root microorganisms is supported by each fertilization regime. Particularly, we noted that Actinobacteria was enriched in the soil microbiome under inorganic NPK fertilizer. Instead, Acidobacteria and Bacteroidetes largely dominated the root microbiome, and were enriched with the addition of organic fertilizer. Firmicutes was enriched under organic fertilization as well. Around 28% fsOTUs identified in root belonged to Acidobacteria, while only half of fsOTUs in soil (14%) were assigned to Acidobacteria.

3.3 Network properties under different fertilization regimes

We constructed co-occurrence networks of bacterial community under each fertilization regime. The overall community taxonomy was changed by fertilization practices (Fig. 3). We further quantified the network stability via modularity, a reflection of how compartmentalized the network is, and cohesion, a metric quantifying the degree of community complexity, respectively.

In both the soil and root microbiome, combined fertilization led to the highest modularity, indicating higher community stability (Fig. 4). Organic fertilizer resulted in higher soil network modularity than inorganic fertilizer. However, the opposite trend was observed in the root microbiome, where bacterial networks showed higher modularity with inorganic fertilizer than organic fertilizer.

Similarly, both the highest negative and positive cohesion metrics were observed with combined fertilization ($p < 0.01$, ANOVA), indicating higher network stability (Fig. 4). However, no statistical differences of cohesion were found between inorganic and organic fertilization. As to the soil microbiome, the differences among fertilization regimes were only found with negative cohesion but not positive cohesion ($p < 0.05$, ANOVA). As like in the root microbiome, combined fertilization showed higher negative cohesion than inorganic NPK or organic manure alone, but no differences were found between organic and inorganic fertilization.

We further carried out canonical correspondence analysis (CCA) to investigate the environmental variables corresponding with fertilization practices. SOM and pH were found significantly correlated with the soil microbiome, while TN was found significantly correlated with the root microbiome (Fig. S11).

3.4 Key drivers in network shifting

The soil and root microbiota under each fertilization regime were combined to construct meta-networks. Consequently,

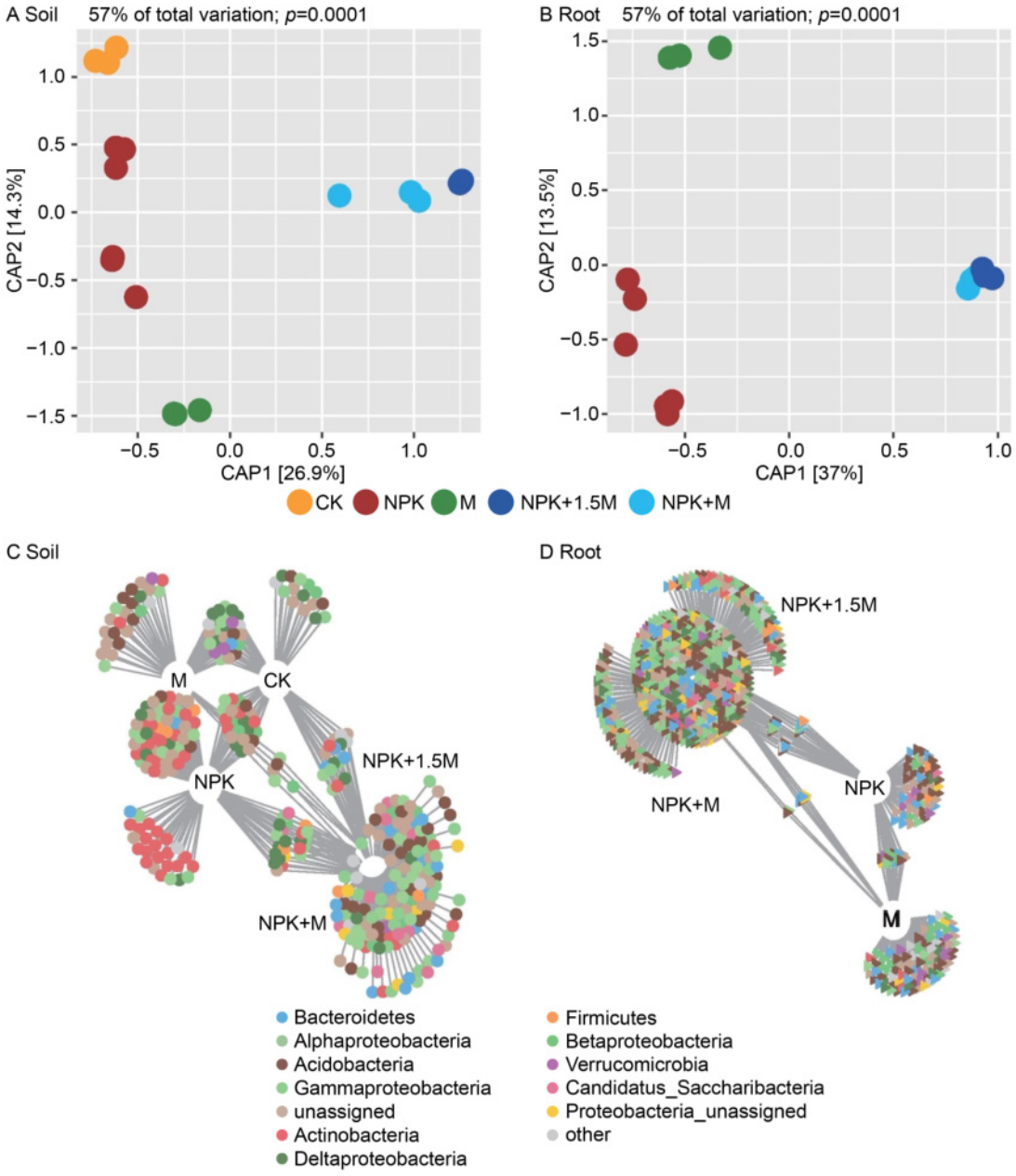

Fig. 2 Fertilization induced differences of soil/root microbiome and fertilization sensitive OTUs. CAP analyses were constrained by the factor "fertilization". The fraction of total variance explained of (A) the soil microbiome and (B) the root microbiome is shown above the plots. The explained fraction of total variation is represented by the percentage of variation given on each axis. Bipartite networks display fertilization system specific OTUs in the soil (C) and root (D) bacterial communities. Circles represent soil bacteria OTUs and triangles represent root bacteria OTUs. OTUs are colored according to their phylum assignment.

we obtained four meta-networks in accordance with the fertilization regime NPK, M, NPK + M, and NPK + 1.5M. We identified a series of module hubs (nodes highly connected to other members in a module) and connectors (nodes linking different modules) based on their within-module connectivity (Zi) and among-module connectivity (Pi), which could be regarded as keystone nodes that play key roles in shaping network structure. (Fig. 5). The number of module hubs were highest under combined fertilization and lowest under inorganic fertilization, which is in line with the results of modularity analysis. Under inorganic fertilization, nearly one fourth of the connector OTUs with NPK were assigned to Acidobacteria, which was less abundant in connector OTUs of other networks. Instead, Firmicutes and Candidatus_Saccharibacteria became prominent as connectors under organic and combined fertilization, whereas they were absent in the connector OTUs with the inorganic fertilizer.

We further explored the potential "driver microbes" in

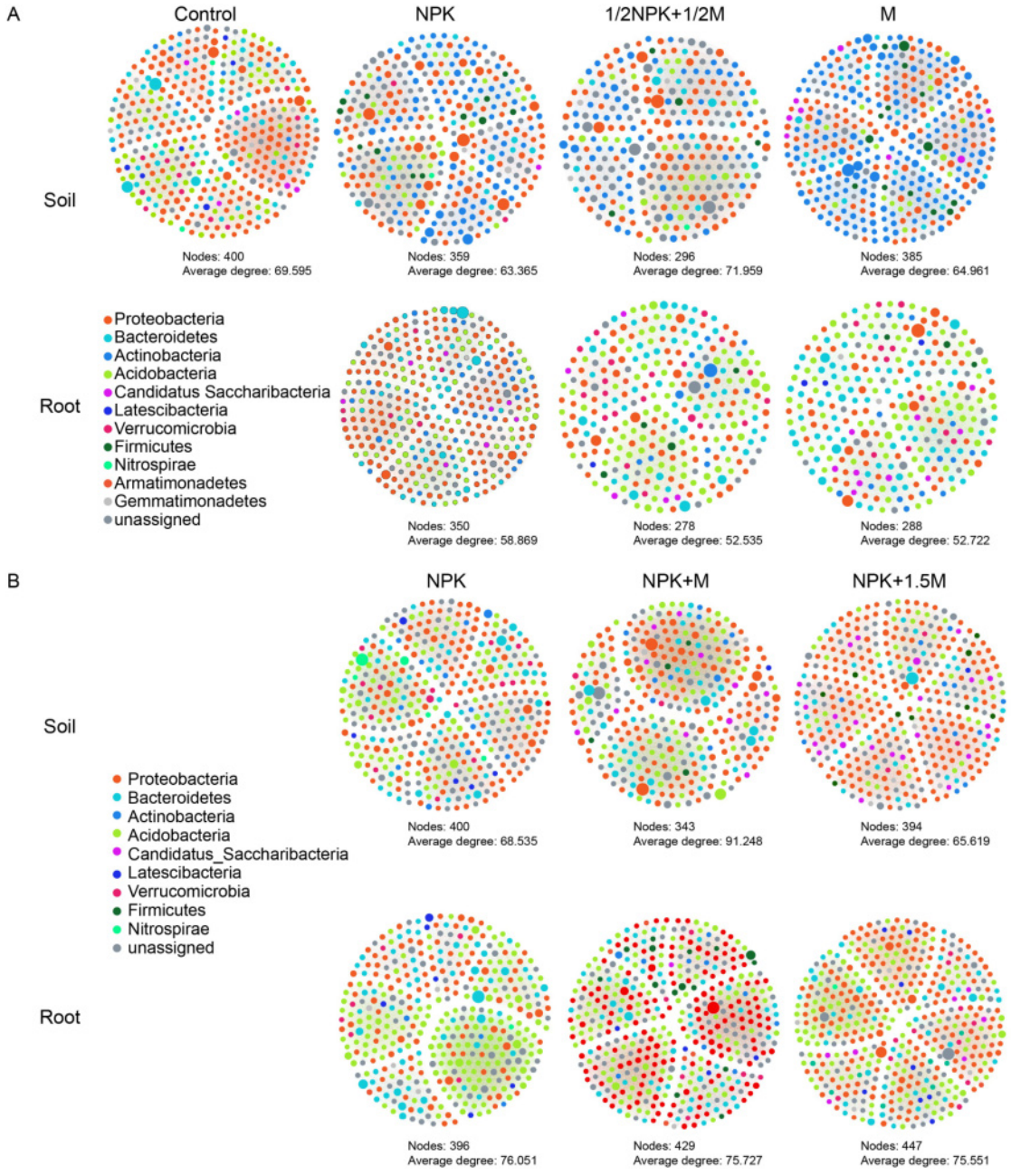

Fig. 3 Co-occurrence networks of soil and root OTUs under each fertilization regime. (A) spring maize, (B) summer maize (wheat maize rotation). Each node (circle) represents an OTU. The color of nodes indicate the assigned phyla. The size of the node were proportional to the relative abundance (25:1).

shaping microbial networks under different fertilization regimes using the newly-developed method "NetShift." The taxon that has a different set of relationships (recognized by a high Neighbor shift (NESH) score) while becoming more relevant for the entire network (identified by a positive delta betweenness (B) score) is predicted as a "driver." Accordingly, we selected top 30 taxa of highest NESH score with positive ΔB values (Fig. 6). In the shift from inorganic (NPK) to organic (M) fertilization, Bacteroidates and Verrumicrobiota stood out as the most prominent drivers, as both of their NESH and ΔB score were high. In comparison with exclusive organic fertilization (M), Bacteroidates, Acidobacteria, Firmicutes, BRC1, and Gammaproteobacteria (Pseudomonadales and Xanthomonadales) contributed as important members in driving network changes under combined fertilization (NPK + M). In the shift of fertilization regime from NPK + M to NPK + 1.5M, *Turicibacter* and *Bacillus* from the phylum Firmicutes were identified as the key drivers with the highest NESH and ΔB score. Besides, increased number of Proteobacteria, particularly Rhizobiales, were found among the driver taxa.

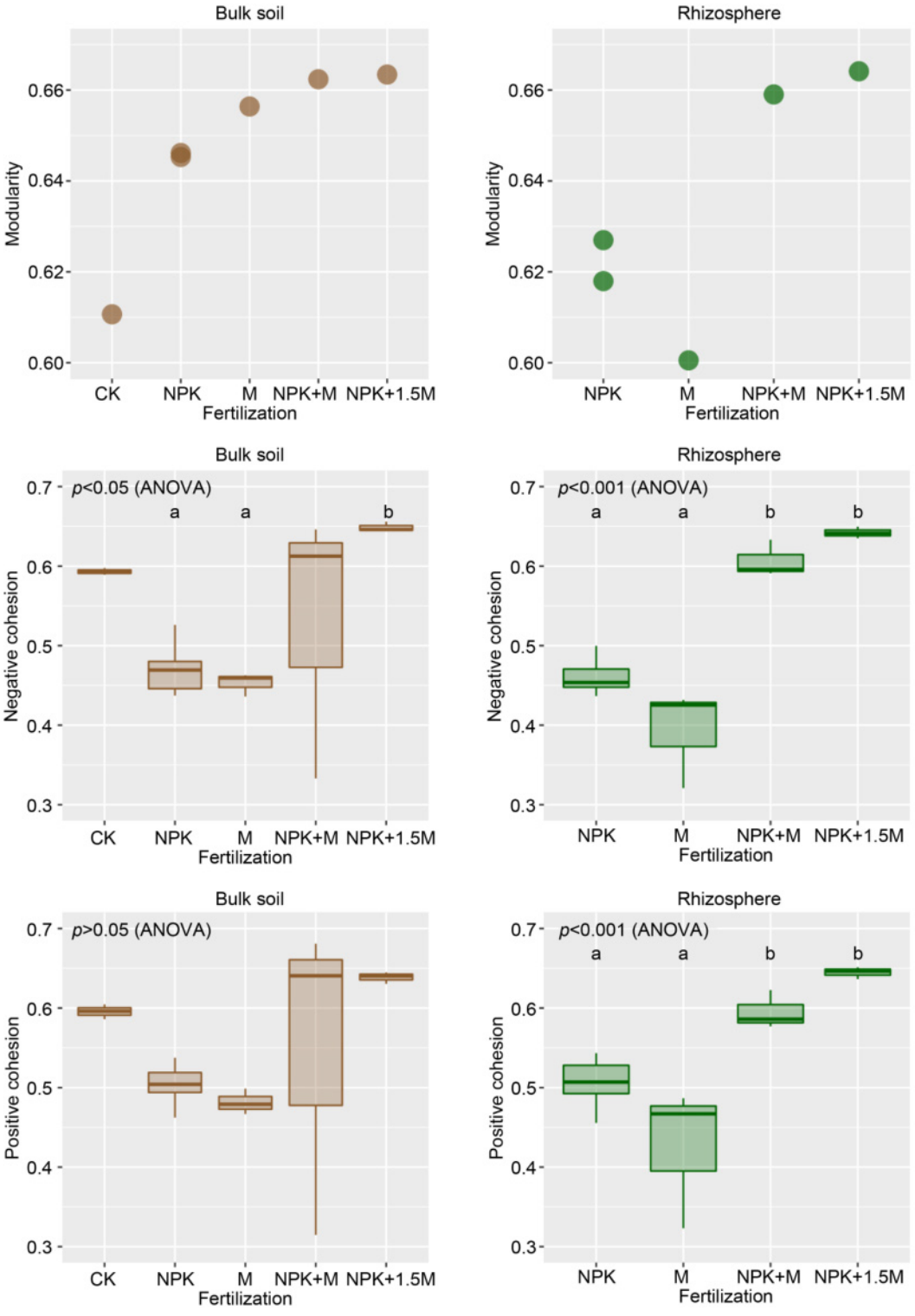

Fig. 4 The modularity, absolute value of negative cohesion and positive cohesion of microbial networks under different fertilization regimes.

4 Discussion

4.1 Combined fertilization leads to higher microbial network stability

In this study, we characterized the soil and root microbiome from 3 different soil types, 4 plant types and 8 fertilization regimes in a long-term field experiment. While soil types could largely determine microbial communities, fertilization practices were found as a primary factor in shaping the soil and root microbiota under the same soil type. Our results indicated that combined organic-inorganic fertilization

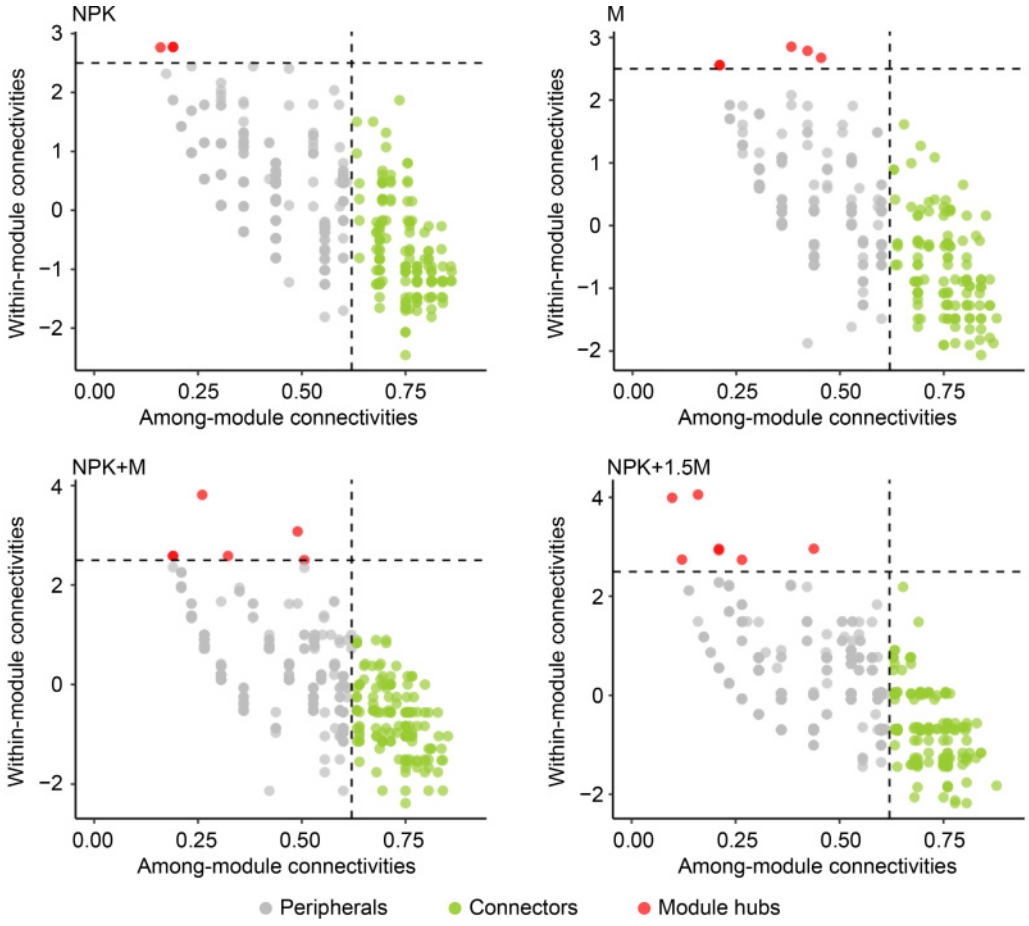

Fig. 5 The Zi-Pi plot of the microbial networks under each fertilization regime. Each node represents an OTU. Zi represent within-module connectivity and Pi represent among-module connectivity. Nodes were separated into four subcategories based on the values of Zi and Pi: peripheral nodes (Zi ⩽ 2.5, Pi ⩽ 0.62), connector nodes (Zi ⩽ 2.5, Pi > 0.62), module hubs (Zi > 2.5, Pi ⩽ 0.62), and network hubs.

resulted higher modularity and cohesion values in both soil and root microbial networks than exclusive inorganic or organic fertilization.

Network complexity relates with the total number of interacting species (network size). The connectance, measuring the proportion of realized interactions among all the possible ones in a network, is also a commonly-used indicator of complexity (Landi et al., 2018). Stability of an ecosystem can be understood as its propensity of returning to its functioning regime after a stress or a perturbation in its biotic components or abiotic components (Landi et al., 2018). The two metric cohesion and modularity together could be used as an indication of microbial stability together (Yuan et al., 2021).

The stability of microbial networks has been successfully predicted using network properties (de Vries et al., 2018; Hernandez et al., 2021; Yuan et al., 2021). Communities with a higher level of modularity, less positive relationships between taxa, and more negative associations between taxa are more stable. By limiting the impact of losing a taxon to its own module, high modularity could help communities stay stable (Stouffer and Bascompte, 2011; Grilli et al., 2016). Negative cohesion (negative relationships) indicates disparate niches and/or negative interactions between taxa, whereas positive cohesion (positive relationships) indicates considerable niche overlap and/or positive interactions between taxa (Herren and McMahon, 2017; Yuan et al., 2021). It is argued that positive associations can lead to dependency and mutual downfall (Coyte et al., 2015). Negative co-occurrences/interactions, on the other hand, may dampen positive feedbacks and hence increase stability (Hernandez et al., 2021).

In this study, we found higher modularity and connectivity (i.e., cohesion) as well as a dominance of negative correlations in microbial networks under combined fertilization, indicating that combined fertilization leads to microbial community with higher network stability. Notably, the response of modularity in root and soil to organic and inorganic fertilizer were opposite. One possible explanation is that the nutrient availability and demands of the two niches are different. The soil microbiota require more carbon to grow, while the rhizosphere microbiota require more nitrogen as a change for the carbon released from plants. Consequently, the organic fertilizer may favor the soil microbiota, whereas the inorganic fertilizer may favor the rhizosphere microbiota (due to the readily-available N). That

Fig. 6 The top 30 taxa of “driver microbes” in microbial networks between the change of fertilization regime. Each dot represents a taxa in the microbial networks. The *X* axis denotes the delta between score (*ΔB*), implying the changes of importance of each taxa in the network in comparison with the former network. The size of the dot corresponds to the NESH score, indicating the changes of node associations. Taxa with high NESH score and positive delta between values were predicted as “driver microbes”.

may also explain why the combined fertilization leads to the highest modularity of both the soil and root microbiome, as combined fertilization satisfied the urgent need of both soil and root microbiota.

A recent study provided evidence that naturally-occurring microbiome display properties characteristic of unstable communities when under persistent stress (Hernandez et al., 2021). Networks with higher stability are more resistant to environmental changes (Santolini and Barabási, 2018). In this sense, our findings may suggest that the microbiota under combined fertilization is more resistant to environmental challenges. In addition, pathogen invasion success in the rhizosphere has been linked to the network structure of resident bacterial populations (Wei et al., 2015). Therefore, the structure and stability of root communities are highly important for the plant health and fitness.

Indeed, our previous results showed combined fertilization resulted higher crop yields than exclusive manure application than solely mineral fertilization (Gai et al., 2018). The yield increase by combined fertilization was also confirmed in other long-term experiments (Wei et al., 2016), with enhanced soil nutrient availability, microbial biomass, enzymatic activities and soil nitrogen processes (Zhao et al., 2016). After 35 years of fertilization, the combination of inorganic fertilizer and cow manure produced the most resistant microbial community, which was related with the lowest relative abundance of possible fungal plant diseases (Fan et al., 2020). In brief, our results are in line with the notion that host can benefit from increased microbiome stability.

4.2 Fertilization had stronger impact on the root microbiome than on the soil microbiome

Interestingly, our results suggested that the influence of fertilization is stronger on the root microbiome, but less significant on the soil microbiome, indicating compartment-specific responses of bacterial communities. Our CAA analysis revealed that TN is the environmental factor responsible for the community variation of the root microbiome, whereas pH and SOM explained the soil community differences. While pH is well-known for its decisive role in selecting bacterial community, it seems that C and N factors drive the soil and root microbiome respectively. Studies have shown that the quantity and quality of SOM following N enrichment were linked with soil microbial communities and the associated enzyme activities (Craine et al., 2007). The organic fertilizer is known to enhance SOM and improve soil fertility (Li et al., 2021). A recent study indicated that the carbon loss under alpine permafrost deterioration is linked to decreasing microbiological stability (Wu et al., 2021). In line with previous reports, our results indicated that organic fertilization and combined organic-inorganic fertilization increased the microbial stability mainly by the increase of SOM, as SOM was found correlated significantly with the soil microbiome under different fertilization regime by CCA analysis.

The root microbiome, however, are dynamically influenced by both the edaphic environment and the host plant. Compared with bulk soil, a higher demand of N is required by plants. The amount of accessible nitrogen that plants can absorb is highly influenced by root-associated microbial guilds (Moreau et al., 2019). For instance, the ability of arbuscular mycorrhizal fungi to transmit nitrogen to plants was recently discovered, and the uptake of nitrogen by the fungal symbiont was facilitated by carbon provided by the host plant (Fellbaum et al., 2012). Mounting evidence reveals that rhizosphere priming appears to be a crucial strategy for plants to receive organic nitrogen (Phillips et al., 2011).

4.3 Microbial shifts in response to different fertilization regimes

We found that Actinobacteria was prominently enriched in the soil microbiome under mineral fertilization. Many members of Actinobacteria were consistently identified as consumers of labile C substrates in stable-isotope probing (SIP) experiments (Kramer et al., 2016). The selective enrichment of Actinobacteria in the NPK fertilized soil indicated a community more reliant on simple C substrates and less adapted to complicated C breakdown and nutrient mineralization.

Our results showed that Bacteroidates and Verrumicrobiota were among the most important drivers in the shift from inorganic fertilization to organic fertilization. Bacteroidetes are primary degraders of biomass derived from complex carbohydrates (Thomas et al., 2011; Lapábie et al., 2019). Members of Verrucomicrobia were identified by SIP experiments as consumers of more complex C compounds, such as cellulose (Pepe-Ranney et al., 2016). Cultured members of these phyla have genetic mechanisms for breaking down complicated plant-derived polysaccharides. Considered together, they may play important roles in the decomposition of complex organic matter, and thereby contribute to the community shift from inorganic to organic fertilization.

With extra manure applied (i.e., NPK + 1.5M), a dominance of Firmicutes was observed as the potential drivers. Firmicutes are likely to increase in nutrient-rich conditions (Fierer et al., 2007). For instance, the increase in Firmicutes in gut microbiota is often correlated with obesity (Ley et al., 2006). In our case, the large dominance of Firmicutes under the fertilization regime NPK + 1.5 M might be an indication of over-fertilization. Fertilization regimes that rely solely on inorganic inputs may cause root selection of microbial communities that are more reliant on readily available C, disrupting the plant's ability to select for a prokaryotic population that mineralizes nutrients from existing organic matter.

5 Conclusions

Overall, we found that fertilization regimes impacted both

the soil and root microbiome. The network analysis with modularity and cohesion indicated a higher stability of microbial networks under organic-inorganic fertilization than those relied solely on inorganic or organic fertilization. In addition, the response of the root microbiome to fertilization was stronger than the soil microbiome and exhibited different patterns. While TN contributed mostly to the variance of the root microbiome, pH and SOM could largely explain the differences in the soil microbiome. Bacteroidetes and Firmicutes were found to be major drivers in the soil and root microbiome when organic fertilizer was added, but Actinobacteria was found to be enriched in the soil microbiome when only inorganic fertilizer was used. Our study implied that combined organic-inorganic fertilization might be a sound practice better than exclusive mineral or organic fertilization. However, the risk of over-fertilization still need to be taken care of.

Statements and declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Electronic supplementary material

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