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Enhancement of beneficial microbiomes in plant–soil continuums through organic fertilization: Insights into the composition and multifunctionality

Wen-Jing Li[1,2,†], Xin-Yuan Zhou[1,2,†], Xin-Li An[1], Li-Juan Li[1,2], Chen-Shuo Lin[1,2], Hua Li[3], Hong-Zhe Li[1,*]

1 Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
2 University of Chinese Academy of Sciences, Beijing 100049, China
3 State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Institute of Environment, Resource, Soil and Fertilizers, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
* Corresponding author. E-mail: hzli@iue.ac.cn (H.-Z. Li)
† These authors contributed equally to this work.
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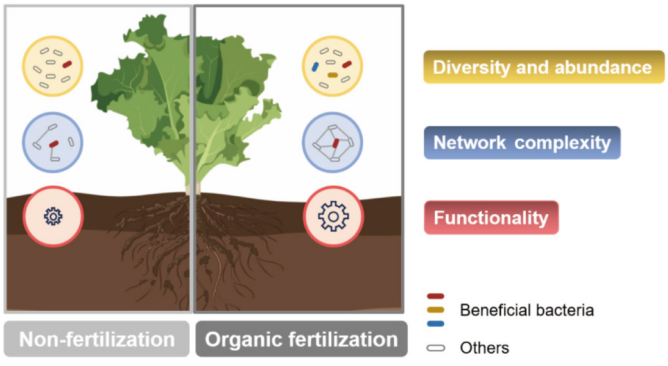
ABSTRACT

• Organic fertilization increased the richness and abundance of beneficial communities.
• The increase in the beneficial communities resulted from soil property optimization.
• Organic fertilization enhanced the functionality of bacterial communities.
• Plants exhibited a preference for internalizing beneficial bacteria.



Soil microbiomes play a crucial role in maintaining ecological functions and are of great importance for soil health. Some of them could bring benefits to plants for growth promotion. Despite numerous studies have focused on specific beneficial bacteria and their interactions with soils and plants, we still lack a comprehensive understanding of beneficial communities in plant–soil continuums and their responses to agricultural activities. To address this gap, we carried out a microcosm experiment using 16S rRNA amplicon sequencing to explore the effects of organic fertilization on beneficial communities in plant–soil continuums and assess their potential multifunctionality. Our findings reveal that organic fertilization had a positive impact on the beneficial functionality of bacterial communities in plant–soil continuums. This improvement was primarily attributed to the optimized soil physicochemical conditions resulting from organic fertilization. Additionally, organic fertilization increased the complexity of bacterial co-occurrence networks in both soils and the endosphere. Keystone taxa in the endosphere undergone a shift of functions toward pathogen suppression as the result of organic fertilization. Furthermore, this study revealed that plants exhibited a preference for internalizing beneficial bacteria over other type of bacteria. We also provided new insights for evaluating the multifunctionality of microbiomes, and found that the functionality of beneficial communities in plant–soil continuums is enhanced by organic fertilization. All these findings suggested that organic fertilization can be an effective strategy for maintaining plant and soil health.

Keywords plant–soil continuums, beneficial bacteria, agricultural activities, multifunctionality, endosphere

1 Introduction

Soil is the loose layer of the planet's surface, harboring a diverse array of microorganisms, including bacteria, fungi, protists, nematodes and viruses. These microorganisms play crucial roles in driving various functions and determining the soil health (Lehmann et al., 2020; Hartmann and Six, 2022). Within the soil microbiome, there exists a subset of microorganisms that provide benefits to plants. These beneficial communities in soils can confer fitness advantages to host plants, such as growth promotion, stress tolerance and disease resistance, consequently promoting plant health and crop production (Trivedi et al., 2020). Several studies have unraveled the molecular mechanisms of interactions between specific beneficial bacteria and host plants (Rudrappa et al., 2008; Yu et al., 2021). However, our understanding of beneficial bacteria with diverse functional traits in response to agricultural activities remains incom-

plete. Additionally, there is a lack of an evaluation system to assess the beneficial functionality of soil microbiomes.

Beneficial bacteria can enhance plant growth via various mechanisms. First, they are able to fix nitrogen, solubilize fixed phosphate, and facilitate the uptake of essential elements for plants, such as iron, thereby promoting the nutrient uptake (Toro et al., 1998; Housh et al., 2021; Zhang et al., 2022a). Additionally, beneficial bacteria can alleviate plant stresses, including drought and salinity, by improving physical and chemical conditions of microenvironments or altering plant physiological traits (Bresson et al., 2013; Li et al., 2021). Moreover, these beneficial bacteria are also capable to suppress plant pathogens either through direct competition for resources or by producing antibiotic compounds (Dowling and O'Gara, 1994; Wei et al., 2015; Kwak et al., 2018). The main source of beneficial bacteria on or in plants is surrounding soils (Xiong et al., 2021a). Specific bacteria in bulk soils, that can take up the metabolite substrates in the root exudate, move toward plants by chemotaxis, and some of them can enter to plants (Sasse et al., 2018; Trivedi et al., 2020). This colonization process becomes noticeable during the period of plants stresses. For instance, a distinct enrichment of *Stenotrophomonas rhizophila*, which was proven to upregulate plant defense signaling, was observed in the rhizosphere and root endosphere of crown rot wheat, with the absolute abundance of 3.7×10^7 cells g^{-1} in the rhizosphere and the relative abundance of 11.4% in the root endosphere (Liu et al., 2021). Amounts of studies have demonstrated that bacterial communities in soils are significantly affected by agricultural activities, thereby agricultural activities potentially influencing the composition and function of beneficial bacteria as well (Zhao et al., 2019; Sun et al., 2021a). Given that bacterial communities in soils act as 'seed banks' for plant recruiting beneficial bacteria, alterations in the composition of beneficial communities in soil can lead to shifts in the assemblages of beneficial bacteria in the rhizosphere and endosphere, which are crucial for maintaining plant health.

It is widely considered that both inorganic fertilizers and organic fertilizers can alter the diversity and composition of bacterial communities in soils (Dai et al., 2021). Compared to inorganic fertilizers, organic fertilizers can avoid the side effects caused by inorganic fertilizers, such as soil degradation and acidification. On the one hand, organic fertilizers can affect the beneficial bacteria associated with plant nutrient uptake, e.g., nitrogen-fixing bacteria (Del Valle et al., 2020). On the other hand, organic fertilizers are also capable to induce pathogen suppression to plants, including fusarium wilt and bacterial wilt, by enriching biocontrol bacteria in soils, e.g., *Pseudomonas* and *Streptomyces* (Chen et al., 2020a; Dong et al., 2020; Deng et al., 2022). All these show that organic fertilization could be an efficient and sustainable strategy for both the yield improvement and health maintenance for crops, thus it is of great importance to investigate organic effects on microbiome in plant–soil continuums. Although many studies have revealed the organic fertilization effects on a specific or a class of beneficial bacteria with a certain function, we still lack a comprehensive exploration of organic fertilization impacts on the composition and multi-functions of beneficial bacterial communities in plant–soil continuums.

In this study, we hypothesized that (1) organic fertilization can alter beneficial microbiomes of soils and plants by optimizing of soil physicochemical conditions, (2) organic fertilization can enhance the potential functionality of beneficial communities in plant–soil continuums. To confirm these hypotheses, we conducted a microcosm experiment by cultivating four common vegetables (radish, lettuce, pakchoi and cabbage), treated with or without organic fertilizers for different durations (14 days, 28 days and 60 days). Based on 16S rRNA amplicon sequencing, we explored the dynamics of plant microbiome in three compartments (bulk soil, rhizosphere soil and endosphere), in response to organic fertilization. There were 26 common plant-beneficial bacteria, proved to be associated with growth promotion, nutrient uptake, pathogen suppressive and stress tolerance, were identified at the genus level, such as Rhizobium with the ability of inducing nodule formation to fix nitrogen and Pseudomonas able to suppress pathogens with characteristics of rapid growth and wide niches (Santoyo et al., 2012; Sun et al., 2023). Here, the purposes of this study were to (1) explore the effects of organic fertilization on the composition of bacterial communities in plant–soil continuums, (2) investigate shifts in the composition and function of beneficial communities in the soil and the endosphere, (3) reveal the mechanisms for changes of beneficial communities induced by organic fertilization, (4) explain the relationships between soil communities and endosphere communities, (5) evaluate the impacts of organic fertilization on multi-functions of plant microbiomes. This work deepens our understanding for the assemblage of plant-beneficial microbiome disturbed by agricultural activities.

2 Materials and methods

2.1 Microcosm experiment

The soil samples were collected from an agricultural filed in Xiamen, Fujian Province, China (24°38′48.4″ N, 118°02′48.1″ E) in September 2020. The basic physicochemical properties of the original soil were as follows: lateritic red soil, pH 6.2, dissolved organic carbon (DOC) 110.9 mg kg^{-1}, dissolved total nitrogen (DTN) 50.1 mg kg^{-1}, total carbon (TC) 18.7 g kg^{-1}, total nitrogen (TN) 1.5 g kg^{-1}.

The soil was passed through a 2 mm sieve to remove plant residues and gravel particles, then mixed. About 2.0 kg soil was packed into each plastic pot. The study included two fertilization treatments: non-fertilization (CK) and organic fertilization (OF). The organic fertilizer was made from chicken manure composts and its basic physicochemical properties were as follows: pH 7.8, DOC 916.5 mg kg^{-1}, DTN 173.1 mg kg^{-1}, TC 179.2 g kg^{-1}, TN 19.5 g kg^{-1}. In organic fertilization treatment (OF), approximately 80 g organic fertilizer was thoroughly mixed with per kg dry soil (Zhang et al., 2019). Before sowing, the water content of soil was adjusted to 70% water holding capacity and incubated at 25°C for a week. Four common vegetables, i.e., radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), pakchoi (*Brassica chinensis*) and cabbage (*Brassica oleracea*) were chosen in this study. Around 10 seeds of each plant were sown in one pot and to ensure 3 seedlings per pot, extra ones were removed after germination. To explore temporal changes of organic fertilization effects on plant microbiomes, we set three growth time, i.e., 14 days (14D), 28 days (28D) and 60 days (60D). Each treatment had three independent replicates, thus there were 72 pots (2 fertilization treatments × 3 growth time × 4 plant types × 3 replicates) in total. All plants grew in the greenhouse with an average temperature of 25°C and watered every evening until harvested at day 14, 28 or 60.

2.2 Soil physicochemical property characterization

The basic physicochemical properties of soil and organic fertilizer samples, including pH, DOC, DTN, TC and TN were measured in this study. pH values were measured in 1:2.5 soil/water and fertilizer/water suspensions used a pH meter (IS126C, Insmark, Shanghai, China). The concentration of DOC and DTN was measured as follows: dried samples were mixed with KCl solutions and vibrated for 2 h, followed with centrifuged. Then, the supernatant was analyzed on a TOC analyzer (TOC-LCPH, Shimadzu, Japan) for DOC and DTN. To measure TC and TN concentrations, samples were air-dried and analyzed on an elemental analyzer (Vario MAX, ELEMENTAR, Germany).

2.3 Sample collection and DNA extraction

Bulk soil, rhizosphere soil, roots and leaves were collected at every sampling time (day 14, day 28, and day 60). Soils in the surface and firmly attached to the roots were regarded to be the bulk soil and the rhizosphere soil, respectively. Briefly, after the bulk soil collection, root tissues were isolated from pots and shaken to remove loose soils. Root tissues were then transferred to the sterile PBS with 0.1% Tween 20 and vortexed at 25°C for 2 h. The suspensions were centrifuged at 8000 × *g* for 20 min and the pellets on the bottom were regarded to be rhizosphere soil samples. To eliminate microbes on the surface and extract microbes in the endosphere, both root and leaf samples were surface-sterilized as follows: washed by sterile water for three times, immersed into 70% ethanol and subsequently 2.5% sodium hypochlorite solution for 3 min and 5min, respectively, and then washed by sterile water for five times. All samples were stored at −20°C until further processing.

DNA was extracted from 0.5 g of each sample using FastDNA Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. The concentration and purity of extracted DNA were measured by Nanodrop 2000 spectrophotometer (Thermo Scientific, USA).

2.4 Amplicon sequencing and sequence processing

To investigate the composition of bacterial communities in soils and plants, the primer set 799F (5′-AACMGGATTAGATACCCKG-3′) and 1193R (5′-ACGTCATCCCCACCTTCC-3′) was used to amplify the V5-V7 region of the 16S rRNA gene. Each PCR reaction system was comprised of 1 μL template DNA, 4 μL FastPfu Buffer, 2 μL dNTPs, 0.8 μL forward primer, 0.8 μL reverse primer, 0.4 μL FastPfu polymerase, 0.2 μL BSA and 10.8 μL ddH_2O. The PCR program was as follows: 95°C for 3 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 45 s, and an extension at 72°C for 10 min. PCR productions were purified using DNA Purification Kit (TIANGEN, China), pooled and sequencing on the Illumina MiSeq PE300 sequencing platform (Illumina, USA).

Low-quality (Q < 20) and short sequences (length < 100 bp) were discarded and the remaining sequences were processed for downstream analysis using Quantitative Insights Into Microbial Ecology2 (QIIME2) pipeline. Sequences were assigned to amplicon sequence variants (ASVs) at 99% similarity threshold using DADA2 and the Greengenes database was used for ASVs taxonomic assignment. To ensure the quality of sequences for downstream analysis, ASVs containing less than 10 sequences, appearing in only one sample, or assigned to plant mitochondria or chloroplast, were discarded. After filtering, amplicon sequencing of bacterial 16S rRNA yielded an average of 2392 ASVs for soil samples, 461 ASVs for endosphere samples, and 227 ASVs for organic fertilizer samples. To focus on fertilization effects on microbiomes, we combine the sequencing data of the four plants and avoid host effects on microbiomes. Raw sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA985575. In this study, we selected 26 common plant-beneficial bacteria, which were reported to be able to exert one or several beneficial functions to host plants, including

growth promotion, nutrient uptake, pathogen suppression, and stress tolerance. The details of these beneficial bacteria are shown in Table S1.

2.5 Statistical analysis

Principal Component Analysis (PCA) and Adonis test were conducted in R 4.0.5. Ordinary least-squares analysis (OLS) was implemented using Excel 2016. OriginPro 2023 was used to draw column, stacked column, box and scatter plots. Venn diagrams showing the shared bacterial taxa between different samples were generated by an online tool (available at the website VIB/UGent−Bioinformatics & Evolutionary Genomic). To identify the taxa with significantly different abundance between fertilized and non-fertilized groups, Linear discriminant analysis Effect Size (LEfSe) was conducted using an online tool (available at the website OmicStudio). Source-tracking analysis was generated to quantify the potential contributions of soil microbes to endosphere microbes based on Bayesian approach in R 1.3.959. The co-occurrence networks based on Spearman correlation were generated on Gephi 0.9.2. In this study, we defined high-degree taxa as the taxa with the top 20% of degree, which are highly connected with other nodes (Zhao et al., 2019). The differences between and among different groups were carried out by the *t*-test and the single-factor analysis of variance (ANOVA), respectively, using SPSS 26.0. All differences were considered to be significant at $P < 0.05$. To identify direct and indirect effects of soil properties and bacterial diversity on beneficial bacterial abundance, the structural equation model (SEM) was generated using SPSS 26.0 and visualized in Amos Graphics 23. The model fit was evaluated by following criteria: Chi-square test ($P > 0.05$), goodness-of-fit index (GFI > 0.9) and the comparative fit index (CFI > 0.9) (Sun et al., 2021a). In this study, we evaluated the functionality of plant bacterial communities using the three metrics: the abundance of beneficial communities, the richness of beneficial communities and the average clustering coefficient of co-occurrence networks. This functionality index and soil physicochemical properties were standardized using Z score transformation (Chen et al., 2020b).

3 Results

3.1 The diversity and composition of bacterial communities

The organic fertilization significantly increased the richness of bacterial communities in soils (throughout the growth period for bulk soil and at D60 for the rhizosphere soil; $P < 0.05$), while with no significant impact on the bacterial community richness in the endosphere ($P > 0.05$; Fig. S2). The bacterial community richness in the endosphere was significantly lower compared to soils ($P < 0.05$). Notably, irrespective of different fertilization treatments and plant compartments, the richness of bacterial communities did not significantly change over time ($P > 0.05$; Table S2). Principal component analysis (PCA) combing with Adonis analysis was conducted to explore the impacts of plant compartments, plant growth time and fertilization treatments on the bacterial community composition (Fig. S3 and Table S3). The three compartments, i.e., bulk soil, rhizosphere soil and endosphere, had significantly different compositions of bacterial communities ($R^2 = 0.31$, $P < 0.001$) and also showed various responses to changes of plant growth time and fertilization treatments. From the soil to the endosphere, the effects of the growth time and fertilization treatments on bacterial communities were gradually weakened. Moreover, compared to plant growth time, bulk soil and rhizosphere soil were more strongly influenced by organic fertilizations, while for the endosphere, growth time had a greater influence on bacterial communities than organic fertilizations. Both of the bulk soil and rhizosphere soil, regardless of different growth time and fertilization treatments, were dominated by *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Actinobacteria* and *Acidobacteria* (Fig. S4a). For endosphere bacterial communities, *Proteobacteria* accounted for over 70%, followed by *Firmicutes* and *Actinobacteria*.

3.2 Effects of organic fertilization on the diversity and composition of beneficial communities

We next investigated how plant beneficial bacterial communities in soils and the endosphere responded to plant growth time and organic fertilization treatments. Similar to whole bacterial communities, the composition of beneficial communities in the bulk soil, rhizosphere soil and endosphere was significantly different ($R^2 = 0.247$, $P < 0.001$; Table 1 and Fig. S5). Meanwhile, both growth time and organic fertilization significantly affected the composition of beneficial communities in these three compartments, while these impacts on beneficial communities diminished from soils to the endosphere. In general, the richness of beneficial communities was significantly increased from 13 to 14 on average due to the organic fertilization ($P < 0.05$; Fig. 1A). The endosphere of plants that were not treated with organic fertilizers, harbored higher beneficial community richness than both the bulk soil and the rhizosphere soil (10 for the bulk soil, 12 for the rhizosphere soil and 14 for the endosphere, respectively). However, organic fertilization greatly increased the richness of beneficial bacterial communities in soils (from 10 to 14 for bulk soil and from 12 to 14 for the rhizosphere soil, respectively, $P < 0.001$), and no significant difference was seen in the richness of beneficial communities in the three compartments with organic fertilization ($P > 0.05$). The rich-

Table 1 The effects of fertilization treatments and growth time on beneficial communities in different compartments based on Adonis test.

	Fertilization		Time		Fertilization × Time	
	R^2	P	R^2	P	R^2	P
Bulk soil	0.425	0.001	0.136	0.001	0.019	0.246
Rhizosphere soil	0.292	0.001	0.135	0.001	0.026	0.498
Endosphere	0.049	0.001	0.064	0.001	0.035	0.030

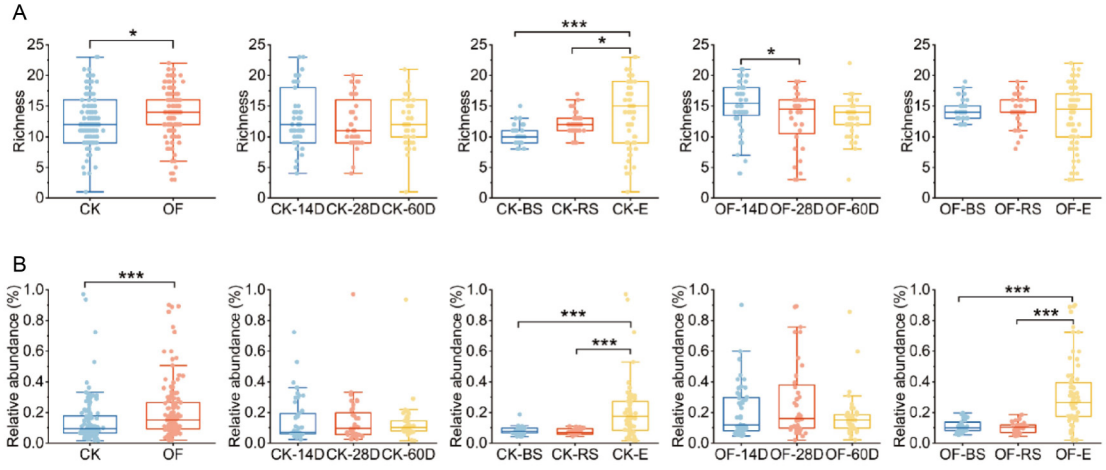


Fig. 1 The richness (A) and abundance (B) of beneficial communities in the bulk soil (BS), rhizosphere soil (RS), and endosphere (E) across different fertilization treatments and growth time. *t*-test and one-way ANOVA are used to test differences between groups. *P* values are indicated by *, eg., * represents $P < 0.05$ and *** represents $P < 0.001$.

ness of beneficial communities in fertilized groups witnessed a decline over time (from 15 at D14 to 13 at D28; $P < 0.05$). Notably, there was no beneficial bacteria detected in the organic fertilizer.

Overall, organic fertilization significantly increased the relative abundance of beneficial bacteria in plant–soil continuums ($P < 0.001$; Fig. 1B). The abundance of beneficial communities in the endosphere was greatly higher than that in soils ($P < 0.001$), while there was no significant difference in beneficial community abundance between the bulk soil and the rhizosphere soil ($P > 0.05$). Notably, the fertilization-caused fold change of beneficial community abundance in the three compartments was different, with the most pronounced rise in the endosphere (increased to 1.7 times; $P < 0.001$), followed by the rhizosphere soil (increased to 1.4 times; $P < 0.05$) and bulk soil (increased to 1.3 times; $P < 0.05$) (Fig. S7). This result suggests that although organic fertilization show a slight effect on the composition of whole bacterial communities in the endosphere, its impact on the abundance of endosphere beneficial communities is of significance. Besides, irrespective of different fertilization treatments, the abundance of beneficial communities in the three compartments did not significantly change over time ($P > 0.5$; Fig. 1B).

To identify the taxa which abundance was greatly different between non-fertilized groups and organic-fertilized groups, we conducted Linear discriminant analysis Effect Size (LEfSe) (Fig. S8). For both bulk soil and rhizosphere soil, there were six beneficial bacteria which abundance increased due to organic fertilization (the taxa sensitive to organic fertilization), i.e., *Devosia*, *Phenylobacterium*, *Cellulomonas*, *Flavobacterium*, *Pseudomonas* and *Pseudoxanthomonas*. For the endosphere, the abundance of the three beneficial taxa (*Devosia*, *Flavobacterium* and *Pseudomonas*), was significantly higher in OF groups than that in CK groups. Notably, these three beneficial taxa in the endosphere were included in the soil sensitive taxa.

3.3 Effects of organic fertilization on functions of beneficial bacteria in co-occurrence networks

We conducted the network analysis to explore changes of bacterial co-occurrence patterns in the bulk soil, rhizosphere soil and endosphere across different growth time and fertilization treatments (Fig. S9 and Fig. 2). We found that organic fertilization increased the number of nodes and edges in the networks of the three compartments, except for the networks in the endosphere at day 28 and day 60 (Fig. 2A and Fig. 2B). Also, the percentage of negative edges increased due to the organic fertilization across the whole plant growth periods (from 15.1% to 21.3% on average) (Fig. 2C). In general, the topology parameters, including

average degree, graph density and average clustering coefficient were higher in networks of OF groups than those in networks of CK groups, with average increases from 19.2 to 23.4 for the average degree, from 0.093 to 0.104 for the graph density and from 0.560 to 0.590 for the average clustering coefficient, respectively (Fig. 2D−F). We also investigated the number of high-degree nodes functioning beneficially to plants (Fig. 2G). We found that organic fertilization increased the number of high-degree beneficial nodes in networks of both bulk soil and rhizosphere soil (average from 4 to 8 for the bulk soil and from 4 to 7 for the rhizosphere soil, respectively), but decreased the number of those in networks of the endosphere (from 12 to 8 on average). Among these functional taxa, the number of high-degree taxa, associating with the nutrient cycling and the pathogen suppression, also witnessed an increase in the soil networks, while with a decrease in the endosphere networks due to the organic fertilization (Fig. 2H−I). However, for the endosphere, the percentage of high-degree taxa related to the pathogen suppression was higher in organic-fertilized networks than in non-fertilized networks (41.7% for non-fertilized networks and 48.0% for organic-fertilized networks, respectively).

3.4 Organic fertilization influencing beneficial bacteria mainly through changing soil properties

Organic fertilizations significantly increased the concentration of DOC, DTN, TC, TN and pH values, while decreased the C/N ratio of both the bulk soil and the rhizosphere soil throughout the whole plant growth period ($P < 0.05$; Fig. S1). We constructed the structural equation model (SEM) to explore the direct and indirect effects of soil properties and bacterial diversity on beneficial bacterial abundance of soils and the endosphere (Fig. 3A). Soil properties had a positive impact on the abundance of beneficial communities in both soils ($\lambda = 0.36$, $P < 0.001$) and the endosphere ($\lambda = 0.29$, $P < 0.01$). Moreover, the abundance of beneficial communities in soils was also positively affected by the diversity of soil bacteria communities ($\lambda = 0.27$, $P < 0.01$).

To further explore the relationships between soil properties (including pH, DOC, DTN, TC, TN and C/N ratio) and beneficial community abundance in soils and the endosphere, we conducted the correlation analysis based on ordinary least-squares analysis (Fig. 3B). For soils, DOC concentrations ($R = 0.330$, $P < 0.001$) had the strongest impacts on beneficial community abundance, followed by pH values ($R = 0.288$,

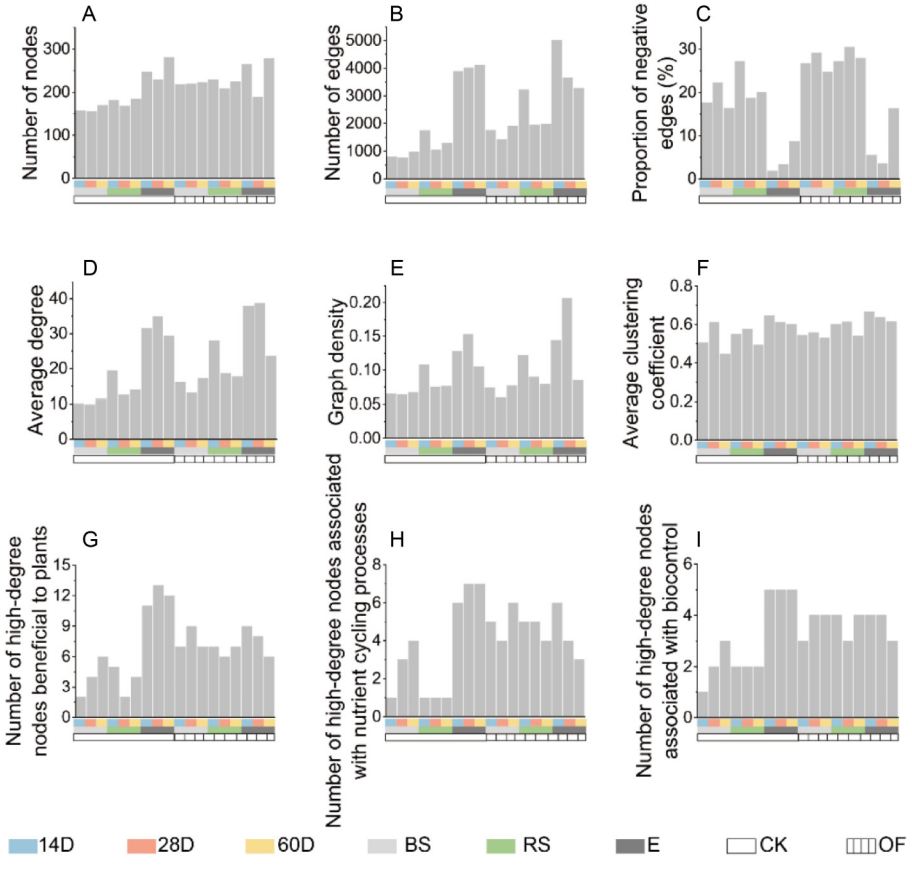


Fig. 2 The number of nodes (A), number of edges (B), proportion of negative edges (C), average degree (D), graph density (E) and average clustering coefficient (F) of bacterial co-occurrence networks in three compartments at different growth stages and with different fertilization treatments. The number of high-degree nodes performing beneficial functions for plants (G) and the number of high-degree nodes associated with nutrient cycling processes (H) and biocontrol (I).

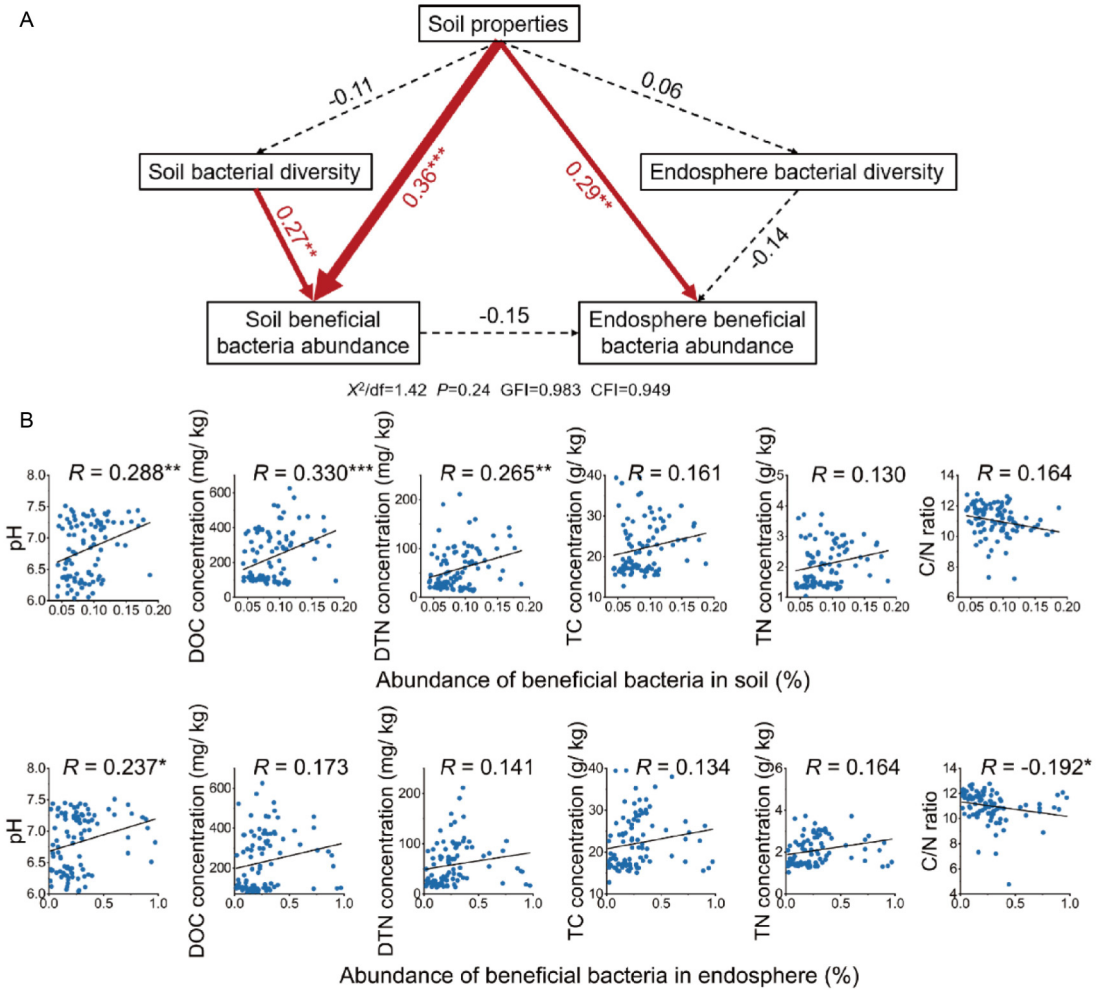


Fig. 3 The structural equation model showing direct and indirect effects of soil properties, soil bacterial diversity and endosphere bacterial diversity on abundance of soil and endosphere beneficial bacteria (A). The relationship between soil physicochemical properties and the abundance of beneficial bacteria in soils and the endosphere based on ordinary least-squares analysis (OLS) (B). *P* values are indicated by *, e.g., * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

$P < 0.01$) and DTN concentrations ($R = 0.265$, $P < 0.01$). For the endosphere, beneficial community abundance was also positive correlated with pH values ($R = 0.237$, $P < 0.05$). Different from soils, there was no significant correlation between beneficial abundance and nutrient concentrations in the endosphere, while with a weak negative correlation with C/N ratios ($R = -0.192$, $P < 0.05$).

3.5 Response of beneficial functionality to organic fertilization

To comprehensively evaluate the potential functions of plant bacterial communities, we constructed the functionality index containing the three metrics: the abundance of beneficial communities, the richness of beneficial communities and the average clustering coefficient of co-occurrence networks. We found that the organic fertilization significantly increased beneficial functionality of bacterial communities in plant–soil continuums ($P < 0.001$; Fig. 4A). Moreover, the ordinary least-squares (OLS) regression model showed that the beneficial functionality was positively corelated with soil physicochemical properties ($R = 0.455$, $P < 0.05$; Fig. 4C). Besides, we also found that the beneficial functionality index of endosphere bacterial communities was significantly higher than the indexes in both the bulk soil and the rhizosphere soil (Fig. 4B).

3.6 Potential contributions of soil microbes to endosphere beneficial bacteria

To determine the potential contributions of soil microbes to endosphere microbes, source-tracking analysis was generated based on a Bayesian approach (Fig. 5A). For the whole communities, most of bacteria (86.1%) in the rhizosphere soil originated from bulk soil, with 5.3% of bacteria in the rhizosphere soil subsequently flowing to the endosphere. For the beneficial communities, bulk soil contributed only 22% to the rhizosphere soil, while with a higher percentage flowing from the rhizosphere soil to the endosphere (12.2%)

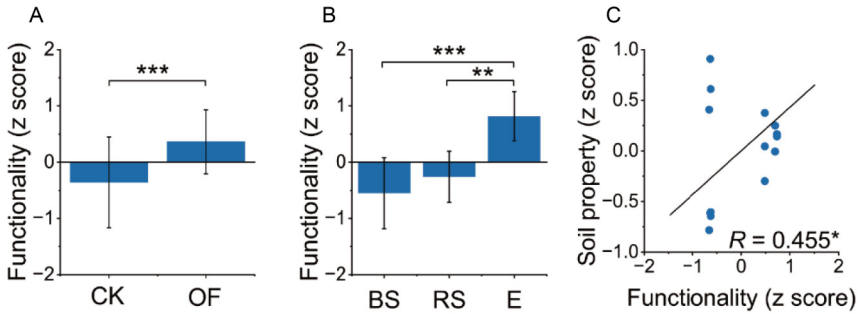


Fig. 4 Beneficial functionality index in response to different fertilization treatments (A) and plant compartments (B). *t*-test and one-way ANOVA are used to test differences between groups. The relationship between beneficial functionality index and soil physicochemical properties fitted by the ordinary least-squares (OLS) regression model (C). *P* values are indicated by *, e.g., * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

compared to the whole communities. We also explored the shared and specific bacterial taxa in the bulk soil, rhizosphere soil and endosphere (Fig. 5B). For the whole communities, there were 62 of 236 genera in the bulk soil and 64 of 239 genera in the rhizosphere soil shared with the endosphere, accounting for 26.3% in the bulk soil and 26.8% in the rhizosphere soil, respectively. However, for the beneficial communities, 8 of 12 genera (accounting for 66.7%) in bulk soil and 10 of 14 genera (accounting for 71.4%) in the rhizosphere soil were shared with the endosphere. This was consistent with the results of source-tracking analysis, indicating that compared with other bacteria, plants prefer to internalize beneficial bacteria.

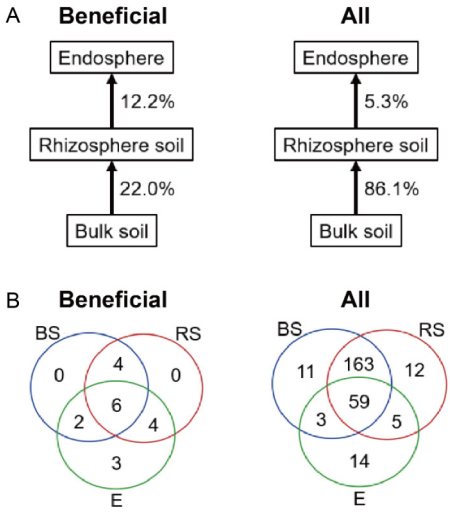


Fig. 5 Source-tracking analysis showing the potential sources of beneficial and all bacterial communities in different compartments (A). Venn diagram showing the shared and specific taxa at a genus level between the bulk soil (BS), the rhizosphere soil (RS) and the endosphere (E) (B).

4 Discussion

The composition and abundance of plant-beneficial communities, and their interactions with other microbes are of great importance for their functions performing in plant–soil continuums (Kwak et al., 2018; Mendes et al., 2018; Deng et al., 2022). Understanding the responses of beneficial communities (traits: growth promotion, pathogen suppression, and stress tolerance) to anthropogenic disturbance is the first step toward predicting and manipulating the assembly of beneficial communities. By conducting a microcosm experiment with two different groups, we found that organic fertilization could significantly increase the abundance of beneficial communities in plant–soil continuums and may greatly enhance the plant health, which could be a guidance for the crop production.

4.1 The relationship between soil communities and endosphere communities

Source tracking analysis showed that a fraction of bacteria in the plant endosphere derived from soils (Fig. 5). Soils have been widely considered to be a vital source for plant microbial selection, with specific bacteria in soils able to enter to plants, and become endophytes (Compant et al., 2005; Xiong et al., 2021a). These endophytes can have beneficial, neutral or potentially harmful impacts on host plants (i.e., phytopathogens) (Adeleke and Babalola, 2021). However, we showed that plants prefer to selectively internalize the bacteria that perform beneficial functions. It can be explained by the three steps for plant uptaking bacteria. First, primary and secondary metabolites in root exudates can attract specific microbes that potentially offer beneficial functions (Vives-Peris et al., 2020). Extensive research has shown that legumes release specific root exudates such as flavonoids, which can attract Rhizobiaceae bacteria to root hairs for nitrogen fixation (Caetano-Anollés et al., 1988). For another example, L-malic acid secreted from *Arabidopsis* roots is able to specifically recruit and enrich *Bacillus subtilis* FB17 in the rhizosphere, which is beneficial bacteria with an ability to suppress pathogens (Rudrappa et al., 2008). Secondly, beneficial bacteria often display high competitive-

ness in successfully colonizing the rhizosphere or rhizoplane, outcompeting other microbes by secreting secondary metabolites, such as antibiotics (Compant et al., 2010). Thirdly, some bacteria are capable to enter plants in a similar way as pathogenic bacteria. For instance, *Bradyrhizobium elkanii* which can form nitrogen-fixing nodules on soybean roots, are capable to enter plants using type 3 secretion systems (T3SSs), which are known to deliver virulence factors by pathogenic bacteria (Okazaki et al., 2013). Additionally, even in the absence of T3SSs, with the enrichment of beneficial bacteria in the rhizoplane, the likelihood of beneficial bacteria passively penetrating plants through root wounds is expected to increase (Compant et al., 2010).

4.2 Effects of organic fertilization on beneficial communities

In our study, we observed that beneficial communities in soils showed a stronger response to organic fertilizers compared to those in the endosphere (Table 1). This finding is consistent with the previous research that fertilization can significantly alter the composition of bacterial communities in soil, but has a weaker impact on bacterial communities in the endosphere (Trivedi et al., 2020; Li et al., 2022). This difference can be attributed to the fact that endophytes are under highly selective pressure caused by the host immune systems, which has a greater impact on the assembly of endosphere microbiomes even than the external disturbance (Xiong et al., 2021b). We also observed that the richness and abundance of beneficial communities in soils significantly increased under organic fertilization (Fig. 1). In this study, organic fertilization led to significant improvement in the soil physicochemical properties, e.g., pH, DOC and DTN. These changes are likely to create more favorable survival environments for certain beneficial bacteria. For instance, with an increase of the soil pH value via organic fertilization, the relative abundance of beneficial bacteria (including *Bacillus*, *Paenibacillus*, *Flavobacterium* and *Pseudomonas*) rises up in the tobacco rhizosphere, with a decline in the abundance of *R. solanacearum* correspondingly (Zhang et al., 2022b). Regarding the endosphere, organic fertilization also led to an increase in the abundance of beneficial communities, and this enrichment was more pronounced compared to soils (Fig. S7). Soil serves as a vital source of beneficial communities for the endosphere, and with the increase of beneficial bacteria in the rhizosphere resulting from improvements in soil conditions, the likelihood of beneficial bacteria entering plants also increases accordingly. Moreover, nutrient inputs can generally improve plant physiologic parameters, such as carbon and nitrogen concentrations, which may provide a better condition for beneficial communities to survive and proliferate in the endosphere (Ren et al., 2011). We also found that the richness of beneficial communities in the fertilized plant–soil continuum declined over time. This could be explained that some beneficial bacteria, such as the bacteria associated with the N-fixation process, are highly-sensitive to the nitrogen input and might gradually decline in the eutrophic environment (Sun et al., 2021b).

There were six beneficial genera that significantly enriched in plant–soil continuums attributing to organic fertilizations (*Devosia*, *Phenylobacterium*, *Cellulomonas*, *Flavobacterium*, *Pseudomonas* and *Pseudoxanthomonas*). The enrichment of beneficial bacteria is likely associated with the specific matters in organic fertilizers. *Pseudomonas* was one of the most abundant beneficial taxa in plant–soil continuums, accounting for 0.11% in soils and 11.70% in the endosphere respectively. *Pseudomonas* is well-known as a biocontrol agent, because of its competitiveness against other microbes and ability to colonize a wide range of niches in plant–soil continuums (Vorholt, 2012; Trivedi et al., 2020; Zhang et al., 2020). The recent research indicated that specific nutrients in organic fertilizers, such as L-arginine, cannot be metabolized by pathogens, e.g., *Ralstonia solanacearum*, but are able to promote the growth of *Pseudomonas* and its suppression of *R. solanacearum* (Zhang et al., 2022b). *Devosia* is the other abundant bacteria exhibited amplified abundance under organic fertilization. With *nodD* and *nifH* genes, *Devosia* spp. can promote nodule formation and nitrogen fixing for plants (Rivas et al., 2002). In a long-term N fertilization field experiment, the abundance of *Devosia* significantly increased with N fertilization and positively correlated with the concentration of organic acids in the rhizosphere soil (Chen et al., 2019). All these suggests that organic fertilization can enhance the growth of beneficial communities in plant–soil continuums, and could be an efficient strategy for promotion of plant health.

4.3 Effects of organic fertilization on the beneficial functionality

We found that the topology parameters, including average degree, graph density and average clustering coefficient, were higher in networks of organic-fertilized groups than those in networks of non-fertilized groups (Fig. 2). This was consistent with the study that compared to the control group, the clustering coefficient of the soil microbial network, including bacteria, fungi and protists, increased with the nitrogen fertilizer addition (Zhao et al., 2019). It could be explained by nutrient inputs through fertilizer application. In our study, a huge amount of carbon and nitrogen was added to soils via organic fertilization, which can increase resource and food availability to microbes in plant–soil continuums, subsequently tighten the interactions between microbes and increase the complexity of networks (Zhou et al., 2011; Shi et al., 2016; Zhao et al., 2019). High-complexity networks are generally considered to be stable for external disturbances, indicating that the organic fertilization is able to

strengthen microbial interactions and improving resistance against pathogen invasions.

In addition, several beneficial bacteria, that also functioned as keystones in networks of plant–soil continuums, have been found in our study. We showed that the number of beneficial bacteria in soils that functioned as keystones in networks increased with the organic fertilization (Fig. 2). Most of these beneficial bacteria are copiotrophic, such as *Pseudomonas* belonging to *Proteobacteria* and *Flavobacterium* belonging to Bacteroidetes (Chen et al., 2023b). This trend was consistent with previous studies that exogenous nutrient inputs can increase the growth and activity of some copiotrophic microbes in soil, including *Proteobacteria*, *Bacteroidetes* and *Firmicutes* (Chen et al., 2023b; Luo et al., 2023). Since copiotrophs are regarded to higher growth rates and lower substrate affinities than oligotrophs, they potentially interact more often and tightly with other microbes, thus likely playing a greater role in soil networks (Chen et al., 2023a). With regard to the endosphere, we found that the percentage of high-degree taxa in the endosphere, related to the pathogen suppression, was higher in organic-fertilized networks than in non-fertilized networks. Organic fertilizers have been indicated to promote the growth of bacterial communities in soil, especially for some copiotrophs. With high growth rates and abilities to secrete antimicrobial compounds, these bacteria can directly compete against or antagonize pathogens, thus protecting plants from diseases (Mendes et al., 2011; Deng et al., 2022).

To comprehensively evaluate the beneficial functionality of plant microbiomes, we integrated the three metrics, i.e., the abundance of beneficial communities, the richness of beneficial communities and the average clustering coefficient of co-occurrence networks (Fig. 4). Our results showed that organic fertilization could enhance the beneficial functionality of plant microbiomes, suggesting that organic fertilization can alleviate the negative impacts of agricultural practices on farmland ecosystems (Chen et al., 2020b). Though, organic fertilizers, especially for animal manures, inevitably harbor microbes that are potentially pathogenic to human, such as *Salmonella* and *Escherichia coli*, the increase in the beneficial functionality of indigenous communities induced by organic fertilization could reduce the risks of these human pathogens (Li et al., 2022). Thus, we consider that organic fertilization could be a green and sustainable strategy, with abilities to both promote the plant growth and the soil conditions.

5 Conclusions

In conclusion, this study indicated that organic fertilization can increase both of abundance and richness of beneficial communities in plant–soil continuums, via optimizing soil physicochemical conditions (pH, DOC, DTN and C/N ratio). This enrichment, induced by organic fertilization, is more obvious in the endosphere compared to soils. Furthermore, organic fertilization can increase the complexity of bacterial co-occurrence networks in soils and endosphere, and the functions of high-degree taxa in the endosphere shifted to pathogen suppression. Besides, compared to other bacteria, plants prefer to selectively internalize the bacteria that perform beneficial functions. In sum, our work provides empirical support that organic fertilization can enhance the beneficial functionality of plant and soil microbiomes and be a vital strategy for improvements in both agricultural productivity and environmental sustainability.

Declaration of competing interest

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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