Effects of Inorganic, Organic and Bio-Organic Fertilizer on Growth, Rhizosphere Soil Microflora and Soil Function Sustainability in Chrysanthemum Monoculture

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Abstract: The production of chrysanthemums is severely hampered by Fusarium wilt, which is exacerbated by monoculture. In this study, the role of inorganic plant nutrition fertilizer (IPN), organic fertilizer (OF) and bio-organic fertilizer (BOF) in avoiding monoculture-related production constraints was evaluated. We conducted a series of greenhouse experiments and studied the growth of chrysanthemum and changes in rhizosphere soil microflora and function. BOF application reduced the incidence of Fusarium wilt by 82.8% and increased the chrysanthemum shoot height and flower ray floret number by 31.4% and 26.1%, respectively. High-throughput Illumina HiSeq2500 sequencing results indicated that BOF and OF treatments increased the values of α-diversity indices of bacteria and fungi. In addition, significant alterations in microbe community structures were found in response to IPN, OF or BOF application. Among the major genera detected after BOF treatments, the levels of Fusarium and Glycomyces decreased while Cladosporium, arbuscular mycorrhizal and endophyte groups increased. In particular, the abundance of Mariniflexile had a positive relationship (R = 0.693, p < 0.05) with the incidence of Fusarium wilt, while Cladosporium showed a significant negative relationship (R = −0.586, p < 0.05). Interestingly, an analysis of microbiomes based on 16S rRNA sequences revealed that the functions of signal transduction, bacterial secretion system, oxidative phosphorylation and the metabolism of carbohydrate, nitrogen and amino acids all increased in both BOF and OF treatments. The results suggested that BOF could be effective for chrysanthemum monoculture soil restoration, potentially by altering the microbial community structures and functions, which affect the physiological and morphological attributes of chrysanthemum in monoculture.

Keywords: chrysanthemum; biofertilizer; Fusarium wilt; soil microbiome; microbial diversity

1. Introduction

The chrysanthemum (Chrysanthemum morifolium Ramat.) is one of the most economically valuable ornamental plants worldwide [1]. It displays a wide range of variability among its cultivars, with many different colors, sizes and flower patterns. However, intensive, year-round cultivation in a closed environment typically results in a rapid decline in soil fertility along with a deterioration in soil physical properties. Hence, the growth and quality of chrysanthemums reduce, while the widespread disease Fusarium wilt [2], caused by a soil-borne pathogen (Fusarium oxysporum f. sp. Chrysanthemum) often increases [3,4].

The optimized application of inorganic plant nutrition fertilizer (IPN) [5], organic fertilizers (OF) or bio-organic fertilizers (BOF) is an important element in chrysanthemum cultivation for plant growth, soil function sustainability, soil-borne disease control and relief...
of monoculture cropping obstacles [2,4,5]. Many researchers have demonstrated that an inorganic plant nutrition fertilizer such as fly-ash increases the yields of various crops and improves the physical and chemical characteristics of the soil [6,7]. Inorganic plant nutrition fertilizer formulations have both soil amending and nutrient enriching properties, which are helpful in enhancing crop growth and yield in low-fertility soils [8]. Silicon–calcium–potassium–magnesium (SCPM) fertilizer is prepared by calcining a mixture of calcium or magnesium-based desulfurized flue gas residue, potassium feldspar, dolomite and so on. Hence, the SCPM fertilizer is rich in available Si, Ca, K, Mg and other micro-nutrients, which can be used as a kind of slightly alkaline soil conditioner, not only improving crop productivity and soil fertility, but also mobilizing macro- and micro-nutrients in the soil [9].

Organic fertilizer application has long been considered important, partially replacing inorganic nitrogen and affording a more sustainable release of nutrients [10]. Its application can promote increased size and volume of plant roots, allowing the plants to obtain greater amounts of water and nutrients [11,12]. It is widely reported that the application of organic fertilizer not only improves soil organic matter content but also provides macronutrients essential for crop growth [13]. In addition, many microbial strains have been isolated from the rhizospheres of various plants and used as inoculants to improve the growth (growth promoting rhizobacteria, PGPR) and health (antagonistic microbes) of other plants. Important genera include Azospirillum [14], Enterobacter [15], Pseudomonas [16], Bacillus [17,18] and Trichoderma [19].

The incorporation of PGPR or antagonistic microbes into organic fertilizers to obtain BOF formulations provides a low cost, environmentally friendly and sustainable agronomic option as they contribute effectively to the mobilization, mineralization and recycling of nutrients [20], increase plant productivity [21] and also prevent soil-borne diseases (Fusarium wilt disease) by modifying soil microbial properties [19].

Soil microorganisms play an important role in the soil nutrient biogeochemical cycle [22] and ecosystem functioning [23] and are also important indicators of soil quality and soil health. Soil microbial community and function are highly complex and dynamic with variations in composition both spatially and temporally and are sensitive to changes in the environment caused, for example, by tillage [24], fertilizer [25], season [26] and plant type [27]. Field studies have shown that management strategies with OF or BOF [28] maintain stable soil structure, improve soil nutrients, build up organisms antagonistic to pathogens [29] and improve soil microbe communities and diversity. Furthermore, amendment with BOF is reported to increase microbial metabolic activity [3] and decrease soil-borne disease incidence in agricultural systems [12].

The application of either IPN or OF/BOF to chrysanthemum monoculture soil is a management strategy commonly used by farmers to improve soil quality and reduce soil-borne diseases. However, little information exists on the influence of different fertilizer management strategies on core soil microbiome community structure and function. In particular, metagenomic analysis of the rhizosphere soil of a continuous chrysanthemum cropping system soil has not been carried out.

The objectives of this study were as follows: (1) to compare and determine how IPN, OF and BOF affect the composition, diversity and functions of soil microbes, and (2) to better understand their impact mechanisms on the soil microbiome of monocultured chrysanthemums.

2. Materials and Methods

2.1. Site Description and Plant Material

The experiment was initiated in June 2018 and the experiment site was located at the Chrysanthemum Germplasm Resources Conservation Center (Nanjing, China) of Nanjing Agricultural University. Prior to the experiment, the field had an eight-year history of continuous chrysanthemum monoculture. The soil (sandy loam) had pH 6.43, EC 467.7 µS·cm⁻¹, organic C 26.20 g·kg⁻¹, available N 0.08 g·kg⁻¹, available K 0.10 g·kg⁻¹ and available P 0.06 g·kg⁻¹. Seedings of the chrysanthemum cultivar Jinba (provided by
Honghua Horticulture Co. Ltd., Shanghai, China) were established in a greenhouse by growing cuttings in perlite for three weeks under the 16 h photoperiod and day/night temperature regime of 28 °C/22 °C.

2.2. Preparation of IPN, OF and BOF

The OF used for the experiment was composted chicken manure purchased from Nanjing Pearl Fertilizer Co. Ltd. (Nanjing, China), which contained 45% organic matter, 2.0% nitrogen, 1.6% P and 1.2% K. BOF was provided by the Jiangsu Provincial Key Laboratory of Organic Solid Waste Utilization. This consisted of a 1:1 mixture of processed oil rapeseed cake and pig manure compost and contained 30.4% organic matter, 2.0% nitrogen, 3.7% P as phosphorus pentoxide and 1.1% potash. *Paenibacillus polymyxa* (strain SQR21) showed high antagonism to *F. oxysporum* [30] and was added to the BOF at a rate of ~5.0 × 10⁹ colony forming units per gram. The IPN (Silicon–Calcium–Potassium–Magnesium fertilizer soil conditioner) was purchased from Sinoma Technology and Equipment group Co., Ltd. (Tianjin 300400, China). The IPN had a pH of 9.0–11.0 and contained SiO₂ ≥ 27.0%, CaO ≥ 25.0%, K₂O ≥ 4.5%, MgO ≥ 4.0%. In addition, it also contained Fe, Zn, B, Mo, Se and other trace elements.

2.3. Experimental Design

Three fertilizer regimes were set up, plus an untreated control, each in triplicate: (1) control, (2) BOF (1.50 kg bio-organic fertilizer per m²), (3) IPN (0.12 kg inorganic plant nutrition fertilizer per m²) and (4) OF (1.50 kg organic fertilizer per m²). The greenhouse experiment was laid out in a randomized complete block design, and each plot had an area of 1.6 m × 1.2 m planted with 48 rooted cuttings. The application amount of treatment was mainly based on the results of a pre-test.

2.4. Chrysanthemum Growth Study and Disease Incidence

Measurements were taken of shoot height and diameter, shoot dry weight, leaf width and length, root fresh and dry weight and flower ray floret number. For these measurements, twelve plants were sampled randomly from each replicate at flowering time (110 days after transplanting). The wilt symptoms were observed in the field and the disease incidence score for each plot was calculated at the same time from the ratio of infected to non-infected plants.

2.5. Soil Sampling of Chrysanthemum Monoculture

Three soil samples were taken from each plot 110 days after transplanting, which was the flowering stage of the chrysanthemum. Whole plants were uprooted, the plant roots were carefully shaken and the trace soil attached to the root was collected. Then, the soil samples were stored at −80 °C for the analysis of microbial community.

2.6. DNA Extraction and PCR Amplification

Genomic DNA was extracted from 250 mg rhizosphere soil samples using a Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA). The concentration and integrity of the resulting DNAs were determined using a NanoDrop 2000 UV spectrometer. Pyrosequencing analyses of the 16S rRNA gene and ITS2 regions were performed to determine the diversity and composition of bacterial and fungal communities, respectively. The V3 + V4 region of the bacterial 16S rRNA gene was amplified using the gene specific primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAAT-3′); the ITS2 region of the fungal DNA was targeted by the primers KYO2F (5′-GATGAAGAACGYAGYRAA-3′) and ITS4R (5′-TCTCTCCGCT TATGGATATGC-3′). The barcode was an eight-base sequence unique to each sample. PCR amplifications of the bacterial 16S rRNA and fungal ITS sequences were performed in triplicate on 50 µL mixture containing 5 µL of 10 × KOD buffer, 5 µL of 2.5 mM dNTPs, 1.5 µL of each primer (5 µM), 1 µL of KOD polymerase and 100 ng of template DNA. Amplicons were extracted from 2%
agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer’s instructions and quantified using an ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, CA, USA).

2.7. Illumina HiSeq Sequencing and Data Analysis

Purified amplicons were pooled equimolarly and paired-end sequenced (2 × 250) on an Illumina platform according to the standard protocols. All library preparation was performed on the Illumina HiSeq2500 platform at Genedenovo Biotechnology Co., Ltd. (Guangzhou, China). Raw reads were filtered using FASTP. Paired-end clean reads were merged as raw tags using FLASH (version 1.2.11), and noisy sequences of raw tags were filtered by the QIIME (version 1.9.1) pipeline under specific filtering conditions to obtain high-quality clean tags. Clean tags were searched against the reference database to perform reference-based chimera checking using the UCHIME algorithm. All chimeric tags were removed and the final effective tags were used for further analysis. The effective tags were clustered into operational taxonomic units (OTUs) of ≥97% similarity using the UPARSE pipeline. A representative sequence from each OTU was selected and Ribosomal Database Project (RDP) classifiers (the RDP Bacterial 16S database for 16S rRNA data and the UNITE Fungal ITS database for ITS data) were used for taxonomy characterization.

2.8. Statistical Analyses and Data Accessibility

The necessary computations were carried out using routines implemented in Microsoft Excel 2017, and the statistical analyses of all parameters were performed using the IBM SPSS statistical software package version 20 (IBM Corporation, New York, NY, USA). The data from each treatment were analyzed by one-way analysis of variance (ANOVA), and the Duncan multiple range test was used to assign significance to differences (p < 0.05) between treatment means. Principal component analyses (PCA) were performed in R with the vegan package (version 3.0.2). Differences in bacterial and fungal communities between treatments were tested by analysis of similarities (ANOSIM). KEGG pathway analysis of the bacterial OTUs was inferred using Tax4Fun (version 1.0) and the fungal OTUs were inferred using FUNGuild. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA573797).

3. Results

3.1. Disease Incidence and Growth Indexes of Chrysanthemum

IPN, OF and BOF applications all significantly decreased the incidence of Fusarium wilt of chrysanthemum when compared with the control (Figure 1). The highest incidence of Fusarium wilt, 20.1%, was found in the control, while the incidences of Fusarium wilt in IPN and OF treatments were 13.2% and 11.8%, respectively. The lowest was found in the BOF treatment, at 3.5%, which was a decrease of 82.8% when compared with the control.

Compared to the control, all the evaluated treatments not only promoted the growth of chrysanthemum but also clearly improved its quality (Table 1). The greatest shoot height, shoot fresh weight, dry weight, leaf width and leaf length were all found in the BOF-treated plants; there were no significant differences of shoot diameter between BOF, IPN and OF treatments. The indices of fresh weight and dry weight of chrysanthemum root were highest for the BOF treatment, followed by the OF and IPN treatments. Meanwhile, the flower ray floret number was increased by all the treatments, and by 26.1% in the BOF treatment compared to the control.
3.2. α-Diversity of Soil Microbiomes

α-Diversity analyses of the soil microbiomes were performed using the OTUs versus the sequences obtained in each treatment. Venn diagrams (Figure 2) show that 690, 434, 1004 and 897 unique bacterial OTUs were obtained from the control, IPN, OF and BOF treatments, respectively; 42, 41, 75 and 69 unique fungal OTUs were found in the control, IPN, OF and BOF. Compared to the control, OF and BOF application increased the unique OTUs of bacteria and fungi, while the number of unique OTUs of soil bacteria and fungi both decreased in the IPN-treated soil.

Richness estimators (Chao1 and ACE) of bacteria were significantly increased in the BOF treatment when compared to the control (Table 2), and there were no significant differences between OF and BOF. The diversity estimators (Shannon–Weaver and Simpson indices) varied among different treatments and the highest indices were recorded for the BOF treatment when compared to the control (Table 2), and there were no significant differences between OF and BOF. Compared to the control, the richness estimator for fungi was increased in the OF and BOF treatments but decreased significantly in the IPN treatment. In addition, the Simpson indexes for bacteria and fungi were positively correlated with both root fresh weight (R = 0.964, p = 0.036) and dry weight (R = 0.973, p = 0.027).
Table 1. The effects of the various soil treatments on plant growth as measured at the time of flowering.

| Treatment | Shoot | Leaf | Root | Flower |
|-----------|-------|------|------|--------|
|           | Height (cm) | Diameter (mm) | Fresh Wt (g/Plant) | Dry Wt (g/Plant) | Width (cm) | Length (cm) | Fresh Wt (g/Plant) | Dry Wt (g/Plant) | Ray Floret Number (No.) |
| Control   | 80.08 ± 2.17 d | 4.15 ± 0.04 b | 75.70 ± 2.54 c | 22.67 ± 0.64 c | 1.47 ± 0.06 b | 2.64 ± 0.02 b | 2.81 ± 0.02 b | 0.55 ± 0.06 c | 162.00 ± 2.65 d |
| OF        | 99.04 ± 1.23 b | 4.69 ± 0.05 a | 94.26 ± 2.04 a | 26.20 ± 0.51 b | 1.63 ± 0.04 b | 3.14 ± 0.02 a | 2.85 ± 0.04 b | 0.88 ± 0.00 a | 172.36 ± 2.37 c |
| IPN       | 91.54 ± 2.42 c | 4.59 ± 0.12 a | 84.70 ± 1.72 b | 24.09 ± 0.98 bc | 1.60 ± 0.03 b | 2.88 ± 0.10 b | 2.68 ± 0.03 c | 0.67 ± 0.02 b | 184.33 ± 1.15 b |
| BOF       | 105.21 ± 0.59 a | 4.74 ± 0.04 a | 98.63 ± 0.80 a | 30.18 ± 0.28 a | 1.96 ± 0.07 a | 3.32 ± 0.10 a | 3.43 ± 0.07 a | 0.86 ± 0.02 a | 204.33 ± 4.01 a |

Each datum is given as mean ± SE (n = 3). Different letters indicate significant differences (p < 0.05) between treatments.

Table 2. α-Diversity indices for the bacterial and fungal components of the rhizosphere soil microbiome under different soil treatments.

| Microbe | Treatment | Chao1 | ACE | Shannon–Weaver | Simpson |
|---------|-----------|-------|-----|----------------|---------|
| Bacteria| Control   | 5715.72 ± 19.34 b | 5714.33 ± 23.56 b | 9.51 ± 0.02 b | 0.994 ± 0.001 b |
|        | OF        | 6048.60 ± 29.30 ab | 6042.41 ± 31.05 ab | 9.51 ± 0.01 b | 0.993 ± 0.000 b |
|        | IPN       | 5643.82 ± 65.03 b | 5672.93 ± 10.39 b | 9.07 ± 0.06 c | 0.992 ± 0.001 c |
|        | BOF       | 6441.25 ± 42.48 a | 6420.13 ± 34.76 a | 10.06 ± 0.02 a | 0.997 ± 0.000 a |
| Fungi   | Control   | 279.82 ± 0.97 bc | 298.55 ± 1.25 bc | 4.36 ± 0.06 c | 0.898 ± 0.006 b |
|        | OF        | 315.26 ± 6.06 a | 332.25 ± 4.89 a | 4.49 ± 0.04 b | 0.918 ± 0.001 a |
|        | IPN       | 270.61 ± 13.16 c | 271.82 ± 13.84 c | 4.21 ± 0.02 d | 0.901 ± 0.001 b |
|        | BOF       | 308.74 ± 10.41 ab | 316.33 ± 7.36 ab | 4.65 ± 0.04 a | 0.913 ± 0.001 a |

Each datum is given as mean ± SE (n = 3). Different letters indicate significant differences (p < 0.05) between treatments.
3.3. β-Diversity of Soil Microbiomes

Principal component analysis (PCA) revealed significant differences in soil microbial communities across treatments; the control, OF, IPN and the BOF treatments were clearly separated along PCA1 for both bacterial and fungal communities (ANOSIM, control vs. BOF vs. IPN vs. OF, p < 0.001) (Figure 3). For bacteria, PCA based on weighted UniFrac distances clearly separated the BOF treatment from IPN and OF (ANOSIM, BOF vs. IPN vs. OF, p < 0.001) along PCA2, whereas the IPN and OF treatments grouped tightly together. For fungi, BOF, IPN and OF treatments showed significant differences based upon the weighted UniFrac distances (ANOSIM, BOF vs. IPN vs. OF, p < 0.001) along PCA1.

![Figure 3. The structure of the rhizosphere soil microbiome as affected by the various soil treatments. PCA of (a) the bacterial and (b) the fungal components.](image)

3.4. Taxonomic Composition of Soil Microbiome

IPN, OF and BOF treatments influenced the phylogenetic composition and community structure of the soil microbiomes. The overall taxonomic complexity of the microbial community at the phylum level is presented in Figure 4. Proteobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes and Planctomycetes were the five most abundant groups across all samples, accounting for more than 75% of the total bacterial sequences (Figure 4a). Actinobacteria were highly abundant in OF, IPN and the BOF treatments, while Proteobacteria were the most abundant in the IPN treatment. Ascomycota, Mortierellomycota and Basidiomycota were the top three most abundant fungal phyla across all treatments (Figure 4b). BOF, OF and IPN application decreased the relative abundance of Basidiomycota and Mortierellomycota, while BOF application increased the relative abundance of Glomeromycota.

![Figure 4. The relative abundance of microbial phyla in the rhizosphere soil as affected by the various soil treatments. (a) Bacterial phyla, (b) fungal phyla. ‘Others’ are low abundance (<0.5%) phyla.](image)

Based on the ten most abundant bacterial and fungal genera, all treatments tested showed different patterns of bacterial and fungal community structure. Fold-change cal-
Calculations were further used to visualize the variations of genera in OF, IPN and BOF. In bacteria, the relative abundances of *Nocardioides* and *Streptomyces* increased while *Mariniflexile* (Figure 5c) decreased significantly in all treatments compared to the control (Figure 5a,b); BOF application decreased the abundance of *Glycomyces* (Figure 5d) significantly. In fungi, the relative abundance of *Arthrobotrys* decreased in all treatments while the abundance of *Cladosporium* increased significantly in BOF (Figure 5e,f). OF and BOF application significantly reduced the abundance of *Fusarium* (Figure 5g), and IPN treatment reduced the relative abundance of *Devriesia* compared to the other treatments (Figure 5h).

The genera of bacteria and fungi that showed the greatest fold-changes were compared in a linear regression model. The results showed that the relative abundance of *Mariniflexile* in the soil sampling of the chrysanthemum monoculture greenhouse had a significantly positive relationship ($R = 0.693, p = 0.012$) with the incidence of *Fusarium* wilt disease (Figure 6), while the relative abundance of *Cladosporium* had a significantly negative relationship ($R = -0.586, p = 0.045$). However, there were no significant relationships between the abundances of *Nocardioides, Streptomyces, Mariniflexile, Glycomyces, Arthrobotrys* and *Devriesia* and the incidence of *Fusarium* wilt disease.
Figure 6. Linear regression analysis illustrating the correlation of the incidence of *Fusarium* wilt with the relative abundance of (a) *Cladosporium*, (b) *Mariniflexile* and (c) *Fusarium* in the soil sampling of chrysanthemum monoculture greenhouse.

3.5. Functional Predictions of Microbial Community Structure

Functional profiles that were predicted from 16S rRNA genes are shown in Figure 7a. Soil microbiome functions increased in the BOF and OF treatments compared to the control, which was related to signal transduction, bacterial secretion system, metabolism of carbohydrate, nitrogen and amino acids and oxidative phosphorylation. The soil microbiome functions that increased in the IPN treatment were related to membrane transport and metabolism of pyruvate, cofactors, amino acids and carbohydrate. Soil microbiome functions related to metabolism and to ribosome and aminoacyl-tRNA biosynthesis were highest in the control. ITS2 gene-predicted functional profiles revealed that arbuscular mycorrhizal and endophyte classes were significantly increased under BOF treatment, while soil saprotrophs were obviously decreased under BOF compared with other treatments (Figure 7b).

Figure 7. Gene-predicted functional profiles obtained with (a) Tax4Fun and (b) FUNGuild.
4. Discussion

The application of OF, BOF or IPN all improved the growth of chrysanthemums, with the best results in greenhouse experiments being from soil treated with BOF. Previous studies have shown that bio-organic fertilizer is beneficial to plant growth and disease resistance by enhancing the uptake of plant nutrients [31], secreting growth hormones [32] and changing the root microbiome [33]. In this study, the results indicate that, compared to IPN and OF, BOF treatment significantly reduced the incidence of *Fusarium* wilt in chrysanthemums. This is in agreement with the results of Lin et al. [30] who showed that the addition of BOF can suppress banana *Fusarium* wilt disease by modifying the rhizosphere microbiome. The effect of organic matter in regulating the soil microbiome is slow and variable [33], and sometimes even conducive to other pathogens [25]; this perhaps explains why, in the short term, the suppression of wilt by the OF treatment was not complete. In addition, no significant differences were observed in disease incidence between the IPN and OF treatments, indicating that neither inorganic plant nutrition nor OF treatment amendment alone were enough to induce soil activity against *Fusarium* wilt disease.

Soil microbial diversity is considered to be critical for the maintenance of soil health and quality and feedback into plant growth [34,35]. In the present study, the highest recorded values of bacterial and fungal Shannon–Weaver and Simpson $\alpha$-diversity indexes were for the BOF treatment. In addition, the bacterial and fungal Simpson indices were significantly positively correlated with fresh weight of roots and dry weight of shoots, respectively. This is consistent with the results of Zhang et al. [19] who showed that BOF treatment significantly increased bacterial $\alpha$-diversity together with a significant positive correlation between bacterial $\alpha$-diversity and *Lobelia chinensis* biomass. However, in Zhang’s research, BOF treatment decreased fungal $\alpha$-diversity, which is contrary to our findings. This may have been caused by the abundance of *Trichoderma* spp. in the bio-organic fertilizer used in Zhang’s research, which primarily accounted for the decrease in fungal $\alpha$-diversity. The bio-organic fertilizer used in our study was enhanced with *Paenibacillus polymyxa*, which is a plant growth-promoting rhizobacterium [36]. Numerous studies have shown that greater biodiversity can lead to more stable systems [37]. BOF treatment has the ability to restore and maintain microbial biodiversity for sustainable soils.

The rhizosphere microbiome contributes significantly to soil health [38]. Previous studies have found that different soil amendments can lead to distinct microbial communities [39–41], which is consistent with our finding that the soil bacterial and fungal community compositions were grouped according to the treatment received. In our study, Proteobacteria and Actinobacteria were the two most abundant bacterial phyla in all soil treatments, as also observed by Zhao et al. [40] and Yu et al. [42], while Ascomycota was the dominant fungal phyla in all soil amendments, in agreement with Shen et al. [43]. However, the compositions of bacteria and fungi following the different soil treatments were significantly altered at the phyla and genera levels. We focused on bacteria and fungi genera that show the greatest fold-changes and have the potential to affect plant growth, such as *Nocardiodes* [44], *Streptomyces* [45], *Mariniflexile* [46], *Glycomyces* [47], *Cladosporium* [48], *Arthrobotrys* [49] and *Fusarium* [50]. In the BOF-treated soil, there was a significant enrichment of beneficial microbial groups such as *Cladosporium* and declines in potentially pathogenic groups such as *Fusarium*, which is similar to the findings of Zhang et al. [32]. The abundance of *Cladosporium* was highest in the BOF treatment. Meanwhile, from the correlation analysis we conducted, the relative abundance of *Cladosporium* was significantly negatively correlated with the incidence of *Fusarium* wilt. This indicates that the *Paenibacillus polymyxa* in BOF not only has direct antagonistic effects on *Fusarium* wilt through its formation of biofilms, the induction of systemic resistance, the promotion of plant growth and the enhancement of siderophore production, but it may also interact with the fungus indirectly to reduce the incidence of disease. This is indicated by the ITS2
gene-predicted functional profiles showing that arbuscular mycorrhizal and endophyte groups were significantly increased by BOF treatment.

Soil micro-nutrients play a key role in regulating plant growth and influencing the associated microbial community [51]. The IPN (SCPM soil conditioner) also contained some trace elements in addition to Si, Ca, K and Mg. In our study, the growth of chrysanthemum was improved by IPN treatment, which is in line with the results of Han et al. [52] who found that the application of SCPM fertilizer improved rice yield. Meanwhile, the abundance of *Arthrobotrys* was significantly decreased and the abundance of *Glycomyces* significantly increased after the IPN treatment. Previous studies have indicated that *Arthrobotrys* provides some biological control of root-knot [49,53]. Shimizu et al. [54] have shown that *Glycomyces* spp. are endophytic actinomycetes, and can be used as biocontrol agents and growth promoters. This explains why IPN soil conditioner can to some extent promote chrysanthemum growth and reduce disease. In addition, according to the 16S rRNA gene-predicted function profiles, after IPN application the microbial community function related to membrane transport predominates. It may be that increases in Ca$^{2+}$ and Mg$^{2+}$ concentrations within a certain range can stimulate membrane phosphorylation, in turn promoting membrane transport [55].

5. Conclusions

In conclusion, these experiments have demonstrated that BOF, IPN and OF treatments improved the growth of chrysanthemum and provided control to some extent over *Fusarium* wilt disease, particularly with BOF. This was achieved by modifying the composition of the rhizosphere soil microbiome of chrysanthemum. The bacterial and fungal Simpson indexes were significantly positively correlated with root fresh weight and chrysanthemum dry weight, respectively. There was significant enrichment in the BOF-treated soil of beneficial microbial groups and a decline in possible pathogen groups such as *Fusarium*. In addition, within the fertilizer treatments, the relative abundance of *Cladosporium* showed a significant negative correlation with the incidence of *Fusarium* wilt. Employing BOF is an excellent strategy for the control of wilt as it is effective, enhances soil general ecological health, and is cheap and easily prepared.

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