Applied Soil Ecology 142 (2019) 136–146

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system

Cece Qiao[a,b], C. Ryan Penton[b], Wu Xiong[a,c], Chao Liu[a], Roufei Wang[a], Zhengyang Liu[a], Xu Xu[a], Rong Li[a,c,*], Qirong Shen[a]

[a] Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing 210095, China
[b] College of Integrative Sciences and Arts, Center for Fundamental and Applied Microbiomics, The Biodesign Institute, Arizona State University, Mesa, AZ, USA
[c] Ecology and Biodiversity Group, Department of Biology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, Netherlands

ARTICLE INFO

Keywords:
Microbial community
Fertilizer treatment
Crop type
Yield promotion
MiSeq platform

ABSTRACT

Characterizing the rhizosphere microbial community composition associated with enhanced crop yield is an important first step towards understanding the role of the microbiota in soil fertility. In the present study, we conducted a two-seasons field experiment in a maize-cabbage cropping system under chemical (CF), organic (OF) and bio-organic (BOF) fertilizer regimes as a model to investigate the combinatory effect of fertilizer treatment and crop type on rhizosphere soil microbiota by targeted sequencing of both the bacterial and fungal communities. The two-seasons sustainable application of bio-organic fertilizer (BOF) containing *Trichoderma* effectively increased maize and cabbage yields, whereas organic fertilizer (OF) increased but not significant, compared to the application of chemical fertilizer (CF). Both fertilizer treatment and crop type induced a significant effect on soil physiochemical properties and were the major factors that impacted the composition of the rhizosphere soil microbiome. Relative abundances of *Trichoderma* were significantly enhanced in the BOF treatment, compared to the OF and CF treatments, and exhibited significant positive relationships with crop yield improvement. The application of bio-organic fertilizer may enhance the growth promotion effect of *Trichoderma* and increase the abundance of potentially beneficial microbial groups such as the genera *Cladorrhinum* and *Massilia*, which were found to be highly correlated to increased crop yields. Overall, the influence of bio-organic fertilizer on crop yield is proposed to be through mechanisms by introduction of the target strain NJAU 4742 and stimulation of a potentially beneficial microbial consortia, in combination with alterations in fungal and bacterial composition and abundance, leading to an enhancement in crop yield.

1. Introduction

In agroecosystems, soil microorganisms represent the largest reservoir of biodiversity (Flint et al., 2007; Johnson et al., 2015). The soil microbiome performs a variety of ecological services such as the mineralization of organic matter, nutrient cycling, and the promotion of plant growth (Nacke et al., 2011; Ru et al., 2012; Mendes and Raaijmakers, 2011). However, soil biological activity is most pronounced in the soil rhizosphere where plant roots and microbes interact (Bakker et al., 2015). This rhizosphere microbiome carries out fundamental processes that contribute to both nutrient cycling and plant health (Zhou et al., 2015; Mendes et al., 2017; Santoyo et al., 2017). Plants directly modify this local soil environment through root exudation and rhizodeposition, leading to alterations in the composition and function of the rhizosphere microbiome (Hertenberger et al., 2015). The influence of individual plant type and agricultural management, combined with nutritional status in rhizosphere soil and other environmental factors, result in combinatorial impacts on the rhizosphere microbiome (Berg and Smalla, 2009; Berendsen et al., 2012; Pérez-Jaramillo et al., 2016). Consequently, the composition and function of the rhizosphere microbial community differs from that associated with the bulk soil (Liu et al., 2016; Fu et al., 2017). In addition, fertilizer amendments are known to have a profound influence on plant nutrition, crop yield, and soil organic matter. These interactions and their downstream influences could reshape the rhizosphere community microbial through the soil microbial community (Shen et al., 2013), and thus can be either beneficial or detrimental to plant growth. Overall, a diverse microbial community that exhibits high biological activity is a

* Corresponding author at: College of Resources and Environmental Sciences, Nanjing Agricultural University, 210095 Nanjing, China.
E-mail address: lirong@njau.edu.cn (R. Li).

https://doi.org/10.1016/j.apsoil.2019.04.014
Received 2 October 2018; Received in revised form 18 March 2019; Accepted 14 April 2019
Available online 02 May 2019
0929-1393/

sensitive biological indicator for soil quality and is thus an area of interest within the context of sustainable agricultural productivity (Bending et al., 2004; Sharma et al., 2010; Bender et al., 2016; Pii et al., 2015). Thus, understanding how the rhizosphere microbiome develops is necessary in order to decipher the underlying mechanisms involved in crop yield enhancement for sustainable agriculture.

The majority of research has focused on alterations in rhizosphere microbial community composition by fertilization regimes alone while little attention has been paid to the interactive effects with variations in crop type. Of the agriculturally important crops, both maize and cabbage are typically cultivated in tropical and subtropical regions globally (Srinivasan, 2012). This maize-cabbage cropping system was previously adopted as a model to investigate the modulation of soil microbial community structure and function in field-based studies (Ai et al., 2015). To date, the majority of research has been devoted to long-term field experiments over five or more years (Calleja-Cervantes et al., 2015). However, in an applied perspective, farmers desire economic benefits from organic amendments applied to soil within a shorter period of time. Thus, a better mechanistic understanding concerning the short-term shifts in the rhizosphere microbial community under different fertilization schemes and crop type is necessary in order to better understand the impact on soil quality as well as to improve agroecosystem productivity.

There is an increasing concern that the over-used of chemical fertilizer has not only resulted in deteriorated soil quality but also has led to large-scale ecosystem degradation and loss of productivity in the long term (Foley, 2005; Shen et al., 2010). To address these concerns, organic amendment substitution for chemical fertilizer is a useful method to increase crop resource use efficiency as well as improve quality of agricultural products (Aparna et al., 2014; Syswerda et al., 2012). Furthermore, bio-organic fertilizers that based on organic amendment substitution, contain microorganisms and specific organic components which, directly or indirectly, increase the mobilization of soil nutrients and positively influence plant health, resulting in enhanced crop yield (Tamreihao et al., 2016; van der Heijden et al., 2008). Novel bio-organic fertilizers have been produced that integrate beneficial microbes with mature composts, resulting in the promotion of yield and/or the control of soil-borne diseases (Asl, 2017; Wang et al., 2016). Among the numerous beneficial microbes, fungi within the genus *Trichoderma* are ubiquitous in both soil and root ecosystems (Hasan, 2012; Li et al., 2013) and are widely recognized for their plant growth-promoting potential (Chen et al., 2011; Viterbo et al., 2010). It is established that strains of *Trichoderma* within the rhizosphere exert beneficial effects on plant growth on and nutrient uptake by mineralizing organic matter with a concomitant reduction in plant disease severity (Martínez-Medina et al., 2014; Saravanakumar et al., 2017; Umadevi et al., 2017). However, there are few reports concerning the specific interactions between *Trichoderma* and the rhizosphere microbiome as well as the impact on nutrient availability and crop productivity in the open-field environment. Therefore, deciphering the impact of fertilization regimes on both the rhizosphere microbiota and *Trichoderma* in the context of plant productivity is essential to understand the influence on the agricultural system.

One *Trichoderma* strain, *Trichoderma guizhouense* NJAU 4742, was previously isolated in our lab and demonstrated a significant ability to promote plant growth (Li et al., 2013; Cai et al., 2014; Li et al., 2015; Jie, 2012). In the present study, we conducted a two-seasons field experiment in a maize-cabbage cropping system under chemical, organic, and bio-organic (organic fertilizer amended with NJAU 4742) fertilizer regimes as a model to analyze the responses of the rhizosphere microbiome by targeted sequencing of both the bacterial and fungal communities. The objectives were to: 1) determine changes in the rhizosphere soil microbial communities due to crop type and fertilizer treatment and their interactive effects, 2) evaluate the impact of different fertilizer schemes on maize and cabbage crop yields, and 3) determine correlations between the composition of the rhizosphere soil microbial community with soil properties and crop yields.

2. Materials and methods

2.1. Site description and experiment layout

The field experiment was located in Libao town of Nantong city, Jiangsu province, China (32º 02' N, 118º50' E, 5.2 m a.s.l). This region has a northern subtropical monsoon climate, with an average annual temperature and precipitation of 14.5 °C and 1025.0 mm, respectively. The soil in this field was characterized as clay loamy typic-hapli-stagnic anthrosol, with a pH of 7.31 (10:1 water to soil ratio), and contains 24.13 g kg^{-1} organic matter, 2.29 g kg^{-1} total N, 4.08 g kg^{-1} total P, 7.94 g kg^{-1} total K and 21.1 mg kg^{-1} available P, 119 mg kg^{-1} available K. Prior to this experiment, the area was cultivated according to traditional Chinese farm management with a maize, cabbage, and tomato rotation amended with chemical fertilizer over a decade.

The field experiment was performed in a completely randomized block design with three replicates for each of the following treatments: 1) CF treatment: soil amended with chemical fertilizer, 2) OF treatment: soil amended with organic fertilizer, and 3) BOF: soil amended with organic fertilizer plus *Trichoderma guizhouense* NJAU 4742. The chemical fertilizer was mineral N, P, K. The organic fertilizer was chicken manure produced by composting at 30–70 °C for 25 days and maintained for 7 days above 55 °C with a pH value of 7.28, with 24.4 g kg^{-1} N, 24.9 g kg^{-1} P_2O_5 and 16.3 g kg^{-1} K_2O. The bio-organic fertilizer was produced by direct inoculation with *Trichoderma guizhouense* strain NJAU 4742 (10^8 CFU g^{-1} dry weight (DW)). Each plot was 4.75 m × 7.50 m and the organic amendments of each treatment (except for the CF plot) were applied at the rate of 200 kg per 667 m^2 in addition to mineral fertilizers (N, 6.68 kg; P_2O_5, 1.09 kg; and K_2O, 0.95 kg) that were added during the cropping season. All treatments received the same levels of nitrogen, phosphorus and potassium (N: 240 kg ha^{-1}, P_2O_5: 120 kg ha^{-1} and K_2O: 90 kg ha^{-1}) during each cropping season. Half of the composts and essential mineral fertilizers were applied as basic fertilizers before planting using a rotary tiller. The remaining organic fertilizer or bio-organic fertilizer and essential mineral fertilizers were applied in panicle and knot bract stages. The maize was planted in May 2015 and harvested in September 2015, followed by cabbage, which was planted in September 2015 and harvested in December 2015.

2.2. Soil sampling, properties analysis and DNA extraction

Five rhizosphere sub-samples were randomly collected from each replicate plot and mixed as a composite soil sample at the harvest time in each season. For rhizosphere soil collection, the root system was first separated from the bulk soil through gentle shaking. Soil still adhered to the roots was considered rhizosphere soil and was removed using sterile saline solution by centrifugation at 12,000 rpm for 10 min. All soil samples were transported to the laboratory, one portion of each sample was stored at −70 °C for subsequent DNA extraction after sifting through a 2-mm sieve and thorough homogenization and the other was air-dried for the determination of physiochemical properties including pH, total organic carbon (TOC), NH_4^+-N, NO_3^--N, available P (AP), available K (AK), total N (TN), total P (TP), total K (TK), according to the method modified from Shen et al. (2013).

Total soil DNA was extracted from 0.25 g soil subsamples using the PowerSoil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, USA), according to the manufacturer's protocol. The concentration and quality (ratio of A260/A280) of the DNA was determined using a spectrophotometer (NanoDrop 2000, ThermoScientific, USA).

2.3. Assay of total soil microbial biomass

Standard curves were generated to estimate the marker gene

abundances of bacteria and fungi using 10-fold serial dilutions of a plasmid containing a full-length copy of the 16S rRNA gene from *Escherichia coli* and 18S rRNA gene from *Saccharomyces cerevisiae* (Fierer et al., 2005). Standard and environmental DNA samples were analyzed on a 7500 Real Time PCR System (Applied BIO-systems, USA), according to a standard procedure (Fu et al., 2017). All presented results were average values of four replicates and were expressed as log (copy numbers g^{-1} dry soil).

2.4. PCR amplification, library preparation and Miseq sequencing

DNA extracted from each soil sample served as a template for 16S rRNA gene and ITS region amplification. Bacterial primers 520F (5′ - AYT GGG YDT AAA GNG - 3′) and 802R (5′ - TAC NVG GGT ATC TAA TCC - 3′) (Claesson et al., 2009) were used to amplify the V4 hypervariable regions of the bacterial 16S rRNA gene, while the ITS1 region of the fungal internal transcribed spacer (ITS) was targeted by ITS1F (5′ - CTT GGT CAT TTA GAG GAA GTA A - 3′) (Gardes and Bruns, 1993) and ITS2 (5′ - GCT GCG TTC TTC ATC GAT GC - 3′) (White, 1990). The primers used for final sequencing consisted of the appropriate Illumina adapter, pad linker, the gene-specific primer, and a 6-nt barcode unique to each sample, attached to the reverse primer. PCR reactions and purification of products were performed according to Fu et al. (2017). PCR products were quantified and pooled in equimolar concentrations to a final concentration of 10 nM followed by Illumina Miseq sequencing at Personal Biotechnology Co., Ltd., Shanghai, China.

2.5. Sequence data processing

After removing the adaptors and primer sequences, the raw sequences were assembled and binned to each sample based on the unique barcode using QIIME (Caporaso et al., 2010). Forward and reverse sequences for each sample were merged using FLASH V1.2.7 (Magoä and Salzberg, 2011). The sequences retained were analyzed using the UPARSE pipeline to generate an OTU table with representative sequences (Edgar, 2013). Sequences with a quality score lower than 0.5 or a length shorter than 200 bp were removed. After discarding the singletons, the remaining reads were assigned to OTUs with 97% similarity for further community analyses. In total, 977,676 16S rRNA sequences comprising 7147 operational taxonomic units (OTUs) and 808,227 fungal ITS sequences comprising 2113 OTUs from all soil samples were obtained. The classification of the representative sequences for each OTU was performed using the RDP classifier against the RDP Bacterial 16S rRNA database (Qiong et al., 2007; Cole et al., 2009) for bacteria and the UNITE Fungal ITS database (Urmas et al., 2013) for fungi, respectively. α- and β-diversities were analyzed in MOTHUR (Schloss et al., 2009). 974,896 16S rRNA sequences were classified to Bacteria. For fungi, 55,073 sequences were returned as unidentified fungi while 150,608 sequences were assigned to five fungal phyla. The numbers of high quality sequences per sample varied from 23,056 to 61,683 for 16S rRNA and 19,437 to 48,583 for fungi. To correct for the unequal number of sequences per sample, a randomly selected subset of 23,056 sequences for 16S and 19,437 sequences for ITS per sample were chosen for further bacterial and fungal community analysis.

2.6. Statistical analysis

An OTU-based analysis was performed to detect the microbial community richness and diversity between fertilizer treatment and crop type. Richness was estimated using Chao index while Shannon diversity index was calculated to estimate the number of observed OTUs that were present.

All statistical tests performed in this study were considered statistically significant at $P < 0.05$. The data were tested for normality and transformed when necessary to meet the criteria for a normal distribution. Duncan and pairwise comparison tests was used to assess the effect of fertilizer treatment on crop yield and microbial community, respectively. Multiple analysis of variance (MANOVA) using the IBM SPSS 22.0 (SPSS Inc., USA) software program was used to determine the effects of fertilizer treatment, crop type, and their interaction on the dependent variables, soil characteristics, gene copy numbers, relative abundances of abundant taxa, and α-diversity index including Chao and Shannon. If the multivariate F was significant, we then proceeded with the individual univariate analysis. In addition, Pearson correlation coefficients between the abundances of selected microbial indicators and enhanced combined yields of OF and BOF as compared to CF were also calculated.

Analysis of similarities (ANOSIM), permutational multivariate analysis of variance (PERMANOVA) and Multiple regression tree (MRT) based on the Bray-Curtis distance were conducted to evaluate community dissimilarities. Differences in microbial community composition among fertilizer treatments were tested by ANOSIM. PERMANOVA was performed to assess the effect of fertilizer treatment, crop type and their interaction on microbial community composition (abundance of OTUs and phyla) using the adonis function of the R (version 3.5.1, R Core Team., 2015) vegan (Oksanen et al., 2018) package with 999 permutations. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance was performed to illustrate β-diversity for bacteria and fungi between individual samples. MRT (De'Ath, 2002) analysis was conducted to evaluate fertilizer treatment and crop type effects on the rhizosphere bacterial and fungal communities using the vegan, mvpart, and MVPARTwrap packages in R. For further visualization of the relationship between the frequencies of samples and environmental variables, redundancy analysis (RDA) was carried out using the rda function and the environmental factors were fitted to ordination plots using the envfit function of the vegan package in R with 999 permutations. Variation partitioning analysis (VPA) was conducted in the vegan package of R after selecting the subset of environmental properties significantly correlated (Pearson correlation) with microbial assemblage dissimilarity to construct the soil property matrix using the BioEnv procedure (Clarke and Ainsworth, 1993).

3. Results

3.1. Impacts of maize and cabbage yields

Compared to the application of chemical (CF) and organic fertilizers (OF), bio-organic fertilizer (BOF) application significantly (Duncan test, $P < 0.05$) increased maize yield by 216 kg $acre^{-1}$ and 151 kg $acre^{-1}$, respectively, and increased the cabbage yield by 4477 kg $acre^{-1}$ and 3241 kg $acre^{-1}$, respectively (Fig. 1). No significant difference was

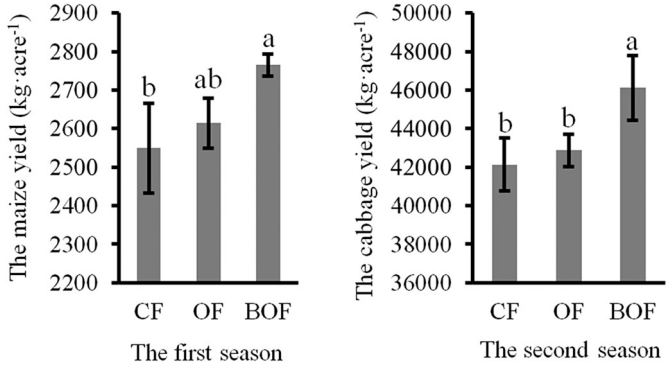

Fig. 1. Crop yield under the fertilizer treatments. Values are means ± standard deviation (n = 3). Fertilizer treatments: CF = chemical fertilizer, OF = organic fertilizer, BOF = organic fertilizer with *Trichoderma guizhouense* NJAU 4742, respectively. Different letters indicate statistically significant differences of each parameter at the 0.05 probability level, according to the Duncan test.

Table 1
Selected physiochemical characteristics for soils according fertilizer treatment, crop type, and their interaction.

Items	pH	TOC (g kg^{-1})	C/N	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})
Fertilizer Treatment§(FT)										
CF	7.53 ± 0.04^{b}	13.90 ± 0.49^{b}	8.25 ± 0.71^{a}	30.72 ± 14.05^{a}	1.22 ± 1.09^{b}	23.68 ± 3.1^{c}	41.67 ± 11.21^{b}	1.61 ± 0.10^{b}	0.65 ± 0.06^{b}	7.68 ± 0.28^{b}
OF	7.75 ± 0.47^{ab}	15.03 ± 0.75^{a}	8.52 ± 0.69^{a}	24.96 ± 2.29^{b}	1.54 ± 1.38^{a}	32.96 ± 3.39^{b}	56.00 ± 10.95^{a}	1.73 ± 0.19^{ab}	0.74 ± 0.05^{a}	7.94 ± 0.14^{a}
BOF	7.81 ± 0.66^{a}	14.85 ± 0.50^{ab}	8.56 ± 0.81^{a}	23.12 ± 4.07^{b}	1.63 ± 1.39^{a}	36.84 ± 2.22^{a}	58.67 ± 3.26^{a}	1.81 ± 0.02^{a}	0.76 ± 0.08^{a}	7.72 ± 0.18^{ab}
Crop Type§(CT)										
Maize	8.01 ± 0.44^{a}	14.70 ± 0.71^{a}	8.31 ± 0.88^{a}	30.44 ± 10.26^{a}	0.29 ± 0.08^{b}	29.76 ± 2.72^{b}	45.78 ± 12.01^{b}	1.74 ± 0.02^{a}	0.72 ± 0.09^{a}	7.83 ± 0.18^{a}
Cabbage	7.38 ± 0.14^{b}	14.73 ± 0.65^{a}	8.57 ± 0.51^{a}	22.09 ± 4.02^{b}	2.63 ± 0.35^{a}	32.56 ± 3.51^{a}	58.44 ± 7.12^{a}	1.69 ± 0.01^{a}	0.72 ± 0.06^{a}	7.74 ± 0.27^{a}
ANOVA *P*-values										
FT	0.04	0.009	NS	NS	< 0.001	< 0.001	< 0.001	NS	0.032	0.029
CT	< 0.001	NS	NS	< 0.001	< 0.001	0.039	< 0.001	NS	NS	NS
FT*CT	< 0.001	NS	NS	NS	0.004	< 0.001	0.002	NS	NS	0.007

Values are means with standard deviations (n = 4 or n = 12).
NS: not significant. Means followed by the same letter for a given factor are not significantly different ($P > 0.05$).
Fertilizer treatments: CF = chemical fertilizer, OF = organic fertilizer, BOF = organic fertilizer with *Trichoderma guizhouense* NJAU 4742, respectively.

observed between the BOF and OF in maize (Duncan test, $P > 0.05$), while a significant difference was present in cabbage (Duncan test, $P < 0.05$).

3.2. Impacts of soil physicochemical characteristics

Both of the organic amendments (OF and BOF) generally resulted in significantly higher levels of soil pH, the concentrations of TOC, TN, TP, TK, NH_4^+-N, AP, AK, and a decrease in NO_3^--N, as compared to the chemical fertilizer amendment (CF) (Table 1). The only significant physiochemical difference between the OF and BOF amendments was observed with AP. Accordingly, soil pH and the concentrations of NO_3^--N, NH_4^+-N, AP, and AK were significantly affected by crop type while soil pH, NH_4^+-N, AP, AK, and TK were significantly affected by the interactions between crop type and fertilizer treatment. Interestingly, NO_3^--N exhibited a clear decrease in cabbage (22.09 mg kg^{-1}) as compared to maize (30.44 mg kg^{-1}) while, conversely, NH_4^+-N was higher in cabbage (2.63 mg kg^{-1}) compared to the maize (0.29 mg kg^{-1}). No significant differences in soil TN and C/N were observed between crop type and fertilizer treatment as well as in their interaction terms.

3.3. Impacts of microbial community abundance

Compared to CF, BOF harbored significantly higher total fungal abundances, and no significant differences for bacteria were observed among the three treatments (Table S1). Similarly, fungi responded with a higher abundance under cabbage than maize, again with no change in bacterial abundances. Both bacteria and fungi exhibited significant differences in total abundances in the interactions between fertilizer treatment and crop type.

3.4. Impacts of microbial community α-diversity

A comparison of bacterial and fungal communities under the different fertilizer treatments and crop types indicated considerable variation in the estimated richness and diversity indices, based on the rarefied sequences (Table S2). For fertilization, BOF resulted in significantly higher richness and diversity for bacteria and higher diversity for fungi as compared to CF, while only higher diversity for fungi was observed in OF as compared to CF. For crop type, only the fungal Chao richness was significantly higher under cabbage than maize.

3.5. Impacts of microbial community β-diversity

Permutational multivariate analysis of variance confirmed that fertilizer treatment and crop type as well as their interaction were significant factors impacting the composition of the both the bacterial and fungal communities, in terms of both the relative abundance of operational taxonomic units (OTUs) and binning at the phylum level (Table 2). The result of pairwise comparison indicted that the fertilization treatment harbored significant effect on fungal community on both crops but not cabbage bacterial communties ($P > 0.05$).

In order to visualize the differences in bacterial and fungal communities composition, non-metric multi-dimensional scaling (NMDS) (Fig. S1) and multivariate regression tree (MRT) analyses (Fig. 2) were performed, respectively. Ordination (NMDS) revealed distinct sample grouping based on both fertilizer treatment and crop type. The bacterial community was separated by crop type by the second component (NMDS2) and the third component (NMDS3) (PERMANOVA, $P < 0.05$, R = 0.12; ANOSIM, $P < 0.05$, R = 0.11) with the BOF/OF samples clearly distinguished from CF. Fungi communities were separated by the first component (NMDS1) and the third component (NMDS3) (PERMANOVA, $P = 0.084$, R = 0.09; ANOSIM, $P = 0.071$, R = 0.12), with the CF and OF samples separated from the BOF.

Multivariate regression tree (MRT) analyses explained 74.24% and

Table 2
Permutational multivariate analyses based on Bray-Curtis dissimilarities.

Source	df	Bacteria				Fungi			
		Abundance of phylum		Abundance of OTUs		Abundance of phylum		Abundance of OTUs	
		Sums of sqs	Pseudo-F	Sums of sqs	Pseudo-F	Sums of sqs	Pseudo-F	Sums of sqs	Pseudo-F
Fertilizer Treatment§(FT)	2	0.43	101.34***	0.18	8.10***	0.03	34.83***	0.34	11.24***
Crop Type§(CT)	1	1.13	19.18***	0.29	2.53**	0.19	3.22*	0.73	2.62**
FT * CT	2	0.31	14.16***	0.07	2.14**	0.09	3.41*	0.44	3.38**
Residuals	18	0.20		0.03		0.36		1.18	

*indicates significant correlations at ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$), respectively.

65.52% of the detected variation in the composition of the soil bacterial and fungal communities, respectively. Bacterial and fungal communities composition were mainly impacted by crop type, which explained 28.26% of the variation in bacterial community composition, followed by organic matter amendment. This was followed by the impact of *Trichoderma* amendment (5.09%) in maize while organic matter (3.48%) and *Trichoderma* amendment (1.40%) impacted the community under cabbage. For the fungal community, 32.30% of the variation was explained by crop type, followed by *Trichoderma* (9.51%) and organic amendment (7.33%) under maize and *Trichoderma* (11.50%) and organic matter amendment (7.33%) under cabbage.

3.6. Linking the microbial communities to crop type and fertilizer treatment

Redundancy analysis (RDA) and variance partitioning analysis (VPA) illustrated the relationships between the major soil characteristics, crop type, and fertilizer treatment, and their relative contributions to the composition of the bacterial and fungal communities.

A Monte Carlo permutation test in RDA resulted in significant correlations between bacterial (Pseudo-F = 6.02, $P = 0.001$) and fungal (Pseudo-F = 3.22, $P = 0.002$) communities and selected soil properties (Fig. 3). The first and second RDA components (RDA1 and RDA2) explained 48.70% and 93.80% of the total variations in bacterial and fungal community compositions, respectively. As illustrated by the close grouping, maize bacterial communities were more correlated to higher soil pH values and NO_3^--N while cabbage were more correlated with soil NH_4^+-N. In fungal community, the communities under cabbage were related to higher soil NH_4^+-N, while the maize communities were associated with higher soil pH values, NO_3^--N and TN. Interestingly, fertilization had stronger impact on maize samples as compare to cabbage samples of both bacteria and fungi, this maybe due to the high pH and total nutrient of maize samples.

A subset of soil environmental parameters (OM, AK, AP, NH_4^+-N and pH) was selected by the BioEnv procedure for VPA which exhibited the highest Pearson correlation to microbial community composition. Results from VPA analysis showed these variables explained 29.54% (bacterial) and 36.87% (fungal) of the total variation (Fig. 4). The highest explanatory factor was crop type that contributed 12.90% ($P = 0.010$) and 17.38% ($P = 0.061$) of the total bacterial and fungal variation, respectively, while 8.89% ($P = 0.022$) of the bacterial and 9.73% ($P = 0.001$) of the fungal total variation was attributed to fertilizer treatment. Soil characteristics exhibited a smaller impact on the composition of both the bacterial and fungal communities.

3.7. Impacts of microbial community taxonomic composition

Classified sequences across all samples were affiliated with 45 bacterial phyla with two candidates and five fungal phyla. The dominant bacterial phyla were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes*, accounting for > 79.00% of the total bacterial 16S rRNA gene sequences (Table S3). *Ascomycota* and *Zygomycota*, at relative average abundances of 66.25% and 11.61%, respectively, were the two most dominant fungal phyla across all samples. The bacterial *Actinobacteria* and *Bacteroidetes* and fungal *Ascomycota* and *Chytridiomycota* relative abundances were significantly ($P < 0.001$) influenced by crop type while *Acidobacteria* and

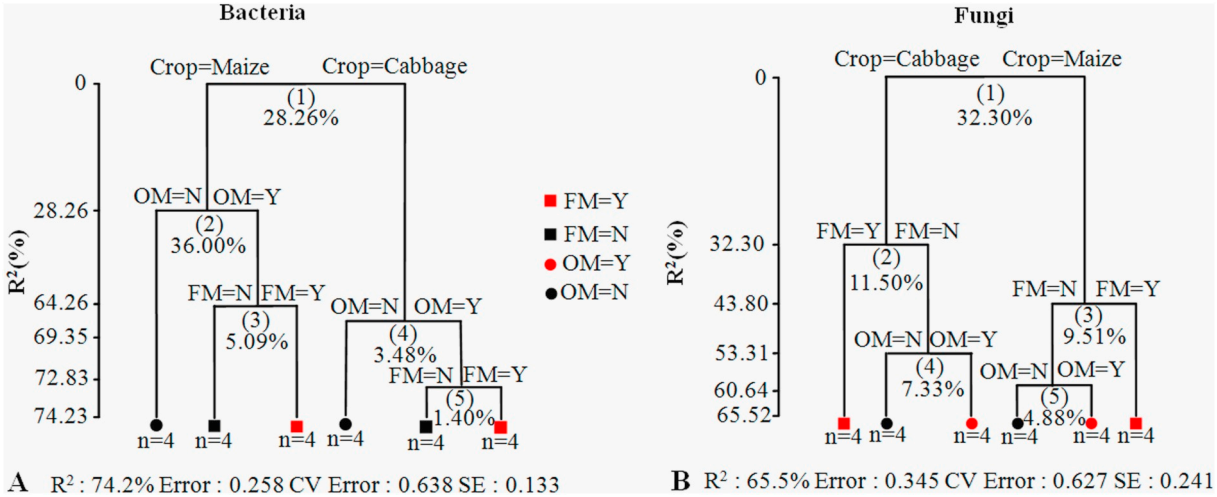

Fig. 2. Multivariate regression tree (MRT) analysis of bacterial (A) and fungal (B) community compositions among all rhizospheric soil samples.
The identity and number of soil samples included in the analysis are indicated by symbols with different shapes and colors under the tree. Numbers under the crosses of each split indicate percentages of variance explained by the split. The R^2, error, cross-validation error (CV Error) and standard error (SE) of MRT analysis are listed under the tree. FM = Y: *Trichoderma guizhouense* NJAU 4742 present, FM = N: no *Trichoderma* detected; OM = Y: organic matter, OM = N: no organic matter. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

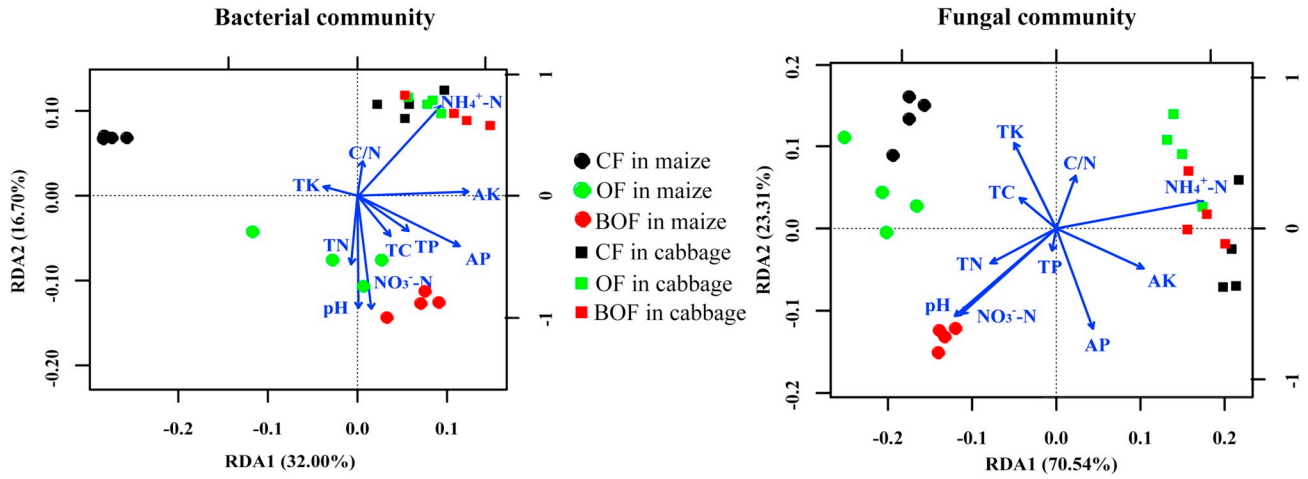

Fig. 3. RDA illustrating the relationship between the major environmental factors within the bacterial and fungal communities.

Chytridiomycota relative abundances were most influenced ($P < 0.001$) by fertilizer treatment. Both fertilizer treatment and crop type as well as their interaction exhibited significant ($P < 0.05$) effects on the bacterial *Acidobacteria*, *Actinobacteria* and *Firmicutes* and the fungal *Chytridiomycota* relative abundances.

For analysis at the genus level, only bacterial and fungal genera with a relative abundance > 1.0% in all combined samples were analyzed (Table 3). Among the bacterial genera, *Massilia*, *Zavarzinella* and *Rubritepida* were significantly enriched in the OF amended rhizosphere soils as compared to those in CF ($P < 0.05$), while *Zavarzinella*, *Rubritepida* and *Bdellovibrio*, were significantly depleted in the BOF treatment rhizosphere soil, as compared to OF ($P < 0.05$). Both crop type and their interaction terms had a significant ($P < 0.05$) impact on all the relatively abundant bacterial genera. The five most abundant fungal genera were *Mortierella* (10.98%), *Humicola* (4.18%), *Derxomyces* (4.03%), *Rhizophydium* (2.71%) and *Massaria* (2.45%). Significantly higher abundances of the fungal genera *Massaria*, *Naumovozyma*, *Cladorrhinum* were observed in the OF treatment, as compared to those in CF ($P < 0.05$) while *Mortierella*, *Humicola*, *Derxomyces*, *Rhizophydium* and *Trichoderma* showed no significant ($P > 0.05$) changes between the OF and CF treatments. The relative abundances of the fungal genera *Humicola*, *Derxomyces*, *Rhizophydium* and *Trichoderma* were significantly increased while *Mortierella* was reduced in the BOF treatment ($P < 0.05$) as compared to the OF treatment. No significant differences of *Trichoderma* relative abundances were observed between crop type and the interaction terms. In addition, we also observed that the relative abundances of *Massaria*, *Naumovozyma* and *Nomuraea* were all in higher relative abundances under cabbage while the *Mortierella* was higher under maize (13.37%).

3.8. Relationships between yield production and the sensitive microbial genera

Pearson correlation coefficients was calculated between the combined improvement crop yields of OF and BOF and the relative abundances of the bacterial and fungal genera that significantly responded to fertilization with BOF (Fig. 5). Improved crop yield was positively correlated with *Cladorrhinum* (r = 0.432; $P = 0.035$) and *Derxomyces* (r = 0.657; $P < 0.001$), *Massilia* (r = 0.585; $P = 0.003$) and negatively correlated with *Nomuraea* (r = −0.510; $P = 0.011$). Relative abundances of *Trichoderma* also exhibited significantly positive relationships with crop yield between BOF and CF (r = 0.625; $P = 0.001$) as well as

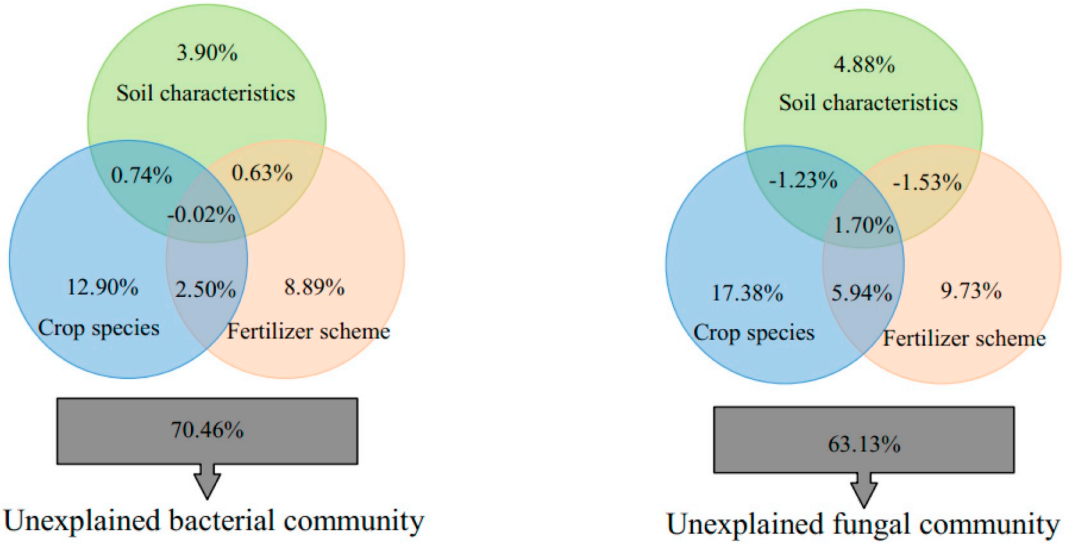

Fig. 4. VPA illustrating effects of soil characteristics (SC), fertilizer treatment (FT), crop type (CT) and their interactions on microbial community composition. Samples from different fertilizer treatments both in under maize (circles) and cabbage (squares) are marked by different colors. Percentage of variation explained by each factor alone is provided within the circles. The percentage of variation explained by interactions between two or three of the factors is shown in the circular junctions. The unexplained variation is depicted as a rectangle on the bottom.

Table 3
Effects of fertilizer treatment, crop type and their interaction on the relative abundance(%) of bacterial and fungal taxa at the genus level.

Taxa/Genus	Bacteria				Fungi							
	Massilia	*Zavarzinella*	*Rubritepida*	*Bdellovibrio*	*Mortierella*	*Humicola*	*Derxomyces*	*Rhizophydium*	*Massaria*	*Naumovozyma*	*Cladorrhinum*	*Nomuraea*	*Trichoderma*
Fertilizer Treatment§(FT)													
CF	1.24 ± 0.46^{b}	0.86 ± 0.19^{b}	0.89 ± 0.42^{b}	1.14 ± 0.59^{a}	12.69 ± 5.56^{a}	3.31 ± 0.95^{b}	3.73 ± 1.19^{b}	2.79 ± 0.69^{b}	2.23 ± 0.37^{b}	2.02 ± 1.01^{b}	1.17 ± 0.71^{b}	2.57 ± 2.05^{a}	0.003 ± 0.003^{b}
OF	1.89 ± 0.31^{a}	2.45 ± 1.35^{a}	1.55 ± 0.29^{a}	1.16 ± 0.24^{a}	11.66 ± 1.40^{a}	4.11 ± 1.08^{b}	3.81 ± 0.61^{b}	4.07 ± 1.56^{b}	2.29 ± 0.71^{ab}	2.97 ± 1.21^{ab}	3.02 ± 1.52^{a}	1.37 ± 0.74^{b}	0.004 ± 0.005^{b}
BOF	1.61 ± 0.87^{a}	1.12 ± 0.72^{b}	1.19 ± 0.89^{b}	1.02 ± 0.41^{b}	8.57 ± 1.08^{b}	5.77 ± 0.73^{a}	5.12 ± 1.81^{a}	1.42 ± 0.70^{a}	2.62 ± 1.24^{a}	3.23 ± 1.04^{a}	2.55 ± 0.99^{a}	1.54 ± 0.47^{b}	0.012 ± 0.009^{a}
Crop Type§(CT)													
Maize	1.16 ± 0.54^{b}	1.88 ± 1.46^{a}	1.95 ± 0.79^{a}	0.73 ± 0.18^{a}	13.37 ± 3.75^{a}	4.49 ± 1.49^{a}	4.19 ± 0.55^{a}	0.67 ± 0.29^{a}	1.82 ± 0.34^{b}	1.60 ± 0.65^{b}	2.31 ± 1.73^{a}	1.02 ± 0.54^{b}	0.007 ± 0.003^{a}
Cabbage	2.22 ± 0.29^{a}	1.08 ± 0.28^{b}	0.49 ± 0.07^{b}	1.48 ± 0.19^{b}	8.58 ± 1.81^{b}	4.16 ± 1.25^{a}	4.17 ± 1.94^{a}	5.16 ± 2.26^{a}	2.97 ± 0.74^{a}	3.88 ± 1.34^{a}	2.16 ± 0.85^{a}	2.74 ± 1.45^{a}	0.005 ± 0.004^{a}
ANOVA *P*-values													
FT	< 0.001	< 0.001	0.015	0.022	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.044	< 0.001	0.002	0.047
CT	0.001	0.005	< 0.001	< 0.001	< 0.001	NS	NS	< 0.001	0.011	< 0.001	NS	< 0.001	NS
FT*CT	< 0.001	0.005	0.003	0.015	< 0.001	NS	< 0.001	< 0.001	< 0.001	NS	< 0.001	< 0.001	NS

Values are means with standard deviations (n = 4 or n = 12).
NS: not significant. Means followed by the same letter for a given factor are not significantly different ($P > 0.05$).
Fertilizer treatments: CF = chemical fertilizer, OF = organic fertilizer, BOF = organic fertilizer with *Trichoderma guizhouense* NJAU 4742, respectively.

between BOF and OF (r = 0.577; $P = 0.019$).

4. Discussion

The first objective was to explore the effects of substituting chemical fertilizer with organic and bio-organic fertilizer on crop yield, and the result proved to harbored an enhanced yield. A similar observation was obtained by Yaduvanshi (2003) and Rong et al. (2018). Considering the environmental implications including soil degradation and accumulation of pesticides of long-term chemical fertilizer application, this result can improve our potential to manage agricultural soils for sustainable productivity by promoting beneficial microorganisms and provide a basis for reducing the application of chemical fertilizers. The following goal of this study was to assess the combinatory influence of fertilizer treatment and crop type on the composition of the rhizosphere soil bacterial and fungal communities in a maize-cabbage cropping system. While considering the effects of agricultural practices on the soil microbial community, previous studies have often been limited to the examination of single factors such as management type (Nacke et al., 2011; Chaudhry et al., 2012), crop type (Larkin and Honeycutt, 2006), soil amendment (Falsaperla and Motta, 2013), or focused solely on either the bacterial or fungal communities (Zhang et al., 2016; Ru et al., 2012). Soil inoculation of microorganism without a suitable organic substrate cannot be expected to be successful due to the absence of nutrients and may lead to poor microbiological activity (Wang et al., 2013). In the present study, a novel fertilization method using *Trichoderma*-enriched bio-organic fertilizer was applied to maize-cabbage cropping systems. Application of the bio-organic fertilizer used in this study was previously focused on economic crops with continuous application that produced cumulative effects that enhanced crop yield when compared to chemical fertilization (Cai et al., 2014). The enhancement of yield is thought to be due to an enhanced availability of soil nutrients and increased abundances and activity of the soil microflora (Cai et al., 2014). Here, in order to further decipher the underlying effects of the bio-organic fertilizer, targeted MiSeq sequencing was used to characterize the bacterial and fungal rhizosphere communities under different fertilizer treatments and crop types.

Overall, the BOF resulted in significantly higher richness and diversity for bacteria and higher diversity for fungi, when compared to the CF alone. This higher abundances tendency corresponded to both the 16S rRNA and ITS gene copy numbers for BOF as compared to CF. Previous research has shown that organic compost amendment enhances bacterial and fungal diversity, as compared with conventional chemical fertilizers (Chaudhry et al., 2012; Mäder et al., 2002), possibly attributed to the increased supply of organic C substrates. Organic amendments may also provide a greater diversity of potential substrates for microbial growth and respiration (Zeng et al., 2007; Chu et al., 2007). However, diversity and richness were not significantly different between the BOF and OF treatments. Therefore, the additional of organic amendment had an positive influence on the rhizosphere microbial community in terms of diversity, richness and abundances that overwhelmed the impact of adding the *Trichoderma* inoculum. As a result, applications of organic manure may play an important role in sustaining a diverse suite of soil microbial populations and corresponding activities (Francioli et al., 2016; Hartman et al., 2018).

Crop type and fertilizer treatment were identified as the major factors that impacted the composition of the bacterial and fungal communities in the rhizosphere soil. This is supported by MRT analysis, NMDS ordination, and VPA where crop type was the most influential determinant. This conclusion is derived consistently from previous observations that crop type exerted greater impacts on soil community composition as compared to fertilizer management (Zhao et al., 2014). Considerable temporal variation including presumably different climatic conditions in soil microbial community has been previously been reported for agricultural soils that impact the rhizosphere soil microbial community (Schutter et al., 2001). In the present study, the greatest

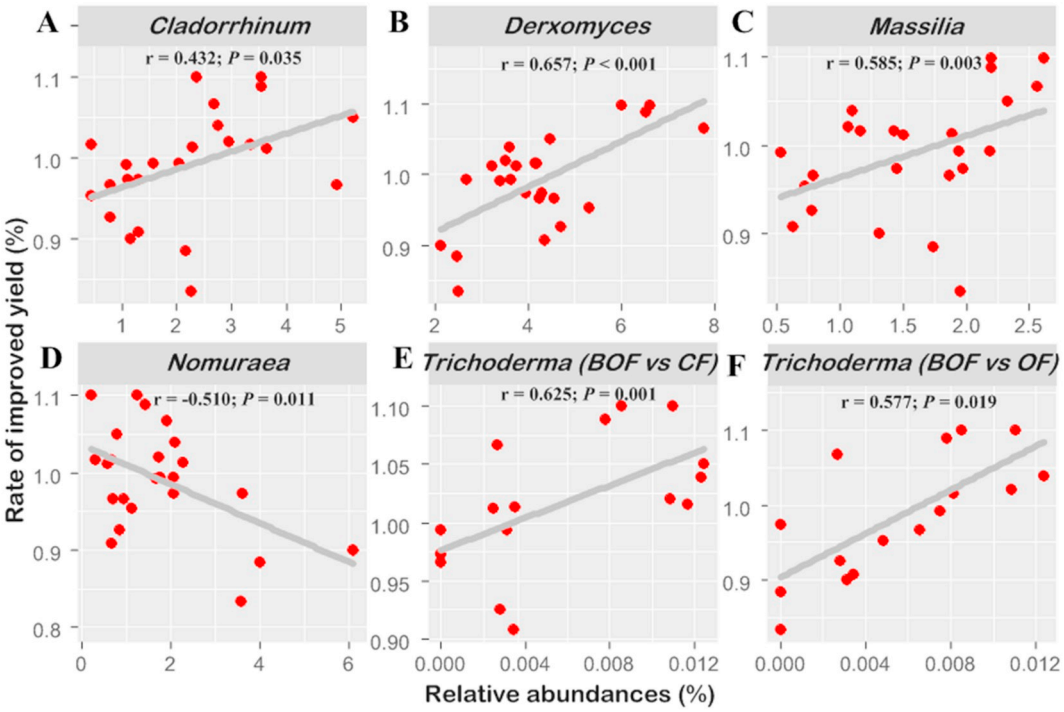

Fig. 5. Pearson correlations (r) between the relative abundances of bacterial and fungal taxa and the rate of improved crop yield. All of the taxa listed in Table 3 were subjected to the Pearson correlation analysis. Taxa with $P > 0.05$ are not shown.

community variation was also found to be crop effect, including the plant species and temporal variation. Further study should divided the two effective factors. The rhizosphere here serves as an important interface for plant-soil-microorganism interactions and signaling and allows for an exchange of both energy and resources. Accumulated evidence suggests that the diversity of plant root exudates and secondary metabolites are the deterministic forces driving the outcomes of interactions in the rhizosphere and, ultimately, plant and soil community dynamics (Srinivasan, 2012; Haichar et al., 2008; Broeckling et al., 2008). It is therefore reasonable to suggest that such maize-cabbage differences in root exudation patterns will be reflected within the rhizosphere microbial community and the biological processes that it regulates.

The second most influential determinant of rhizosphere soil bacterial and fungal community composition was fertilizer regime, as determined by NMDS, MRT, and PERMANOVA. Previous findings have recognized that fertilization is an important factor in shaping the soil microbial community by comparing chemical, organic and bio-organic fertilizers (Sun et al., 2015; Shen et al., 2013; Falsaperla and Motta, 2013). As discussed previously, of particular importance is the application of organic manure as it supports soil organic matter accumulation and the development of soil microbial communities with greater biodiversity. For instance, Gu et al. (2009) observed that changes in soil bacterial community composition were more noticeable in soils subjected to organic manure applications than in the soils treated with mineral fertilizer. It is well established that long-term organic manure application improves various aspects of soil fertility (Liang et al., 2012). In line with this concept, our results showed that the organic and bio-organic fertilizer both provide more available nutrients (e.g. NH_4^+-N, available P (AP), available K (AK)), which may promote microbial growth. Conversely, in some cases, inorganic or organic fertilizers have relatively little or no impact on soil fungal diversity and activities (Dong et al., 2014). In our study, fertilizer treatment explained 9.73% ($P = 0.001$) of the total fungal variation, which was more than that of bacteria at 8.89% ($P = 0.022$). This may be attributed to the bio-organic fertilizer containing the "functional microorganism" *Trichoderma*. As *Trichoderma* has been shown to have the ability to solubilize phosphate by secreting organic acids (Li et al., 2015), bio-organic fertilizer application may enhance the growth promotion effect of the organic manure by further influencing the soil microbiome (Xiong et al., 2017). This is supported by higher available phosphorus in the BOF treatment along with a non-statistically significant increase in crop yield over the organic manure amendment alone. In addition to phosphorus, other soil available nutrients such as NH_4^+-N and available K (AK) were significantly higher in the OF and BOF treatments, compared to the CF treatment. However, our inoculant *Trichoderma guizhouense* NJAU 4742 has been shown not to perform direct N fixation or K dissolution under laboratory conditions (data not shown). Therefore, the resulting enhancement of available N and K in the soil could be attributed to other effects including bacteria (Schmalenberger and Fox, 2015), fungi (Cai et al., 2014), protists (Trap et al., 2016) and/or nematodes (Gebremikael et al., 2016). Nutrient contents in soil are considered a good indicator of soil productivity, and our findings demonstrate the potentially positive effects of treatments OF and BOF on soil nutrient conditions. Similar conclusions were drawn by Marinari et al. (2006) and Nautiyal et al. (2010), who argued that these improvements were a consequence of the enhancement of soil organic matter that supplies substrates and nutrients for mineralization. In our study soil pH was higher in the OF and BOF treatments, which may be facilitated by the application of organic manure that may decrease soil acidity by increasing soil organic matter while enhancing soil base saturation (Advan et al., 2006; Zhao et al., 2014).

Overall, the BOF treatment positively impacted the rhizophere soil microbial community in all regards, though the differences, when compared to OF, were usually not statistically significant, pointing to the overwhelming short-term influence that the manure had on the microbial community. While the relationship between soil microbial community composition and function is not straightforward due to the complexity of the soil system (Nannipieri et al., 2010), some microbes can stimulate plant growth by releasing phytohormones and stimulation of both the induced systemic resistance and systemic-acquired resistance components of the plant immune system (Lugtenberg and

Kamilova, 2009; Ent et al., 2010). As such, changes in the composition of the rhizosphere microbial community with organic fertilizer treatments observed in our experiment were likely accompanied by changes in the function of the community, though this remains unresolved.

The fungal genus *Trichoderma* is particularly present within the plant rhizosphere (Mendes et al., 2013) and various strains from the genus *Trichoderma* have been widely used for their promotion of plant growth and biocontrol abilities (Hermosa et al., 2012; Contreras-Cornejo et al., 2009). *Trichoderma guizhouense* NJAU 4742 is among these various beneficial types (Zhang et al., 2016; Xiong et al., 2017; Cai et al., 2014). In the present study, the relative abundances of *Trichoderma* were significantly enhanced in the BOF treatment when compared to OF and CF treatments, and showed significantly positive relationships with the rate of improved yield (Table 1) between BOF and CF ($r = 0.625$; $P = 0.001$), and BOF and OF ($r = 0.577$; $P = 0.019$). As discussed previously, the plant growth promotion effect between BOF and CF suggested higher available nutrients. However, the relative abundance of the genus *Trichoderma* was not as high as we expected, thus suggesting that in addition to the inoculated *Trichoderma*, there were other indigenous and beneficial microbial groups involved in plant growth promotion. Among these, higher abundances of *Cladorrhinum*, *Derxomyces* and *Massilia* were observed in the BOF and OF treatments as compared to the CK treatment (Table 3) and were positively correlated with yield improvement (Fig. 5). The genus *Cladorrhinum* is generally reported to constitute a fungal group of prime importance for agriculture with some species exhibiting high biocontrol potentials and plant growth promotion (Carmarán et al., 2015). *Massilia* has been reported to colonize and proliferate on roots (Ofek et al., 2012), and a high variation in *Massilia* abundance was found to be exhibited in vitro related to plant growth promotion, including IAA (Kuffner et al., 2010) and siderophore production (Hrynkiewicz et al., 2010). However, for the sake of practical investigation, there are few reports concerning the effect of *Derxomyces* on plant growth promotion. Furthermore, BOF has achieved improved yield and higher relative abundances of *Trichoderma* when compared to OF, indicating the function of biological input. The application of organic fertilizer and bio-organic fertilizer could effectively stimulate the potentially beneficial microbial consortia such as Cladorrhinum and Massilia over chemical fertilizer alone. When inoculated *Trichoderma guizhouense* NJAU 4742, BOF harbored significantly more *Trichoderma guizhouense* NJAU 4742 as compared to OF and CF, this is the main difference when compare BOF to OF. Therefore, we surmise that enrichments of *Trichoderma*, *Cladorrhinum* and *Massilia* in the rhizosphere soil of BOF may be associated with plant growth promotion and crop yield enhancement.

5. Conclusion

This study investigates the effect of fertilizer treatment and crop type on rhizosphere microbiota in a maize-cabbage cropping system. Crop type and fertilizer treatment were identified as the major factors that impacted the composition of the microbial communities in rhizosphere soil. Our study also demonstrated that the sustainable application of bio-organic fertilizer containing *Trichoderma guizhouense* NJAU 4742 could effectively increase maize and cabbage yields over chemical fertilizer alone. The influence of bio-organic fertilizer on crop yield and rhizosphere microbial community composition is proposed to be through mechanisms such as: 1) altering the rhizophere soil microbial community composition; 2) introduction of the target strain, *Trichoderma guizhouense* NJAU 4742, resulting in P solubilization directly or indirectly and impacts on the total rhizophere soil microbial community; and 3) stimulation of a potentially beneficial microbial consortia such as *Cladorrhinum* and *Massilia*. The induction of these potentially beneficial microbial cohorts remains a subject of future study in order to elucidate their role in plant growth promotion in terms of the design of beneficial bio-organic fertilizers and their use in sustainable strategies for plant growth promotion.

Acknowledgements

This research was supported by the National Key Research and Development Program (2016YFD0200305), the National Key Basic Research Program of China (2015CB150506), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the 111 project (B12009), Postgraduate Research & Practice Innovation Program of Jiangsu Province (CX(15)100606), the Top-notch Academic Programs Project of Jiangsu Higher Education Institution (PPZY2015A061), and the China Scholarship Council (award to Rong Li for 1 year's abroad study). The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.04.014.

References

Advan, D., Ojde, V., Korthals, G., Ahcvan, B., 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. Appl. Soil Ecol. 31, 120–135.
Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., He, X., 2015. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biol. Biochem. 80, 70–78.
Aparna, K., Pasha, M.A., Rao, D.L.N., Krishnaraj, P.U., 2014. Organic amendments as ecosystem engineers: microbial, biochemical and genomic evidence of soil health improvement in a tropical arid zone feld site. Ecol. Eng. 71, 268–277.
Asl, A.N., 2017. Effects of nitrogen and phosphate biofertilizers on morphological and agronomic characteristics of sesame. Open J. Ecol. 07, 101–111.
Bakker, M.G., Chaparro, J.M., Manter, D.K., Vivanco, J.M., 2015. Impacts of bulk soil microbial community structure on rhizosphere microbiomes of *Zea mays*. Plant Soil 392, 115–126.
Bender, S.F., Wagg, C., Mg, V.D.H., 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol. Evol. 31, 440.
Bending, G.D., Turner, M.K., Rayns, F., Marx, M.C., Wood, M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol. Biochem. 36, 1785–1792.
Berendsen, R.L., Pieterse, C.M., Bakker, P.A., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486.
Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.
Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008. Root exudates regulate soil fungal community composition and diversity. Appl. Environ. Microbiol. 74, 738–744.
Cai, F., Chen, W., Wei, Z., Pang, G., Li, R., Ran, W., Shen, Q., 2014. Colonization of *Trichoderma harzianum* strain SQR-T037 on tomato roots and its relationship to plant growth, nutrient availability and soil microflora. Plant Soil 388, 337–350.
Calleja-Cervantes, M.E., Irigoyen, I., Fernández-López, M., Aparicio-Tejo, P.M., Menéndez, S., 2015. Thirteen years of continued application of composted organic wastes in a vineyard modify soil quality characteristics. Soil Biol. Biochem. 90, 241–254.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Knight, R., 2010. Qiime allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.
Carmarán, C.C., Berretta, M., Martínez, S., Barrera, V., Munaut, F., Gasoni, L., 2015. Species diversity of *Cladorrhinum* in Argentina and description of a new species, *Cladorrhinum australe*. Mycol. Prog. 14, 1–11.
Chaudhry, V., Rehman, A., Mishra, A., Chauhan, P.S., Nautiyal, C.S., 2012. Changes in bacterial community structure of agricultural land due to long-term organic and chemical amendments. Microb. Ecol. 64, 450–460.
Chen, L., Yang, X., Raza, W., Li, J., Liu, Y., Qiu, M., Zhang, F., Shen, Q., 2011. *Trichoderma harzianum* SQR-T037 rapidly degrades allelochemicals in rhizospheres of continuously cropped cucumbers. Appl. Microbiol. Biotechnol. 89, 1653–1663.
Chu, H., Lin, X., Fujii, T., Morimoto, S., Yagi, K., Hu, J., Zhang, J., 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol. Biochem. 39, 2971–2976.
Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PLoS One 4, 66–69.
Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure to environmental variables. Mar. Ecol. Prog. Ser. 92, 205–219.
Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., Mcgarrell, D.M., Marsh, T., Garrity, G.M., 2009. The Ribosomal Database Project: Improved Alignments and New Tools for rRNA Analysis.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Cortés-Penagos, C., López-Bucio, J., 2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol. 149, 1579–1592.
De'Ath, G., 2002. Multivariate regression trees: a new technique for modeling species-environment relationships. Ecology 83, 1105–1117.
van der Heijden, M.G., Bardgett, R.D., Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310.
Dong, W., Zhang, Y., Dai, X., Fu, X., Yang, F., Liu, X., 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. Appl. Soil Ecol. 84, 140–147.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996.
Ent, S.V.D., Wees, S.C.M.V., Pieterse, C.M.J., 2010. Cheminform abstract: jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochem. 41, 1581.
Falsaperla, P., Motta, S., 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. Biol. Fertil. Soils 49, 723–733.
Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71, 4117–4120.
Flint, H., Duncan, S.K., Louis, P., 2007. Interactions and competition within the microbial community of the human colon: links between diet and health. Environ. Microbiol. 9, 1101–1111.
Foley, A.J., 2005. Global consequences of land use. Science 309 (5734), 570–574.
Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., Reitz, T., 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. Front. Microbiol. 7, 1446.
Fu, L., Penton, C.R., Ruan, Y., Shen, Z., Xue, C., Li, R., Shen, Q., 2017. Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil Biol. Biochem. 104, 39–48.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.
Gebremikael, M.T., Steel, H., Buchan, D., Bert, W., De Neve, S., 2016. Nematodes enhance plant growth and nutrient uptake under C and N-rich conditions. Sci. Rep. 6, 32862.
Gu, Y., Zhang, X., Tu, S., Lindström, K., 2009. Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping. Eur. J. Soil Biol. 45, 239–246.
Haichar, F.E.Z., Marol, C., Berge, O., Rangelcastro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2, 1221.
Hartman, K., Heijden, M.G.A.V.D., Wittwer, R.A., Banerjee, S., Walser, J.C., Schlaeppi, K., 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6, 14.
Hasan, S., 2012. *Trichoderma* - a promising plant growth stimulator and biocontrol agent. 3, 524–531.
Hermosa, R., Viterbo, A., Chet, I., Monte, E., 2012. Plant-beneficial effects of *Trichoderma* and of its genes. Microbiol 158, 17–25.
Hertenberger, G., Zampach, P., Bachmann, G., 2015. Plant species affect the concentration of free sugars and free amino acids in different types of soil. J. Plant Nutr. Soil Sci. 165, 557–565.
Hrynkiewicz, K., Baum, C., Leinweber, P., 2010. Density, metabolic activity, and identity of cultivable rhizosphere bacteria on Salix viminalis in disturbed arable and landfill soils. J. Plant Nutr. Soil Sci. 173, 747–756.
Jie, H., 2012. Study on growth-promoting effect of *Trichoderma harzianum* strain SQR-T037 on Eggplant Seedlings. J. Anhui Agri. Sci. 40, 15671–15673.
Johnson, D.R., Helbling, D.E., Lee, T.K., Park, J., Fenner, K., Kohler, H.P., Ackermann, M., 2015. Association of biodiversity with the rates of micropollutant biotransformations among full-scale wastewater treatment plant communities. Appl. Environ. Microbiol. 81, 666–675.
Kuffner, M., Maria, S.D., Puschenreiter, M., Fallmann, K., Wieshammer, G., Gorfer, M., Strauss, J., Rivelli, A.R., Sessitsch, A., 2010. Culturable bacteria from Zn- and Cd-accumulating Salix caprea with differential effects on plant growth and heavy metal availability. J. Appl. Ecol. 108, 1471–1484.
Larkin, R.P., Honeycutt, C.W., 2006. Effects of different 3-year cropping systems on soil microbial communities and rhizoctonia diseases of potato. Phytopathol. 96, 68.
Li, R., Tan, P., Jiang, Y., Hyde, K.D., Mckenzie, E.H.C., Bahkali, A.H., Kang, J., Wang, Y., 2013. A novel *Trichoderma* species isolated from soil in Guizhou, *T. guizhouense*. Mycol. Prog. 12, 167–172.
Li, X., Cai, F., Pang, G., Shen, Q., Li, R., Chen, W., 2015. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. PLoS One 10, 0130081.
Liang, Q., Chen, H., Gong, Y., Fan, M., Yang, H., Lal, R., Kuzyakov, Y., 2012. Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain. Nutr. Cycl. Agroecosyst. 92, 21–33.
Liu, H., Chen, D., Zhang, R., Hang, X., Li, R., Shen, Q., 2016. Amino acids hydrolyzed from animal carcasses are a good additive for the production of bio-organic fertilizer. Front. Microbiol. 7, 1290.
Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 541–556.
Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. Science 296, 1694.
Magoä, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Marinari, S., Mancinelli, R., Campiglia, E., Grego, S., 2006. Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy. Ecol. Indic. 6, 701–711.
Martínez-Medina, A., Alguacil, M.D.M., Pascual, J.A., Wees, S.C.M.V., 2014. Phytohormone profiles induced by *Trichoderma* isolates correspond with their biocontrol and plant growth-promoting activity on melon plants. J. Chem. Ecol. 40, 804–815.
Mendes, R., Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease- suppressive bacteria. Science 332, 1097–1100.
Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. 37, 634–663.
Mendes, L.W., Raaijmakers, J.M., Hollander, M.D., Mendes, R., Tsai, S.M., 2017. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISME J 12, 212–224.
Nacke, H., Thürmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schöning, I., Schrumpf, M., Daniel, R., 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. PLoS One 6, 17000.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2010. Microbial diversity and soil functions. Eur. J. Soil Biol. 54, 655–670.
Nautiyal, C.S., Chauhan, P.S., Bhatia, C.R., 2010. Changes in soil physico-chemical properties and microbial functional diversity due to 14 years of conversion of grassland to organic agriculture in semi-arid agroecosystem. Soil Tillage Res. 109, 55–60.
Ofek, M., Hadar, Y., Minz, D., 2012. Ecology of root colonizing *Massilia*, Oxalobacteraceae. PLoS One 7, 40117.
Oksanen, J., Guillaume, B.F., Michael, F., Roeland, K., Pierre, L., Dan, M., Peter, R.M., O'Hara, R.B., Gavin, L.S., Peter, S., Henry, M., Eduard, S., Helene, W., 2018. vegan: Community Ecology Package. https://cran.r-project.org/web/packages/vegan/index.html.
Pérez-Jaramillo, J.E., Rodrigo, M., Raaijmakers, J.M., 2016. Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol. Biol. 90, 635.
Pii, Y., Borruso, L., Brusetti, L., Crecchio, C., Cesco, S., Mimmo, T., 2015. The interaction between iron nutrition, plant species and soil type shapes the rhizosphere microbiome. Plant Physiol. Biochem. 99, 39–48.
Qiong, W., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73 (16), 5261–5267.
R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rong, Q.L., Ruo-Nan, L.I., Huang, S.W., Tang, J.W., Wang, L.Y., 2018. Soil microbial characteristics and yield response to partial substitution of chemical fertilizer with organic amendments in greenhouse vegetable production. J. Integr. Agric. 17, 1432–1444.
Ru, L., Khafipour, E., Krause, D.O., Entz, M.H., Kievit, T.R.D., Fernando, W.G.D., 2012. Pyrosequencing reveals the influence of organic and conventional farming systems on bacterial communities. PLoS One 7, 51897.
Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., Hernández-León, R., 2017. The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review. Spanish J. Agri. Res. 15, 0301.
Saravanakumar, K., Li, Y., Yu, C., Wang, Q., Wang, W., Sun, J., Gao, J.X., Chen, J., 2017. Effect of *Trichoderma harzianumon* maize rhizosphere microbiome and biocontrol of Fusarium Stalk rot. Sci. Rep. 7, 1771.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75 (23), 7537–7541.
Schmalenberger, A., Fox, A., 2015. Bacterial mobilization of nutrients from biochar-amended soils. Adv. Appl. Microbiol. 94 (1), 109–159.
Schutter, M., Sandeno, J., Dick, R., 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biol. Fertil. Soils 34, 397–410.
Sharma, S.K., Ramesh, A., Sharma, M.P., Joshi, O.P., Govaerts, B., Steenwerth, K.L., Karlen, D.L., 2010. Microbial Community Structure and Diversity as Indicators for Evaluating Soil Quality. 5. Springer, Netherlands, pp. 317–358.
Shen, W., Lin, X., Shi, W., Min, J., Gao, N., Zhang, H., 2010. Higher rates of nitrogen fertilization decrease soil enzyme activities, microbial functional diversity and nitrification capacity in a Chinese polytunnel greenhouse vegetable land. Plant Soil 337 (1–2), 137–150.
Shen, Z., Zhong, Z., Wang, Y., Wang, B., Mei, X., Li, R., Ruan, Y., Shen, Q., 2013. Induced soil microbial suppression of banana fusarium wilt disease using compost and biofertilizers to improve yield and quality. Eur. J. Soil Biol. 57, 1–8.
Srinivasan, R., 2012. Assessment of soil quality, residual effect and yield of organic and inorganically grown cabbage-baby corn cropping system. J. Exp. Zool. 90, 229–265.
Sun, R., Guo, x., Wang, D., Chu, H., 2015. Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. Appl. Soil Ecol. 95, 171–178.
Syswerda, S.P., Basso, B., Hamilton, S.K., Tausig, J.B., Robertson, G.P., 2012. Long-term nitrate loss along an agricultural intensity gradient in the Upper Midwest USA. Agric. Ecosyst. Environ. 149, 10–19.
Tamreihao, K., Ningthoujam, D.S., Nimaichand, S., Singh, E.S., Reena, P., Singh, S.H., Nongthomba, U., 2016. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiol. Res. 192, 260.
Trap, J., Bonkowski, M., Plassard, C., Villenave, Cécile, Blanchart, E., 2016. Ecological

importance of soil bacterivores for ecosystem functions. Plant Soil 398 (1–2), 1–24.
Umadevi, P., Anandaraj, M., Srivastav, V., Benjamin, S., 2017. *Trichoderma harzianum* MTCC 5179 impacts the population and functional dynamics of microbial community in the rhizosphere of black pepper (*Piper nigrum L*). Braz. J. Microbiol. 49, 463–470.
Urmas, K.L., Henrik, N.R., Kessy, A., Leho, T., Taylor, A.F.S., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Viterbo, A., Landau, U., Kim, S., Chernin, L., Chet, I., 2010. Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. FEMS Microbiol. Lett. 305, 42.
Wang, B., Yuan, J., Zhang, J., Zhong, S., 2013. Effects of novel bioorganic fertilizer produced by bacillus amyloliquefaciens W19 on antagonism of Fusarium wilt of banana. Biol. Fertil. Soils 49 (4), 435–446.
Wang, L., Yang, F., Yaoyao, E., Yuan, J., Raza, W., Huang, Q., Shen, Q., 2016. Long-term application of bioorganic fertilizers improved soil biochemical properties and microbial communities of an apple orchard soil. Front. Microbiol. 7.
White, T.J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols.
Xiong, W., Guo, S., Jousset, A., Zhao, Q., Wu, H., Li, R., Kowalchuk, G.A., Shen, Q., 2017. Bio-fertilizer application induces soil suppressiveness against Fusarium wilt disease by reshaping the soil microbiome. Soil Biol. Biochem. 114, 238–247.
Yaduvanshi, N.P.S., 2003. Substitution of inorganic fertilizers by organic manures and the effect on soil fertility in a rice-wheat rotation on reclaimed sodic soil in india. J. Agric. Sci. 140, 161–168.
Zeng, L., Liao, M., Chen, C., Huang, C., 2007. Effects of lead contamination on soil enzymatic activities, microbial biomass, and rice physiological indices in soil-lead-rice, Oryza sativa L system. Ecotoxicol. Environ. Saf. 67, 67–74.
Zhang, J., Bayram, A.G., Atanasova, L., Rahimi, M.J., Przylucka, A., Yang, D., Kubicek, C.P., Zhang, R., Shen, Q., Druzhinina, I.S., 2016. The neutral metallopeptidase NMP1 of *Trichoderma guizhouense* is required for mycotrophy and self-defence. Environ. Microbiol. 18, 580–597.
Zhao, J., Ni, T., Li, Y., Xiong, W., Ran, W., Shen, B., Shen, Q., Zhang, R., 2014. Responses of bacterial communities in arable soils in a rice-wheat cropping system to different fertilizer regimes and sampling times. PLoS One 9, 85301.
Zhou, X., Guan, S., Wu, F., 2015. Composition of soil microbial communities in the rhizosphere of cucumber cultivars with differing nitrogen acquisition efficiency. Appl. Soil Ecol. 95, 90–98.