Plant Soil (2018) 423:229–240
https://doi.org/10.1007/s11104-017-3504-6

REGULAR ARTICLE

Continuous application of different organic additives can suppress tomato disease by inducing the healthy rhizospheric microbiota through alterations to the bulk soil microflora

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Received: 30 May 2017 / Accepted: 14 November 2017 / Published online: 1 December 2017

Abstract
Aims Bio-organic fertilizer and different additives are widely applied to suppress soil-borne diseases. However, how different additives alter bulk soil microflora and thereby induce the healthy rhizospheric microflora remains unclear.

Methods A 3-season field experiment containing four fertilization management programs (chemical fertilizer, organic fertilizer, amino acid organic fertilizer, and bio-organic fertilizer was conducted in a tomato production agroecosystem with high disease incidence to evaluate the induced efficacy. The bacterial and fungal microflora of bulk and rhizosphere soil influenced by different management programs were performed on the Illumina MiSeq platform. Principal coordinate analysis based on the Bray-Curtis distance metric was performed to compare the similarities and differences of the bacterial and fungal community compositions among all soil samples.
Results Soil amended with organic fertilizer, amino acid organic fertilizer, and bio-organic fertilizer progressively and significantly suppressed tomato diseases in comparison with chemical fertilizer, and bio-organic fertilizer presented the best efficacy in all seasons. Interestingly, rhizospheric and bulk soil bacterial and fungal communities of the different fertilization management programs were separated from each other. Six bacterial and 10 fungal rhizospheric genera positively correlated with the same genera observed in bulk soil showing significant relationships with tomato disease incidence were observed, and functional strain SQR9 can be detected in the bulk and rhizosphere soils of bio-organic fertilizer treatments. Additionally, the redundancy analysis results showed the genera in treated chemical fertilizer bulk soil were dominated by *Ralstonia* and *Fusarium*, the abundances of which were highest and lowest in treated chemical fertilizer and bio-organic fertilizer rhizosphere, respectively.

Responsible Editor: Birgit Mitter

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-017-3504-6) contains supplementary material, which is available to authorized users.

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Conclusions This study provided insights into soil-borne disease suppression by bulk soil management and confirmed that alterations to the bulk soil microbiota by different organic additives played distinct roles in the formation of rhizospheric soil microflora for the suppression of disease.

Keywords Bio-organic fertilizer · Bulk and rhizosphere soils · Disease incidence · Tomato · Monoculture

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated worldwide (Singh and Siddiqui 2015). However, bacterial and Fusarium wilts caused by *Ralstonia solanacearum* (Smith) (Wei et al. 2011) and *Fusarium oxysporum* f. sp. *lycopersici* (Shanmugam et al. 2015), respectively, are two common diseases throughout tomato growing areas. More seriously, these two pathogens often infect tomato roots concurrently under field conditions, resulting in complex disease occurrence and severe yield loss every year (Liu et al. 2012).

The main cause for soil-borne disease incidence is an unbalanced soil microecosystem (Avis et al. 2008). Most soil-borne pathogens are adapted to survive in bulk soil, while the rhizosphere is the playground and infection court where pathogens establish a parasitic relationship with the plant (Raaijmakers et al. 2009). The pathogens need to grow saprophytically in the rhizosphere to reach their host or to achieve sufficient numbers on their host before they can infect host tissue (Berendsen et al. 2012). It has been reported that plants actively recruit beneficial soil microorganisms in their rhizosphere to counteract pathogen assault (Mendes et al. 2011) and that rhizospheric microbial communities are influenced by soil type, plant development, fertilizer management, and other environmental factors (Chaparro et al. 2014; Horwath et al. 1998). The outcome of these factors is the development of a rhizospheric microbial community that differs markedly from the source communities in bulk soil (Minz et al. 2013). Moreover, Bakker et al. (2015) has demonstrated that different soil amendments could manipulate different bulk microbial communities, which further shows that legacy effects of prior selections in microbiotas may continue to influence rhizospheric microbial community composition. Thus, there is a need to study the relationship between the legacy effects from bulk soil to the rhizosphere on plant disease.

Chemical soil fumigation, organic amendments, and biocontrol have been suggested as control methods for bacterial and Fusarium wilts (French 1994; Bonanomi et al. 2010). Chemical soil fumigation can reduce the abundance of pathogens quickly, but pathogens might rebound to a higher abundance again (Gamliel et al. 2000). An application of organic amendments could effectively control most soil-borne pathogens (Bailey and Lazarovits 2003), however, an increase in disease incidence was also observed in several other cases after the addition of organic amendments (Mazzola et al. 2001). Recently, more studies have been performed to examine the relationships between rhizospheric microorganisms and plant diseases, which recently became popular in plant pathology (Cai and Liao 2003). Our previous study revealed that application of bio-organic fertilizer could control tomato disease via the manipulation of the rhizospheric microbial communities (Wang et al. 2015). However, how bio-organic fertilizer alters bulk soil microflora and induces the development of rhizospheric microflora remains unclear.

In this study, a 3-season field experiment was conducted in a tomato production agroecosystem with high disease incidence to evaluate the suppression of tomato disease by the following four multi-level fertilization management programs: chemical fertilizer, organic fertilizer, amino acids organic fertilizer, and bio-organic fertilizer. Afterwards, bulk and rhizospheric soil microbiotas were surveyed using Illumina-MiSeq sequencing to seek out whether rhizospheric microbiotas affected by the different fertilizer management programs exhibited varying disease suppression and how these effects were induced by bulk microbiotas alterations.

Materials and methods

Field site and experiment description

The field experimental site was located at the Nanjing Institute of Vegetable Science, Nanjing, China (31°43′ N, 118°46′ E). This region has the tropical monsoon climate with an average annual temperature and precipitation of 15.4 °C and 1106 mm, respectively. Tomato has been continuously cropped in the field for several years and has suffered from high bacterial and Fusarium wilt disease presence. The oven-dried soil had a pH

value of 7.08, and the contents of organic matter, total N, NH_4-N, NO_3-N, available P, and available K were 28.4 g/kg, 2.04 g/kg, 52.7 mg/kg, 544 mg/kg, 110 mg/kg, and 277 mg/kg, respectively.

A 3-season field experiment was performed from March 2014 to June 2015 and included the following four treatments: (1) CF treatment, soil amended with chemical fertilizer; (2) OF treatment, soil amended with organic fertilizer (chicken manure compost); (3) AOF treatment, soil amended with amino acids organic fertilizer; and (4) BF treatment, soil amended with bio-organic fertilizer. Each treatment had three randomized independent replications. The chicken manure compost was produced by Nantong Huinong Co. Ltd., Jiangsu, China by composting chicken manure at 30–70 °C for more than 20 days. The bio-organic fertilizer was produced by solid state fermentation according to Liu et al. (2016). In brief, pre-compost matured chicken manure added with 0.2 ml g^{-1} of compound liquid amino acids for 4 days (the pH of the mixture was 5.0–6.0), then antagonistic strain *Bacillus amyloliquefaciens* SQR9 (Cao et al. 2011) was inoculated into the mixture for a secondary fermentation with 4 days. After fermentation, the SQR9 cell concentration in the bio-organic fertilizer was greater than 1×10^8 CFU g^{-1} dry weight. The fertilizer without antagonistic bacteria inoculation and produced with the same procedure was regarded as amino acids organic fertilizer. All treatments were adjusted to the same amount of N (225 kg/ha), P (65 kg/ha) and K (150 kg/ha) for each season using mineral fertilizers as necessary. The N (urea) and K (K_2SO_4) fertilizers were applied as basal and supplementary fertilizer, while the P (calcium superphosphate), organic fertilizer, amino acids organic fertilizer and bio-organic fertilizer were only used as basal fertilizers. A detailed fertilization scheme is shown in Table S1.

Assay of tomato disease incidence and yield

Two months after the tomato plantlets were transplanted into the field, a bioassay for disease incidence including bacterial wilt disease, Fusarium wilt disease and the two-wilt disease complex was performed until the end of the experiment and was based on observations of typical wilt symptoms, including foliage chlorosis, necrosis and drooping of the leaves. Disease incidence was expressed as the percentage of diseased plants per total number of plants. For total tomato fruit yield of each plot, all mature tomato fruits were harvested and weighed. The disease incidence and fruit yield of each crop season (1st: spring season; 2nd: autumn season; 3rd: spring season) were analyzed in this study.

Soil sampling, DNA extraction, real-time PCR assay and soil chemical analysis

Soil sampling was performed in June 2015 during tomato harvesting. Briefly, 6 healthy tomato plants were randomly selected in each replicate plot, 3 of which were pooled to minimize the community variation. The bulk and rhizospheric soil samples were obtained according to Bakker et al. (2015). Thus, 6 bulk and rhizospheric soil samples were collected for each treatment. Finally, 5 bulk and rhizospheric soil samples for each treatment were randomly chosen for subsequent DNA extraction using the UltraClean Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, USA) according to the manufacturer's protocol. The quality and concentration of the DNA samples were determined using a spectrophotometer (NanoDrop 2000, USA). Total numbers of bacteria and fungi were quantified by Real-Time PCR with primers Eub338 and Eub 518 and primers ITS1f and 5.8 s, respectively, according to Liu et al. (2016). The copy numbers of SQR9 was also quantified by Real-Time PCR with primers SQR9F (5′-CATGAGATGGCGGGCTTT-3′) and SQR9R (5′-CGCATCCTCCCTGTCTTTG-3′) according to Qiu et al. (2014). Each sample was performed in three replicates, and the results were expressed as log (copies g^{-1}) dry soil. All bulk soil chemical properties were determined according to Bao (2010).

Illumina MiSeq sequencing

The DNA of each soil sample served as the template for the amplification of the 16S rRNA gene and the ITS region. The V4 region of the bacterial 16S rRNA gene was amplified using primers 520F (5′-AYTGGGYDTAAAGNG-3′) (Ahmed et al. 2009) and 802R (5′-TACNVGGGTATCTAATCC-3′) (Ahmed et al. 2009), and ITS1F (5′-CTTG GTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns 1993) and ITS2 (5′-GCTGCGTTCTTCAT CGATGC-3′) (White et al. 1990) were used for the ITS1 region of the fungal ITS gene. The barcodes of

soil samples used to distinguish each sample in the pyrosequencing programs in this study are provided in Table S2. The programs of amplification and sequencing of the 16S and ITS genes were performed at Personal Biotechnology Co, Ltd. (Shanghai, China) on the Illumina MiSeq instrument (USA). All sequences were deposited in the NCBI Sequence Read Archive database with the accession number (SRP067366).

Bioinformatics analysis

Quality control and annotation of the raw sequences were performed according to Liu et al. (2016). A total of 36,473 16S rRNA and 41,614 ITS gene sequences for each sample were randomly selected for further bacterial and fungal microbial community analysis, respectively. To compare the similarities and differences of the bacterial and fungal community compositions among all soil samples, principal coordinate analysis (PCoA) based on the Bray-Curtis distance metric was performed using Mothur (Liu et al. 2016) and analysis of molecular variance (AMOVA) was performed to evaluate the significant differences in bacterial and fungal community structures among the four fertilizer treatments. AMOVA was used to compare the relative abundance of different groups according to the ordination base on OTU. In addition, Pearson's correlation coefficient was used to evaluate the correlation between selected rhizospheric soil genera (relative abundance >0.1%) and tomato disease incidence. Afterwards, the relative abundances of genera that showed significant differences with disease incidence were further compared among the different fertilizer management programs in bulk and rhizospheric soil. Furthermore, Pearson's correlation coefficient was used to evaluate the correlation between the relative abundances of these genera in bulk and rhizospheric soil samples. In order to examine the relationships among the bulk soil bacterial and fungal genera, samples and environmental variables, redundancy analysis (RDA) was carried out via the vegan package of R (version 3.2.2), bioEnv procedure was performed to select the best subset of environmental variables. In addition, the Mantel test was used to calculate the correlation between the selected soil characteristics and the selected microbial genera.

Statistical analysis

The differences among the different treatments were assessed using a one-way ANOVA analysis, and the calculated means were subjected to Duncan's multiple range test at $P < 0.05$. All analyses were performed in SPSS v18.0 (SPSS Inc., USA).

Results

Effects of different fertilization management programs on disease incidence and tomato yield

Disease incidence in the spring crop season in 2014 and 2015 was significantly higher (20–75%) than that in the autumn season (0–10%) in 2014 (Fig. 1a), and the disease incidence trends of all the seasons was similar [DI (CF) > DI (OF) > DI (AOF) > DI (BF)]. In the third season, the OF, AOF and BF treatments significantly ($P < 0.05$) reduced disease incidence to 30%, 16% and 6%, respectively. These results indicated that the bio-organic fertilizer application more effectively controlled the outbreak of wilt disease in tomato plants compared to the other treatments. In contrast to the disease incidences, tomato yields with the different fertilization treatments were significantly higher than CF in all crop seasons (Fig. 1b). For the third crop season, the application of organic fertilizer, amino acid organic fertilizer and bio-organic fertilizer significantly ($P < 0.05$) increased yield by 13%, 45% and 70%, respectively, compared to the CF treatment. These results indicated that the fertilization treatments (OF, AOF, and BF) progressively suppressed disease incidence and increased tomato crop yields compared to the CF treatment.

Total bacterial, fungal abundances and SQR9 copy numbers

Compared to the CF treatment, the treatments of BF, AOF, and OF significantly ($P < 0.05$) increased bulk soil bacteria abundances (Fig. 2a). Similarly, rhizospheric bacteria abundances were also significantly ($P < 0.05$) enhanced. Moreover, no significant differences with fungi were observed in bulk or rhizospheric soils, regardless of fertilization management program (Fig. 2b). These results showed that the management programs that contained organic fertilizer (organic fertilizer, amino acid organic fertilizer and bio-organic fertilizer) had a positive effect on the abundance of bulk and rhizospheric bacteria rather than fungi compared to the CF treatment. The copy numbers (Log10

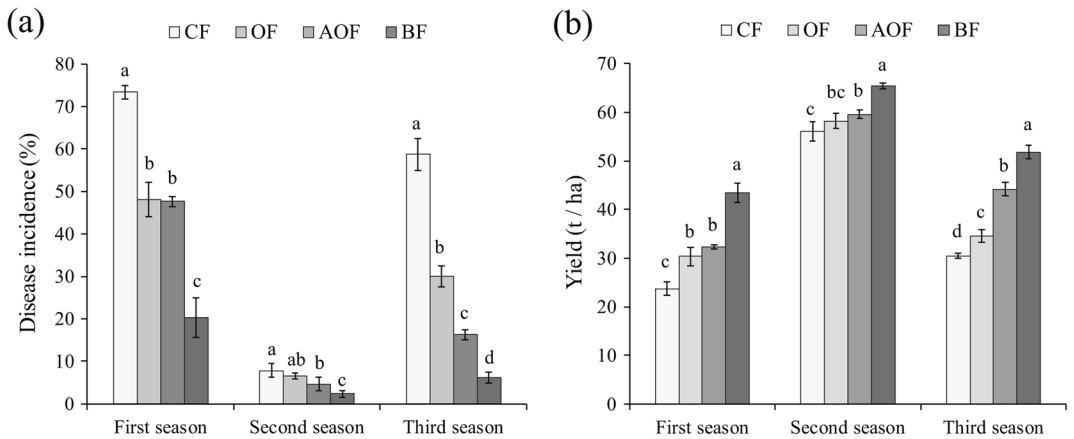

Fig. 1 Effects of different fertilization management programs on disease (**a**) and yield (**b**) of tomato. Fertilization management programs: chemical fertilizer (CF), organic fertilizer (OF), amino acids organic fertilizer (AOF) and bio-organic fertilizer (BF)

copies) of SQR9 in bulk and rhizosphere soils of BF treatment were 4.48 and 5.17 (Fig. 2c), respectively, while it cannot be detected in the other treatments of CF, OF and AOF.

Sequencing results

As shown in Table S3, after basal quality control, a total of 2,171,613 16S rRNA and 3,548,914 ITS sequences were obtained for all soil samples. The number of high quality sequences per sample varied from 36,743 to 127,938 for bacteria and from 41,614 to 141,100 for fungi. Moreover, at the 97% similarity cut-off level, 7579 bacterial and 4517 fungal OTUs were obtained.

Microbial community composition

PCoA based on the Bray-Curtis distance metric revealed that the bulk soil bacterial (Fig. 3a) and fungal (Fig. 3b) communities significantly differed ($P < 0.001$) from those in the rhizosphere along the first component. Interestingly, the rhizospheric soil bacterial communities of all treatments were distinct ($P < 0.05$) from each other along the second component, showing the same separation tendency ($P < 0.05$) as the bulk soil. Similarly, the rhizospheric soil fungal communities for the different fertilization managements were all separated ($P < 0.05$) from each other along the second component with the same alteration trends ($P < 0.05$) for rhizospheric and bulk soil samples.

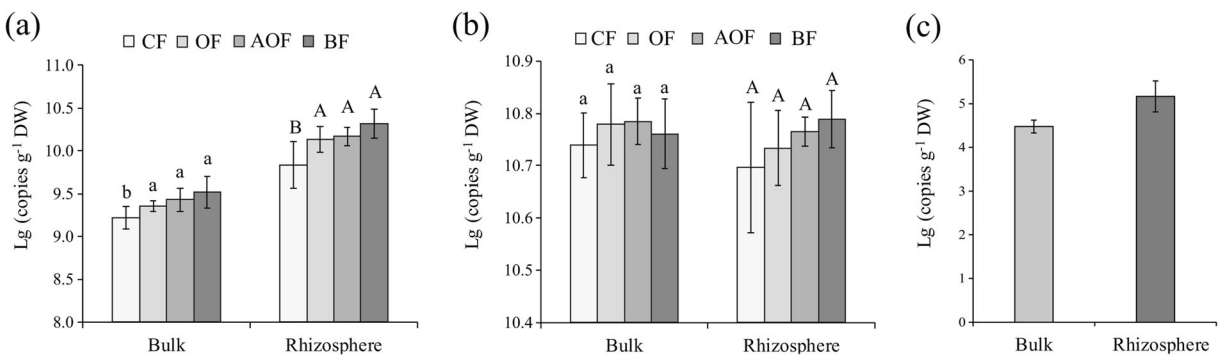

Fig. 2 Total bacterial (**a**) and fungal (**b**) microbial copies in bulk and rhizospheric soil with the different fertilization management programs. The copy numbers of SQR9 in the bulk and rhizosphere soil of BF treatment (**c**). Bars with different letters indicate significant differences as defined by Duncan's test ($P < 0.05$). Fertilization management programs: chemical fertilizer (CF), organic fertilizer (OF), amino acids organic fertilizer (AOF) and bio-organic fertilizer (BF)

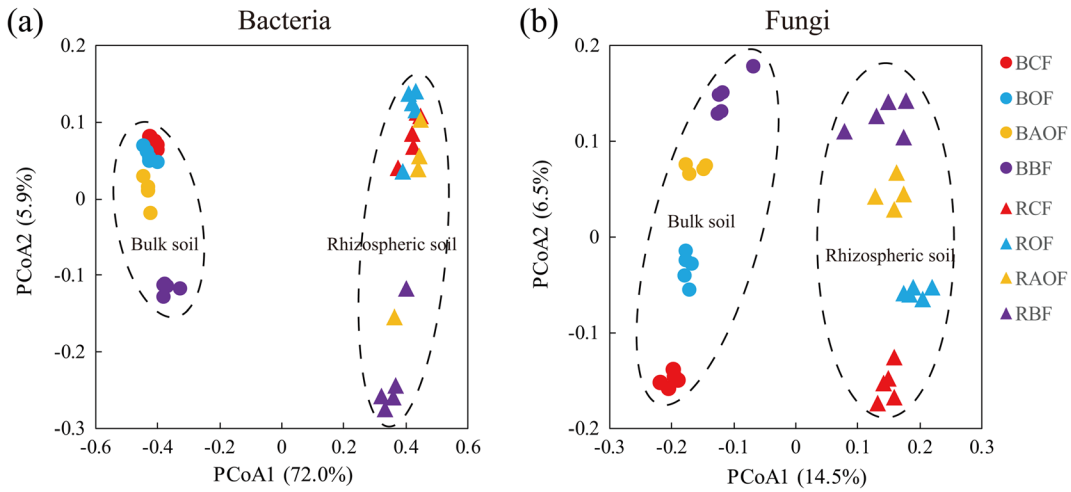

Fig. 3 The bacterial (**a**) and fungal (**b**) microbial community compositions of the different treatments. Fertilization management programs: chemical fertilizer (CF), organic fertilizer (OF), amino acids organic fertilizer (AOF) and bio-organic fertilizer (BF); bulk soil amended with chemical fertilizer (BCF), organic fertilizer (BOF), amino acids organic fertilizer (BAOF) and bio-organic fertilizer (BBF); rhizosphere soil amended with chemical fertilizer (RCF), organic fertilizer (ROF), amino acids organic fertilizer (RAOF) and bio-organic fertilizer (RBF)

Genera abundance analysis

Clear positive correlations between disease incidence and the relative abundances of *Bacillus* ($P < 0.05$), *Ralstonia* ($P < 0.05$), *Fimetariella* ($P < 0.01$), *Fusarium* ($P < 0.01$), *Gliomastix* ($P < 0.01$), *Guehomyces* ($P < 0.01$), *Humicola* ($P < 0.01$), *Penicillium* ($P < 0.01$), and *Trichoderma* ($P < 0.01$), were observed. In contrast, negative correlations were observed for the genera *Chitinophaga* ($P < 0.05$), *Enterobacter* ($P < 0.05$), *Pseudomonas* ($P < 0.05$), *Pseudoxanthomonas* ($P < 0.05$), *Debaryomyces* ($P < 0.01$), *Phialemonium*, and *Purpureocillium* ($P < 0.01$) (Table 1).

Table 1 Spearman's rank correlation coefficient between rhizosphere abundant genus and disease incidence

	Disease incidence
Bacillus	0.51*
Chitinophaga	−0.64**
Enterobacter	−0.46*
Pseudomonas	−0.46*
Pseudoxanthomonas	−0.54*
Ralstonia	0.56*
Debaryomyces	−0.83**
Fimetariella	0.57**
Fusarium	0.83**
Gliomastix	0.78**
Guehomyces	0.88**
Humicola	0.63**
Penicillium	0.81**
Phialemonium	−0.47*
Purpureocillium	−0.71**
Trichoderma	0.66**

Statistical significance at *P < 0.05 and **P < 0.01

Among these genera, the treatment of application of PGPR-containing organic fertilizer (BF) significantly ($P < 0.05$) increased the abundance of *Chitinophaga*, *Pseudomonas*, *Debaryomyces*, *Phialemonium* and *Trichoderma* and decreased the abundance of *Humicola* compared to the other treatments (CF, OF and AOF) in bulk soil (Fig. S1). Moreover, the lowest value of *Fusarium* was observed in the BF treatment, which was significantly ($P < 0.05$) lower than that in CF. Additionally, the AOF treatment showed the lowest relative abundance of *Ralstonia* and the highest relative abundance of *Penicillium* and *Purpureocillium* among all treatments.

Similar to bulk soil, the same variation trends of the relative abundances of *Chitinophaga*, *Pseudomonas*, *Pseudoxanthomonas*, *Debaryomyces*, *Fusarium* and *Guehomyces* were observed in the BF treated rhizosphere, and the highest and lowest values of the former four and latter two were observed. The AOF treatment significantly ($P < 0.05$) enriched the relative abundances

of *Debaryomyces* and *Purpureocillium* compared to the CF and OF treatments. In addition, the organic fertilizer containing treatments (OF, AOF and BF) significantly reduced the relative abundance of *Fusarium* compared to CF and amended with bio-organic fertilizer showed significantly lower relative abundances of *Ralstonia* and *Fusarium* compared to other treatments.

Although the correlation coefficients of *Ralstonia* and *Fusarium* between bulk and rhizospheric soils were not significant ($P > 0.05$), the two genera including pathogens still showed a positive relationship (Fig. 4). Particularly, 8 genera of *Chitinophaga*, *Pseudomonas*, *Pseudoxanthomonas*, *Debaryomyces*, *Guehomyces*, *Humicola*, *Phialemonium,* and *Purpureocillium* in bulk soil had a significant ($P < 0.01$) and positive relationship with the corresponding genera in rhizosphere soil, and no genus showed a significantly ($P < 0.05$) negative relationship between the bulk and rhizospheric soils, indicating that the bulk microbiota is crucial and critical to the development of the rhizospheric microflora and suppressing tomato disease.

Effects of soil chemical properties on bacterial or fungal taxa

The three crop seasons with the different fertilization management programs changed the soil chemical properties (Table 2). The fertilization management programs containing organic fertilizer (OF, AOF and BF) significantly ($P < 0.05$) increased soil OM, TP and AP contents compared to the CF treatment. In addition, fertilizer management programs containing amino acids (AOF and BF) significantly ($P < 0.05$) enhanced soil EC and decreased soil TK and NH_4-N concentrations compared to the fertilizer management programs that did not contain amino acids (CF and OF). Moreover, the BF treatment showed significantly ($P < 0.05$) higher NO_3-N, TN, and AK contents and a lower pH value compared to the other treatments.

The Mantel test based on the selected soil chemical properties and the abundances of the analyzed microbial genera revealed that the selected soil chemical properties were significantly correlated with variations in the

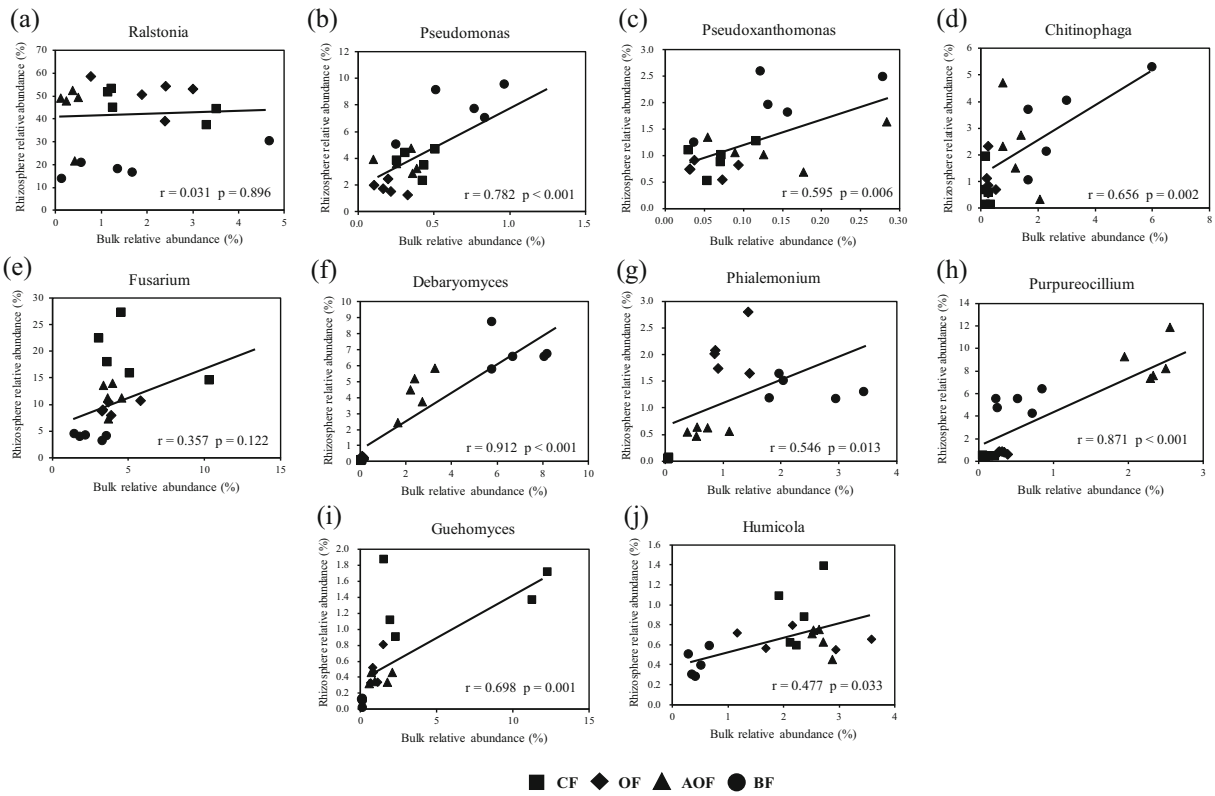

Fig. 4 Spearman's rank correlation coefficient between rhizospheric abundant genera and the same bulk abundant genera. Fertilization management programs: chemical fertilizer (CF), organic fertilizer (OF), amino acids organic fertilizer (AOF) and bio-organic fertilizer (BF)

Table 2 Physicochemical properties of bulk soil samples under the different fertilization treatments

	BCF	BOF	BAOF	BBF
pH	7.64 ± 0.16 a	7.81 ± 0.18 a	7.72 ± 0.10 a	7.18 ± 0.08 b
EC (μS/cm)	309 ± 11 c	341 ± 8 c	531 ± 33 b	806 ± 66 a
NH_4-N (mg/kg)	13.8 ± 0.9 b	15.1 ± 0.6 a	11.5 ± 0.7 c	12.9 ± 0.9 b
NO_3-N (mg/kg)	229 ± 12 b	235 ± 9 b	237 ± 9 b	256 ± 6 a
Total N (g/kg)	1.97 ± 0.04 b	1.97 ± 0.01 b	1.94 ± 0.02 b	2.15 ± 0.05 a
Organic Matter (g/kg)	24.6 ± 0.4 c	28.1 ± 0.3 b	28.3 ± 0.5 ab	29.0 ± 0.8 a
Total P (g/kg)	0.54 ± 0.08 c	0.61 ± 0.02 b	0.60 ± 0.03 b	0.64 ± 0.02 a
Available P (mg/kg)	106 ± 7 b	116 ± 3 a	117 ± 6 a	114 ± 7 a
Total K (g/kg)	5.33 ± 0.28 a	5.27 ± 0.18 a	5.00 ± 0.12 b	4.88 ± 0.30 b
Available K (mg/kg)	155 ± 12 b	159 ± 14 b	164 ± 2 b	194 ± 9 a

Values (means ± SD, $n = 5$) within the same row followed by different letters are significantly different at $P < 0.05$ according to Duncan's test. Fertilization management programs: chemical fertilizer (CF), organic fertilizer (OF), amino acids organic fertilizer (AOF) and bio-organic fertilizer (BF); bulk soil amended with chemical fertilizer (BCF), organic fertilizer (BOF), amino acids organic fertilizer (BAOF) and bio-organic fertilizer (BBF)

selected bacterial and fungal genera ($r = 0.85$, $P < 0.001$). The redundancy analysis performed to examine the relationship between the analyzed genera from bulk soil and soil chemical properties showed that the two components could explained 49.07% of the total variation (Fig. 5). The second component (RDA2), which explained 16.36% of the variation, separated BF from the other treatments. As shown in Fig. 5, the microbial genera in the BF soil samples were dominated by *Trichoderma*, *Pseudomonas*, *Chitinophaga* and were related to TN, EC and OM contents, while the genera in the CF soil samples were dominated by *Ralstonia* and *Fusarium*. Moreover, the microbial community in the AOF were dominated by *Penicillium*, *Purpureocillium* and *Fimetariella*; and in OF soil samples were dominated by *Bacillus*, *Humicola* and *Fimetariella*.

Discussion

Applications of organic fertilizer, amino acid organic fertilizer, and bio-organic fertilizer progressively and significantly suppressed plant disease and improved fruit yield compared to the chemical fertilizer. These results were in agreement with previous studies suggested that organic amendments could be used to control diseases caused by soil-borne pathogens (Hoitink and Fahy 1986; Szczech 1999). More importantly, the most efficacious disease suppression was observed in the BIO treatment, which is in accordance with previous reports where bio-organic fertilizer acted as both an organic fertilizer and a biocontrol agent for different soil-borne diseases in tomato (Wang et al. 2015), banana (Shen et al. 2013), and watermelon (Ling et al. 2014) when compared to organic amendments and biocontrol agents applied alone.

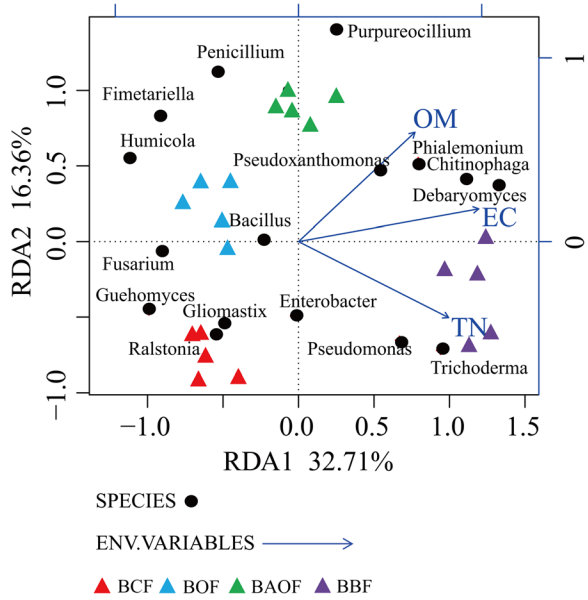

Fig. 5 Redundancy analysis of the relationship between the analyzed bulk genera, samples and environmental variables. Fertilization management programs: chemical fertilizer (CF), organic fertilizer (OF), amino-acids organic fertilizer (AOF) and bio-organic fertilizer (BF). Bulk soil amended with chemical fertilizer (BCF), organic fertilizer (BOF), amino acids organic fertilizer (BAOF) and bio-organic fertilizer (BBF)

The fertilizer management programs (OF, AOF and BF) all significantly increased bacterial abundances when compared with CF and was similar to previous studies where higher populations of bacteria in organic treated soils were observed compared with that from a chemical fertilizer treatment (Witter et al. 1993). Interestingly, bacterial abundances in rhizospheric soils of these treatments were also higher compared to CF, indicating that bulk soil management induced microbial abundance variation in the rhizosphere. Functional strain SQR9 can be detected in the bulk and rhizosphere soils of BF treatments, suggesting that SQR9 could survive in the tomato bulk soil and further colonize in the rhizosphere soil. This result was in accordance with the other report as SQR9 could effectively colonize in the rhizosphere soil and suppress the cucumber plant disease (Qiu et al. 2014).

PCoA results revealed that the bacterial and fungal communities in the bulk soils of the different treatments were well differentiated from the rhizospheric communities along the first component, which were mainly due to the ability of root exudates to influence the composition of the rhizospheric microbial communities (Chaparro et al. 2014). Interestingly, in both bulk and rhizospheric soils, bacterial and fungal microbial communities of the different fertilization management programs were well separated from each other along the second component. In accordance with our results, Bonanomi et al. (2010) also reported that an application of organic amendments significantly shifted the soil microbial community. Moreover, amino acids are excellent C and N sources for microbes (Ge et al. 2009), and may induced microbial communities variation. Lots of researches have confirmed that the PGPR-containing bio-organic fertilizers had a considerable effect on shaping the microbial community (Wang et al. 2015; Shen et al. 2013; Ling et al. 2014). The same alteration trends for the bacterial and fungal communities for rhizospheric and bulk soil samples were observed. Ridder-Duine et al. (2005) also demonstrated that the rhizosphere microbial composition of the wild plant *C. arenaria* was largely dependent on the microbial composition of the bulk soil. Hence, we can deduce that the different fertilization management programs altered the microbial community in bulk soil first, inducing their specific rhizospheric microbiotas.

The rhizospheric genera *Ralstonia*, *Fusarium*, *Bacillus*, *Fimetariella*, *Gliomastix*, *Guehomyces*, *Humicola*, *Penicillium*, and *Trichoderma* were positively and significantly ($P < 0.05$) correlated with tomato disease incidence. In contrast, a significantly ($P < 0.05$) negative relationship was observed for genera *Chitinophaga*, *Enterobacter*, *Pseudomonas*, *Pseudoxanthomonas*, *Debaryomyces*, *Phialemonium*, and *Purpureocillium*. Because all the tested plants in the different treatments were healthy, the healthy plants in high disease incidence treatments contained more relative abundances of the rhizospheric genera *Ralstonia* and *Fusarium*, increasing the risk of plant illness (Wei et al. 2011; Shanmugam et al. 2015). Moreover, studies have confirmed that the genera *Bacillus* (Singh and Siddiqui 2015), *Penicillium* (Sabuquillo et al. 2006), and *Trichoderma* (Blaya et al. 2013) act as biocontrol agents could induce a resistance capacity of plants that provides protection against of the microbial pathogens (Ent et al. 2009). *Pseudomonas* was reported to suppress plant disease in several studies (Dowling and O'Gara 1994; Singh and Siddiqui 2015). *Enterobacter* has been reported for bioactivity against *Fusarium* (Al-Mughrabi 2010). *Pseudoxanthomonas* is known as a biocontrol agent against *Xanthomonas* (Al-Saleh 2014). *Debaryomyces* has been reported as a biocontrol agent against *Penicillium expansum* (Singh et al. 2011), and *Purpureocillium* has been found exhibiting bio-control of *Meloidogyne incognita* (Singh et al. 2013). However, the role played by these three genera for suppressing *Ralstonia* and *Fusarium* are still unclear. Xue et al. (2015) reported that the relative abundance of *Bacillus* was negatively correlated with banana disease incidence, while in this study, the relative abundance of *Bacillus* was positively correlated with pathogen abundance and disease incidence as well as *Trichoderma*. Previous study observed when plant infected by pathogens, plant roots would secrete more citric acid and fumaric acid to stimulate the chemotaxis response of plant growth promoting rhizobacteria (Liu et al. 2014). This may be the reason why more *Bacillus* and *Trichoderma* occurred in the higher plant disease incidence rhizosphere soil. Furthermore, of the 16 rhizospheric genera, 8 showed a significant ($P < 0.01$) positive relationship to the abundances of the same genera in bulk soil, while none of them showed a significantly ($P < 0.05$) negative relationship. Similar dynamics were observed across the communities, such as the relative abundance of Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes and Acidobacteria, as the rhizosphere developed from bulk soil communities (Bakker et al. 2015). Therefore, our results suggested

that the bulk microbial community played the crucial and critical role in manipulating the rhizospheric microflora and suppressing plant disease.

The fertilizer management programs containing organic fertilizer (OF, AOF and BF) significantly ($P < 0.05$) increased soil OM compared to CF, which were consistent with a previous study (Haynes and Naidu 1998). The fertilizer management programs containing amino acids (AOF and BF) significantly ($P < 0.05$) enhanced soil EC and decreased NH_4-N compared to the fertilizer management programs containing no amino acids (CF and OF). Amino acids can form complexes with cations via carboxylate (−COO) and amine ($-NH_2$) groups (Dalir and Khoshgoftarmanesh 2014) and might be the reason for the enhancement of soil EC. Yadessa et al. (2010) also found that there were significantly ($P < 0.01$) negative correlations between tomato bacterial wilt disease incidence and EC. The higher soil NH_4-N content in the fertilizer treatments with no amino acids may be due to the application of the chemical nitrogen (urea breaks down to NH_4-N) fertilizer (Shen et al. 2013). Moreover, the BF treatment showed significantly ($P < 0.05$) higher soil NO_3-N content than the CF, OF and AOF treatments. Huber and Watson (1974) reviewed that tomato root disease caused by *Fusarium* could be reduced by decreased NO_3-N and increased NH_4-N contents. In contrast, it was reported that bacterial wilt of tomato increased as NO_3-N increased and was reduced as NH_4-N was reduced (Yadessa et al. 2010). Although the processes of disease suppression were different, the possible key factor of disease suppression was to decrease the rhizospheric population of pathogens (Nel et al. 2007). Additionally, the genera in CF bulk soil were dominated by *Ralstonia* and *Fusarium*. While, this phenomena was not observed in OF, AOF and BF treatments. Those results also suggested that soil amended with organic fertilizers have a high efficacy for suppressing the diseases. Strikingly, the application of bio-organic fertilizer showed the highest efficacy.

Conclusion

In the present study, the application of different additives in a three-season field experiment showed fertilization management programs (organic fertilizer, amino acid organic fertilizer and bio-organic fertilizer) progressively and significantly decreased soil-borne diseases and enhanced fruit yields of tomato compared to the CF treatment. Bulk soil microbial compositions in agroecosystems were significantly affected by the different fertilizer management programs, and the altered bulk soil microbial communities played a crucial role in manipulating rhizospheric soil microflora. Compared to the CF treatment, OF, AOF and BF had a high efficacy for suppressing the tomato disease, while BF possessed the highest efficacy. This study provides insights into the soil-borne disease suppression by bulk soil management to induce healthy rhizospheric soil microflora.

Acknowledgements This research was supported by the National Key Basic Research Program of China (2015CB150506), the National key research and development program (2016YFD0800605, 2016YFE0101100 and 2016YFD0200106), the National Natural Science Foundation of China (41571242), Jiangsu Science and Technology Program (BE2014398, BY2016077-05), the Natural Science Foundation of Jiangsu Province, China (BK20160710 and BK20150059), the Priority Academic Program Development of the Jiangsu Higher Education Institutions (PAPD), 111 project (B12009), and Jiangsu Key Laboratory for Solid Organic Waste Utilization (BM2011013).

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