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ORIGINAL ARTICLE

Intercropping with marigold promotes soil health and microbial structure to assist in mitigating tobacco bacterial wilt

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Abstract

Marigold is reported to have antibacterial activity, and effectively protect crops against soil-borne diseases. However, it is not known whether and how tobacco bacterial wilt (TBW) could be mitigated via intercropping with marigold under field conditions. In this study, a field experiment was performed to measure and compare the occurrence of TBW, the soil chemical properties, and soil microbial composition and diversity between a tobacco-marigold intercropping system and a tobacco monocropping system. At 100 days (d) post-transplantation, the incidence (*I*) and disease index (*DI*) for the tobacco-marigold intercropping system were 30.12% and 58.25% lower than that for tobacco monocropping system, respectively. The results showed that Sobs, Shannon and Chao 1 index of soil bacterial communities in the tobacco-marigold intercropping system were 10.34%, 1.41% and 5.13% higher than that in the tobacco monocropping system at 100 d post-transplantation, respectively. It exhibited a higher richness and diversity of soil bacterial communities in the tobacco-marigold intercropping system. The relative abundance of some beneficial genera in tobacco-marigold intercropping system, such as *Lysobacter*, *Burkholderia*, *Trichoderma*, *Mortierella*, *Chaetomium, Penicillium*, was 1.50, 1.61, 3.35, 1.67, 4.40 and 4.50 fold higher than that in tobacco monocropping system. The presence of the intercropping system inhibited soil acidification and loss of soil calcium ions. The redundancy analysis (RDA) indicated that soil pH and exchange Ca^{2+} were the main environmental factors which seemed to influence the bacterial and fungal community. The results from this study provided valuable insight into the possible mechanisms enhancing soil health in the tobacco-marigold intercropping system.

Keywords Marigold · Tobacco bacterial wilt · Intercropping · Soil chemical properties · Microbial communities

Introduction

Tobacco bacterial wilt (TBW), caused by *Ralstonia solanacearum* phylotype I, is one of the major soil-borne diseases affecting cultivated tobacco (*Nicotiana tabacum*) (Liu et al. 2013). *R. solanacearum* is considered the most important and destructive bacterial plant pathogen (Mansfied et al. 2012) for its devastating lethality (Yabuuchi et al. 1995), wide host range (Denny 2006), and worldwide distribution (Elphinstone 2005; Liu et al. 2009). As such, TBW caused by *R. solanacearum* results in serious yield and economic losses (Elphinstone 2005; Yuliar et al. 2015).

It was previously reported that the frequency of TBW has increased with the persistence of monocropping (Shiomi et al. 1999; Niu et al. 2017). Many studies have indicated that use of the monocropping system, a very common worldwide agricultural practice, can inhibit plant growth and is associated with the development of serious soil-borne diseases, particularly bacterial wilt (Hiddink et al. 2009; Larkin et al. 2011; Yang et al. 2012; Zhang et al. 2013; Guo et al. 2014; Liu et al. 2014). Studies have shown that, by maintaining multiple crop species in an ecosystem, intercropping can help in increasing the activity and diversity of rhizosphere soil microorganisms (Li et al. 2014), optimizing microbial community structure (Wu et al. 2018; Tian et al. 2019), and then preventing crop

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vulnerability to biotic stresses (Flombaum and Sala 2008; Newton et al. 2009). Some soil-borne diseases, such as watermelon fusarium wilt (Ren et al. 2008; Su et al. 2008; Hao et al. 2010), soybean red crown rot (Gao et al. 2014), tomato bacterial wilt (Michel et al. 1997; Yu 1999), peanut root rot (Li et al. 2014), faba bean fusarium wilt (Dong et al. 2016), konjac soft rot (Wu et al. 2018), could be in some cases effectively prevented and/or controlled by intercropping. As a consequence, intercropping is now more widely utilized in Asia, Latin America, America and Africa, and is roundly popular with farmers worldwide (Ratnadass et al. 2012; Boudreau 2013).

Marigold (*Tagetes erecta* L.) is a multipurpose crop used for ceremonial, ornamental, medical and pharmaceutical purposes, which has antimicrobial properties (Gómez et al. 2003). There are many examples of the successful use of marigold in controlling crop diseases. In particular, marigolds are well-known for their ability to suppress 14 genera of plant-parasitic nematodes (Hooks et al. 2010). As reported, nematode infestations in tomato, okra, eggplant, angelica and soybean could effectively be reduced by intercropping, rotating or covering with marigold (El-Hamawi et al. 2004; Kumar et al. 2005; Hooks et al. 2010; Xie et al. 2017). Moreover, the early blight disease of tomato could be alleviated by intercropping with marigold (Gómez et al. 2003). Importantly, marigolds have been in one case reported to successfully suppress *R. solanacearum* when used as a rotational or intercropping plant under greenhouse conditions (Terblanche 2007). In order to better understand the TBW suppressing effects of a tobacco-marigold intercropping, in this study apart from disease monitoring, soil samples were collected from tobacco farmlands and analyzed by 16S rRNA and internal transcribed spacer (ITS) gene sequencing. This is to explore microbial changes that may underlie the mechanism of enhanced soil health and disease suppressing characteristics in the tobacco-marigold intercropping system.

Materials and methods

Field experiment and study site

Field experiments were carried out in the Xuan'en area (29.97°N, 109.38°E), Hubei province, China. The field, 792 m^2 in size, used in this study had a 15 year history of continuous tobacco cultivation, and incidence of TBW was higher than 95% every year for the past five years before this study. The experimental design consisted of three blocks, each 264 m^2 in size. Each block was divided into two plots of 132 m^2, representing the two plantation systems. Treatments levels included (1) tobacco monocropping system (C-field) where tobacco (cv. Yunyan87) was planted 0.55 m apart in a row and 1.2 m between rows, and (2) tobacco-marigold intercropping system (I-field) for 3 years (from 2015 to 2017) where the planting density of tobacco was same as tobacco monocropping system and marigolds were planted in ridges between two rows of tobaccos. 200 tobacco plants were in per plot.

Monitoring disease occurrence

Tobacco seedlings were cultivated in greenhouse and sterile tobacco plants with 4–5 leaves were transplanted into field on April 30th, 2017. Symptoms of TBW across the C-field and I-field were monitored at five separate sites in each plot at 50 d and 100 d post-transplantation, respectively. 24 plants were monitored in each separate site, thus 120 plants were monitored in each plot. The following scale (Li et al. 2016b) was used for disease recording per plant: 0 = plants without visible symptoms; 1 = presence of occasional chlorotic spots on stems, or less than half of the leaves wilted on unilateral stems; 3 = presence of a black streak less than half the height of the stem, or between half to two-thirds of the leaves wilted on unilateral stems; 5 = presence of a black streak over half the length of the stem, but not reaching the top of the stem, or more than two-thirds of the leaves wilted on unilateral stems; 7 = presence of a black streak reaching the top of the stem, or all leaves wilted; and 9 = dead plant. Based on the number of plants in each rating scale, incidence (*I*) and disease index (*DI*) of TBW were calculated as $I = n'/N \times 100\%$ and $DI = \sum(r \times n)/(N \times 9) \times 100$, where n' is the total number of infected tobacco plants, r is the rating scale of disease severity, n is the number of infected tobacco plants with a rating of r, and N is the total number of tobacco plants tested.

Soil sampling

Rhizosphere soils (soil around the plant roots) were sampled at five separate sites in each plot at 50 d and 100 d post-transplantation when recording the disease occurrence. Then the soil samples from the five separate sites were mixed to one soil sample for each plot. The above samples were denoted as C_50 and C_100 for C-field, and I_50 and I_100 for I-field. Before transplantation (0 d), soil samples from cultivated soil (10–25 cm soil layer) were gathered similar to the same sampling method used for rhizosphere soils, which were named as C_0 for C-field and I_0 for I-field, respectively. In total, 18 samples, each of 100–150 g, were collected. Each soil sample was partitioned into two sub-samples of 50–75 g, one was stored at −80 °C for further DNA analysis, and the other was air-dried for testing of chemical properties.

Determination and differential analysis of soil chemical properties

The analysis of soil chemical properties, including soil pH, organic matter (OM), hydrolysable nitrogen (HN), available phosphorous (AP), available potassium (AK), exchangeable calcium (Ca^{2+}) and exchangeable magnesium (Mg^{2+}), was performed according to Li et al. (2015). Using SPSS Statistics 22.0 (SPSS, Chicago, Illinois, USA), the differences of soil chemical properties between the C-field and I-field were compared at 0 d, 50 d and 100 d post-transplantation by Student's *t* test, respectively.

Soil DNA extraction

Soil microbial genomic DNA was extracted from all 18 soil samples using the FastDNA Spin Kit (MP Biomedicals, USA), according to the manufacturer's instructions. The quantity and purity of the DNA samples were determined using Thermo Scientific™ NanoDrop™ One.

Microbial rRNA gene amplification and Illumina sequencing

The extracted soil genomic DNA was used as template to amplify 16S rRNA and internal transcribed spacer (ITS) rRNA genes, respectively. The V4 region of the 16S rRNA gene was amplified using primers 515 forward (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806 reverse (5′-GGACTACHVGGGTWTCTAAT-3′), and the ITS1 region of ITS rRNA gene was amplified using primers ITS5–1737 forward (5′- GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2–2043 reverse (5′-GCTGCGTTCTTCATCGATGC-3′). Sequencing libraries were generated using the TruSeq DNA PCR-Free Library Preparation Kit for Illumina following the manufacturer's recommendations. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Termo Scientifc) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina HiSeq platform at the Novogene Bioinformatics Institute, Beijing, China, and 250 bp paired-end reads were generated.

Microbial community analysis

All effective tags of all samples were clustered using Uparse software (Version 7.0.1001). Sequences with ≥99.5% identity for 16S rRNA and sequences with ≥97% identity for ITS were assigned to the same OTUs (operational taxonomic units). For each representative 16S rRNA sequence, the Silva Database (http://www.arb-silva.de/) and the Mothur algorithm was used to annotate the taxonomic information for bacteria. For each representative ITS sequence, the Unite Database (http://unite.ut.ee/) and Blast algorithm, which was calculated by QIIME software (Version 1.7.0), were used to annotate taxonomic information for fungi. Three indices, 1) species observed (Sobs), 2) the Shannon diversity index and 3) the Chao1 richness index (Hill et al. 2003), were calculated to evaluate the richness and diversity of the soil microbial community. The difference in these indices between C-field and I-field were analyzed using Student's *t* test and a value of $P < 0.05$ was considered as statistically significant. The principal coordinates analysis (PCoA) with the weighted Unifrac distance was carried out using R (Version 2.15.3).

At phylum level, the relative abundances of bacterial phyla and fungal phyla in each sample were analyzed. Venn diagrams were drawn to highlight the number of common and special genus between the different analyzed samples. At the genus level, hierarchical cluster (Heat-map) analyses based on the microbial community profiles were generated using the gplots package of R (Version 2.15.3), which described the similarity and distinction between the samples according to the color difference in the Heat-map. On the basis of the annotation for bacteria at species level, the relative abundance of *R. solanacearum* in C-field and I-field were analyzed.

Redundancy analysis (RDA) and Spearman correlation using the vegan package in R (Version 2.15.3) was performed to analyze the relationships between microbial community structure and environmental variables.

Results

The incidence/severity of TBW

Symptoms of TBW were recorded at 50 d and 100 d post-transplantation, and the incidence (*I*) and disease index (*DI*) were calculated. At 50 d post-transplantation, the *I* and *DI* for the I-field were 68.31% and 75.54% lower than that for C-field ($P < 0.01$), respectively. And at 100 d post-transplantation, the *I* and *DI* for the I-field were 30.12% and 58.25% lower than that for C-field ($P < 0.01$), respectively (Table 1),

Table 1 The occurrence of tobacco bacterial wilt in marigold-tobacco intercropping system (I) and tobacco monocropping system (C)

	50 days post-transplantation		100 days post-transplantation	
	Incidence(%)	Disease Index	Incidence(%)	Disease Index
C-field	22.78 ± 2.55A	3.27 ± 0.28A	97.78 ± 0.96A	37.41 ± 0.96A
I-field	7.22 ± 0.96B	0.80 ± 0.11B	68.33 ± 2.89B	15.62 ± 1.12B
p	0.004	0.002	0.001	0.000

For the same index, different capital letters in the same column represented the incidence and disease index of tobacco bacterial wilt showed significant differences at $p < 0.01$ based on T-test between C-field and I-field

indicating that 3 years of intercropping effectively restrained the incidence and severity of TBW.

Soil chemical properties

Seven chemical properties of soil from the C-field and I-field at 0 d, 50 d and 100 d post-transplantation were analyzed (Supplementary Table 1). There was no significant difference in hydrolysable nitrogen (HN), available phosphorous (AP), available potassium (AK) and exchangeable magnesium (Mg^{2+}) content between the two fields.

Soil organic matter (OM) is the fraction of the soil that consists of plant or animal tissue in various stages of breakdown. The OM in soils from the I-field was significantly higher ($P < 0.01$) 45.28% and 34.48% than that from the C-field at 50 d and 100 d post-transplantation, respectively. Interestingly, from 0 d to 100 d post-transplantation, there was almost no change of the soil pH in the I-field, while the soil pH in the C-field decreased by 0.32. At 100 d post-transplantation, the soil pH in the I-field was significantly higher ($P < 0.01$) 0.57 than that in the C-field. The exchangeable calcium (Ca^{2+}) in the I-field was significantly higher 20.26% ($P < 0.05$) than that in the C-field at 100 d post-transplantation. It should be noted that though Ca^{2+} declined in both fields from 50 d to 100 d post-transplantation, the rate of decline in the I-field (12.84%) was much slower than that in the C-field (22.11%). It has been suggested that soil acidification and loss of Ca^{2+} may be inhibited in the intercropping system. Therefore, maintenance of soil pH, Ca^{2+} and OM may play an important role in regulation of TBW.

Bacterial and fungal diversity in soil

A total of 51,162 and 54,481 bacterial OTUs (operational taxonomic units, based on 99.5% identity) were identified across soil samples from the tobacco monocropping system and the tobacco-marigold intercropping system, respectively (Supplementary Table 2). For the bacterial community, the difference of Sobs, Shannon and Chao1 index between C_field and I_field were analyzed by Student's *t* test (Table 2). At 50 d post-transplantation, the Sobs, Shannon and Chao 1 index from I_50 were higher 16.85% ($P < 0.01$), 2.27% and 11.33% ($P < 0.01$) than that from C_50, respectively. At 100 d post-transplantation, the three indexes from I_100 were higher 10.34%, 1.41% and 5.13% than that from C_100, respectively. These results indicated that the soil bacterial diversity and richness in the I-field could be higher than that in the C-field. This suggested that the intercropping performed in this study might improve bacterial community diversity. The principal co-ordinates analysis (PCoA) with the weighted Unifrac distance was carried out, and PC1 and PC2 explained 50.47% of the total bacterial community (Fig. 1a). Bacterial community from tobacco monocropping system and the tobacco-marigold intercropping system at the same period were clustered together (I_0 and C_0, I_50 and C_50, I_100 and C_100) at PC1 axis, while the bacterial communities of I_50 and C_50 were separated from others at PC2 axis. Therefore, the results of the PCoA suggested tobacco growth period had greater effects on the bacterial communities in the soil than the monocropping system and the intercropping system.

A total of 5645 and 6067 fungal OTUs (based on 97% identity) were identified across soil samples from the tobacco monocropping system and the intercropping system, respectively (Supplementary Table 2). The difference of Sobs, Shannon and Chao1 index of fungal community between C_field and I_field were also analyzed (Table 2). At 50 d post-transplantation, the Sobs and Chao 1 index of fungal community from I_50 were higher 12.78% and 26.83% ($P < 0.05$) than that from C_50, respectively; Shannon index from I_50 was lower 6.74% ($P < 0.05$) than that from C_50. At 100 d post-transplantation, the three indexes from I_100 were lower 4.71%, 5.72% and 11.43% than that from C_100, respectively. These results implied that intercropping with marigold might play an important role in restraining fungal communities diversity. According to PCoA analysis, PC1 and PC2 explained 40.52% of the total fungal community (Fig. 2a). The fungal community of I_100 and C_100 were separated from others at PC1 axis, suggesting tobacco growth period showed main effects on the fungal communities at PC1 axis. While tobacco monocropping system (C_0, C_50 and C_100) and the tobacco-marigold intercropping system (I_0, I_50 and I_100) were located in positive and negative coordinate axis at PC2 axis, suggesting the intercropping system showed main effects on the fungal communities on the PC2.

Bacterial community structure in soils

A total of 46 bacterial phyla were identified from all soil samples in the two cropping systems. Among the 10 predominantly present bacterial phyla (Fig. 1b), *Proteobacteria* were dominant (34.88~50.22%), followed by *Acidobacteria* (7.98~17.59%), *Actinobacteria* (6.81~18.23%), *Gemmatimonadetes* (6.21~11.80%), *Chloroflexi* (5.89~7.80%), *Bacteroidetes* (3.19~7.82%), *Firmicutes* (0.27~7.12%), *Verrucomicrobia* (1.18~4.35%), *Planctomycetes* (0.95~3.81%) and *Rokubacteria* (0.59~3.97%). Among the 10 predominantly present bacterial phyla, the relative abundance of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadete* and *Chloroflexi* in all samples was all higher than 5% for each pylum and totaled up to 72.92%~87.62% of the 46 bacterial phyla. Compared with C_100, the relative abundances of *Proteobacteria* in I_100 decreased by 12.66%, while *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes* and *Chloroflexi* in I_100 increased by 31.28%, 14.62%, 18.15%

Table 2 Observed species richness (Sobs), Shannon diversity and Chao1 richness of bacteria and fungi community in marigold-tobacco intercropping system (I) and tobacco monocropping system (C)

	Bacterial			Fungal		
	Observed_species (Sobs)	Shannon	Chao1	Observed_species (Sobs)	Shannon	Chao1
I_50	13,003.00 ± 271.40A	8.10 ± 0.17	19,976.00 ± 305.66A	1053.00 ± 41.24	5.64 ± 0.06b	1461.12 ± 66.45a
C_50	11,128.00 ± 622.20B	7.92 ± 0.03	17,943.00 ± 329.45B	933.67 ± 94.24	6.05 ± 0.18a	1151.99 ± 93.74b
p	0.009	0.149	0.001	0.176	0.036	0.019
I_100	10,284.00 ± 361.72	7.56 ± 0.05	15,609.00 ± 557.30	937.67 ± 50.08	5.64 ± 0.37	1071.87 ± 49.00b
C_100	9320.30 ± 1134.60	7.46 ± 0.21	14,848.00 ± 1348.60	984.00 ± 25.50	5.98 ± 0.46	1210.19 ± 39.99a
p	0.234	0.452	0.417	0.308	0.457	0.036

Observed species richness (Sobs), Shannon diversity and Chao1 richness of bacteria and fungi community in soils are presented as the mean ± SE. Different lowercase letters (Student' s *t*-test, $p < 0.05$) or capital letters (Student' s *t*-test, $p < 0.01$) in the same column indicate statistically significant differences between C-field and I-field at 50 d and 100 d post-transplanted, respectively

and 7.30%, respectively, demonstrating that the intercropping system had an effect on the bacterial community structure.

Venn diagram revealed that the sum of total taxa at the bacterial genera level was 1375 (Fig. 1c). 896, 1082, 981 genera were identified for I_0, I_50, I_100 and 1054, 1032, 961 genera for C_0, C_50, C_100. 51 and 42 specific genera were found in I_50 and I_100, respectively. Based on the microbial community profiles at the genus level, hierarchically clustered analysis (Heatmap) was used to identify the different composition of these bacterial community structures (Fig. 1d). In the Heatmap, relative abundance of soil bacterial community from high to low was represented by red through white to blue. The results showed that C_50 and I_50, C_100 and I_100 were clustered together, and it was also testified by the PCoA, suggesting there were clear distinctions of bacterial community structure among different tobacco growth periods. Although group C_100 and I_100 had similar bacterial community structure, some bacterial genus (such as *Lysobacter, Burkholderia-Paraburkholderia, unclassified_f__Micrococcaceae*) in the Heatmap showed difference between these two. Among them, the relative abundances of *Lysobacter* and *Burkholderia-Paraburkholderia* in I_100 were 1.50 and 1.61 fold higher than that in C_100, respectively.

Fungal community structure in soils

A total of 6 known fungal phyla were identified from all soil samples, including *Ascomycota* (55.53~72.37%), followed by *Zygomycota* (8.98~25.74%), *Basidiomycota* (4.39~20.31%), *Chytridiomycota* (1.60~10.66%), *Glomeromycota* (0.09~2.23%) and *Neocallimastigomycota* (0.01~0.24%) (Fig. 2b). Among the 6 predominant fungal phyla, the relative abundance of *Ascomycota*, *Zygomycota* and *Basidiomycota* totaled up to 85.75~98.00%. Compared with C_100, the relative abundances of *Ascomycota* and *Zygomycota* in I_100 increased by 6.75% and 32.09%, respectively, while *Basidiomycota* in I_100 decreased by 37.72%, demonstrating that the intercropping system had effect on the composition of fungal community.

Venn diagrams revealed that the sum of total taxa at the fungal genera level was 511 (Fig. 2c). A total of 339, 283, 234 genera were identified for I_0, I_50, I_100 respectively and 271, 232, 252 genera for C_0, C_50, C_100 respectively. 40 and 23 specific genera were found in I_50 and I_100, respectively. In the Heatmap for fungal community structures (Fig. 2d), C_0 and I_0 had similar fungal community structure, while C_50 and I_50, C_100 and I_100 showed clear distinctions of fungal community structure. In I_100, the relative abundance of *Chaetomium*, *Monographella*, *Mortierella*, *Trichoderma*, *Scopulariopsis*, *Penicillium*, *Myrothecium*, *Pseudeurotium* and *Entoloma* were 4.40, 2.78, 1.67, 3.35, 2.36, 4.50, 5.84, 8.58 and 116.69 fold higher than that in C_100, respectively. Especially, *Chaetomium* in I_100 showed significantly higher ($P < 0.01$) relative abundance than that in C_100.

The relative abundance of *R. solanacearum*

The relative abundance of *R. solanacearum* in the soil from the intercropping system and the monocropping system were analyzed. Compared with C_0, the relative abundance of this pathogen in C_100 increased by 184.21%; while compared with I_0, the relative abundance of this pathogen in I_100 decreased by 39.53%. The relative abundance of this pathogen in I_50 was lower 39.74% than that in C_50, and the relative abundance in I_100 was lower 51.85% than that in C_100 (Fig. 3).

Relationships between microbial community structure and environmental variables

The relationships between bacterial microbial community composition and soil chemical properties were analyzed

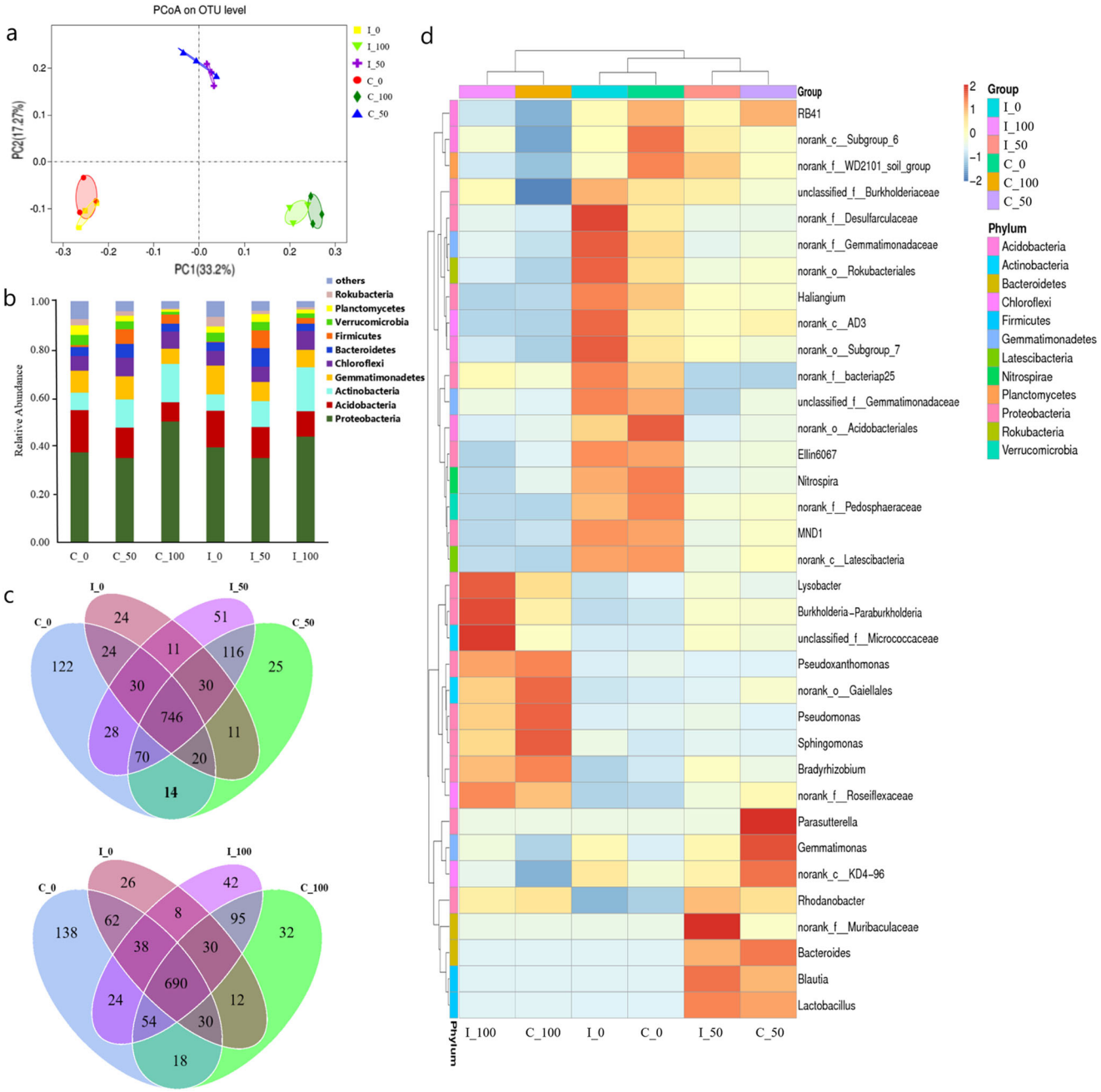

Fig. 1 Soil bacterial community in tobacco monocropping system and tobacco-marigold intercropping system. **a** The principal co-ordinates analysis (PCoA) with the weighted Unifrac distance. **b** The relative abundance of bacterial phyla in soil samples. **c** Venn diagrams based on total taxa at the bacterial genera level. **d** Hierarchical cluster analysis of 35 predominant bacterial communities among the six samples. Legends showed the Z-scores, demonstrating all samples were represented by the median-centered Z-scores as the relative abundance levels

by redundancy analysis (RDA). The results showed that 72.19% and 73.40% of community variation could be explained for I-field and C-field, respectively (Fig. 4). In RDA, the longer the arrows of environmental factors showed, the greater the impact of the factor on the microbial community composition was. For I-field, pH had the shortest arrow, and it was not significantly correlated with the relative abundances of all of bacterial phyla (Supplementary Table 3). For C-field, pH showed long arrow, and it especially had significant correlated with *Actinobacteria* and *Gemmatimonadetes*.

The relationships between fungal microbial community composition and soil chemical properties were also analyzed by RDA. 84.09% and 70.90% of community variation could be explained for I-field and C-field, respectively (Fig. 5). For I-field, pH and exchangeable Ca^{2+} showed the shortest arrows, and most of soil chemical properties including pH and exchangeable Ca^{2+} were not significantly correlated with

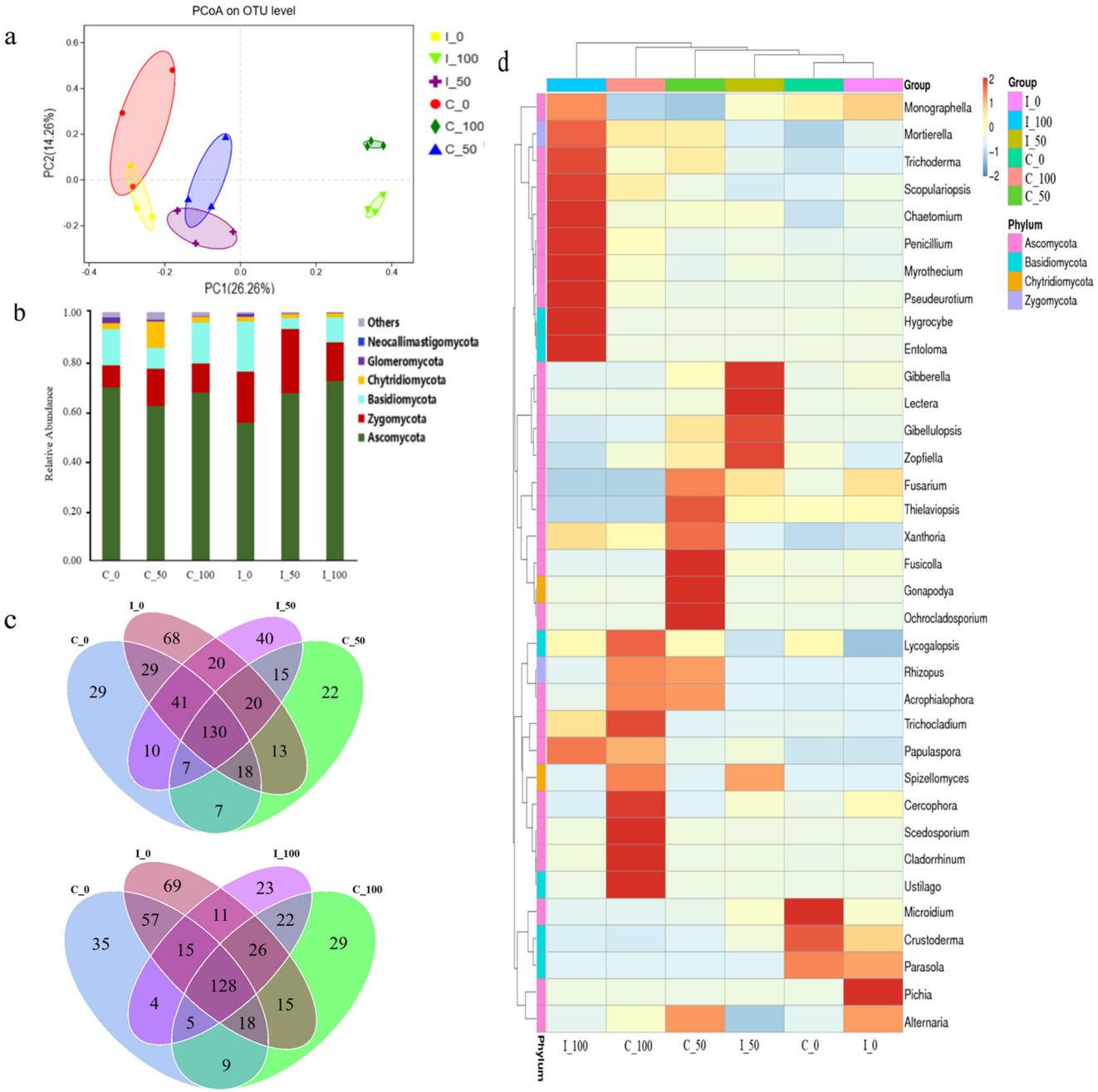

Fig. 2 Soil fungal community in tobacco monocropping system and tobacco-marigold intercropping system. **a** The principal co-ordinates analysis (PCoA) with the weighted Unifrac distance. **b** The relative abundance of fungal phyla in soil samples. **c** Venn diagrams based on total taxa at the fungal genera level. **d** Hierarchical cluster analysis of 35 predominant fungal communities among the six samples. Legends showed the Z-scores, demonstrating all samples were represented by the median-centered Z-scores as the relative abundance levels

fungal phyla. With regard to C-field, pH and exchangeable Ca^{2+} showed significantly correlated with *Glomeromycota* and *Basidiomycota,* respectively (Supplementary Table 4).

Discussion

This study focused on the incidence and severity of TBW, soil chemical properties, and soil bacterial and fungal communities in the tobacco-marigold intercropping system. At 100 d post-transplantation, the incidence (*I*) and disease index (*DI*) of TBW for tobacco-marigold intercropping system was 30.12% and 58.25% lower than that for tobacco monocropping system ($P < 0.01$), respectively. Therefore, the use of marigolds as intercropping plants could possibly aid in suppressing TBW under field conditions.

It is widely recognized that the mechanisms underlying the effect of intercropping on disease dynamics can involve the

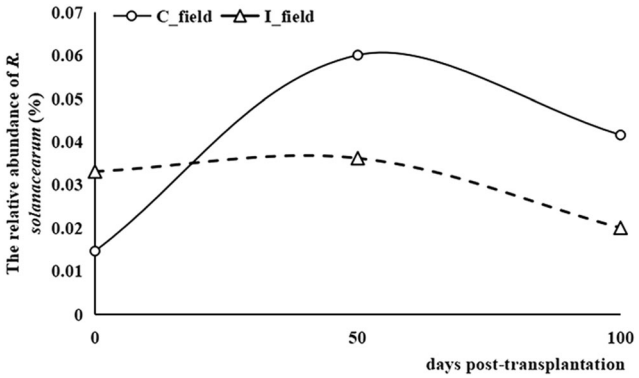

Fig. 3 The relative abundance of *R. solanacearum* in soils from tobacco monocropping system (C) and tobacco-marigold intercropping system (I)

alteration of wind and rain, modification of microclimate (especially temperature and moisture), changes in host morphology and physiology, and direct pathogen inhibition (Boudreau 2013). Recently, more focus has been placed on how intercropping that could alter the belowground environment. Aboveground plant diversity is closely linked to belowground biodiversity (Li et al. 2014, 2016a; Dong et al. 2016; Wu et al. 2018). In this study, compared with tobacco monocropping system, the Sobs, Shannon and Chao1 index of soil bacterial communities during the incidence of TBW, were increased in tobacco-marigold intercropping system. The results suggested that the richness and diversity of bacterial communities could be important biological indicators for distinguishing the tobacco monocropping system and the tobacco-marigold intercropping system, which was also mentioned by previous studies (Kennedy and Smith 1995; Avidano et al. 2005; Zhou and Wu 2012).

In this study, the results of PCoA (Figs. 1a and 2a) indicated that the interactions between crop species and crop growth stage could influence microbial communities in rhizosphere soil, which was the similar results found in previous studies (Wieland et al. 2001; Song et al. 2007). Certain soil microorganisms (*Actinobacteria, Acidobacteria, Proteobacteria* and *Chloroflexi* etc.), can respond quickly to environmental perturbations, such as alternative cropping regimes that result in dynamic changes in microbial biomass, activity, abundance, composition and structure (Li et al. 2016a; Li and Wu 2018). In this study, the 10 predominantly bacterial phyla and 6 known fungal phyla were all found in tobacco monocropping system and tobacco-marigold intercropping system. However, some antagonistic bacterial phyla that contain antagonistic species/strains (Berg et al. 2006; Costa et al. 2006) showed higher relative abundances in the intercropping system. For example, the relative abundance of *Actinobacteria* and *Gemmatimonadetes* in the intercropping system were 14.62% and 18.15% higher than that in the monocropping system at 100 d post-transplantation, respectively. It is possible that an increased production of antibiotics by some species of these phyla inhibited the colonization of soil-borne pathogens (such as *R. solanacearum*), and enhanced plant health (Barka et al. 2016). Among the fungal phyla, the relative abundance of *Ascomycota* in the intercropping system was 6.75% higher than that in the monocropping system at 100 d post-transplantation, which could have been beneficial to promote the C circulation in soil and the nutrient absorption in plant (Unterseher et al. 2013; Purahong et al. 2016).

The relative abundance of beneficial bacteria and fungi decreases in long-term continuous cropping system, while increases in intercropping system (Jiang et al. 2017; Lian et al. 2018; Zhang et al. 2018; Wu et al. 2018). In this study, for bacterial genus, *Lysobacter* showed higher relative abundance in tobacco-marigold intercropping system

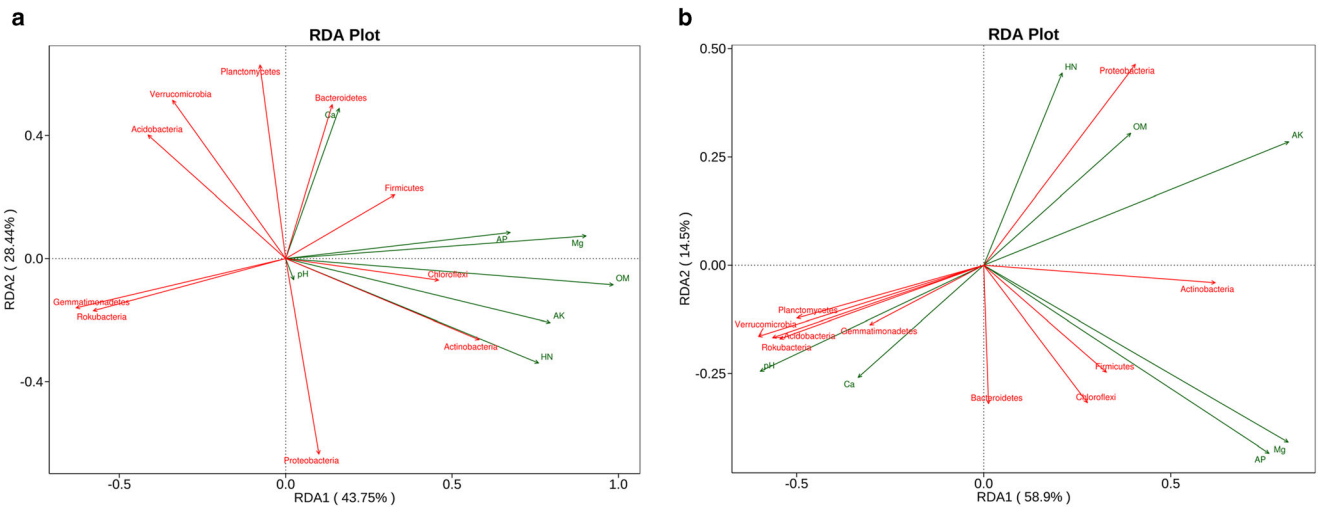

Fig. 4 Redundancy analysis (RDA) of the relationship between bacterial community structure and soil chemical properties. **a** tobacco-marigold intercropping system; **b** tobacco monocropping system. The soil chemical properties are indicated with green arrows, and bacterial community are indicated with red arrows. The percentage of variation is indicated by each axis

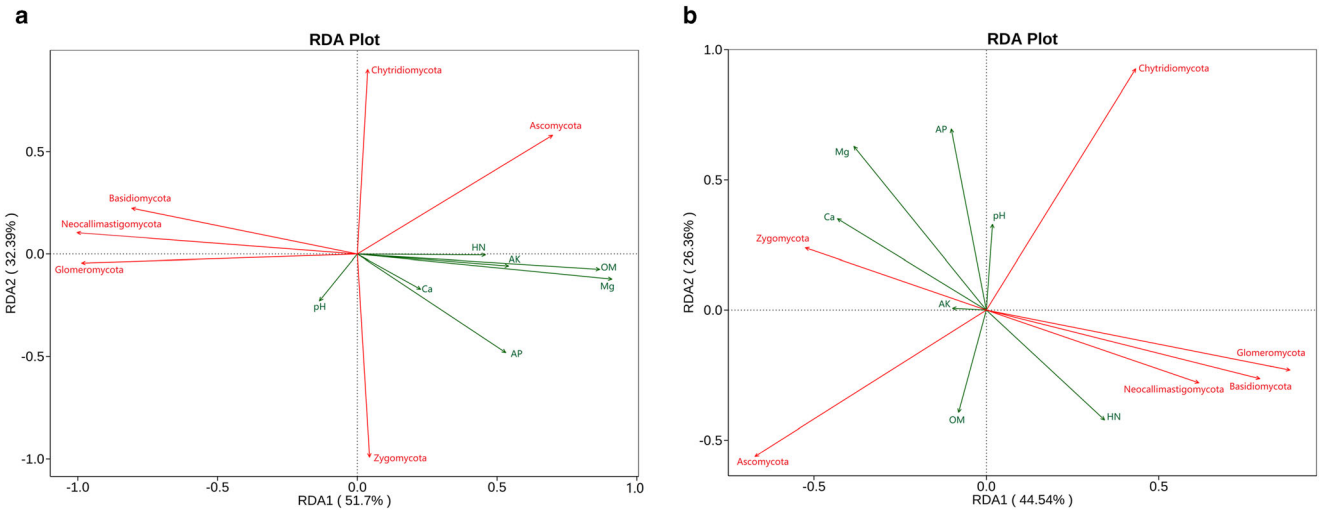

Fig. 5 Redundancy analysis (RDA) of the relationship between fungal community structure and soil chemical properties. **a**. tobacco-marigold intercropping system; **b**. tobacco monocropping system. The soil chemical properties are indicated with green arrows, and fungal community are indicated with red arrows. The percentage of variation is indicated by each axis

(1.50 fold) than that in the monocropping system, which could mitigate many soil-borne diseases such as pepper phytophthora blight, sugar beet and cucumber damping-off by secreting multiple antibiotics (Folman et al. 2003; Islam et al. 2005; Kobayashi and Yuen 2005). Similar as some species of *Lysobacter, Burkholderia* which is well documented as beneficial rhizosphere microorganisms for promoting plant growth and health (Badri et al. 2009), was present at higher relative abundance in the intercropping system (1.61 fold) than that in the monocropping system. The activity and effects of beneficial fungal genera, such as *Trichoderma*, *Mortierella*, *Chaetomium* and *Penicillium*, on mitigating plant disease and promoting plant growth are well documented (Silva et al. 2019; DiLegge et al. 2019; Meng 2009; Larena et al. 2003). *Trichoderma* could directly interact with roots and produce bioactive substances such as fungal cell wall degrading enzymes and secondary metabolites, which can promote plant growth and resist biotic and abiotic stress (Silva et al. 2019). *Mortierella* has effect on reducing root galls and mitigating the symptoms of root knot nematode (DiLegge et al. 2019). *Chaetomium* has a positive effect on reducing *Verticillium dahliae*, *Diaporthe phaseolorum* f. sp. *meridionalis*, *Colletotrichum falcatum* and so on by secreting multiple antibiotics such as chaetomin and chaetoglobosin (Meng 2009). By adding 10^6~10^7 CFU/g of *Penicillium* to the seedling substrate and rhizosphere soil, tomato fusarium wilt and verticillium wilt could be effectively controlled (Larena et al. 2003). At 100 d post-transplantation, the relative abundance of the above beneficial fungal genera in the intercropping system was 3.35, 1.67, 4.40 and 4.50 fold higher than that in the monocropping system in this study. There are therefore indications tobacco plants in the intercropping system recruit beneficial members of the soil microbiome to protect themselves from infection. Rehabilitating the microbial community by intercropping to prevent plant diseases may be more effective than only repressing pathogen populations (Shi et al. 2019).

Reports have verified that cinnamic, myristic, fumaric, oxalic, malic and citric acids in tobacco root exudates can promote *R. solanacearum* colonization, increase its biofilm biomass and exacerbate the incidence of bacterial wilt (Wu et al. 2014; Li et al. 2017). It was reported that the roots of marigolds contain and excrete thiophenes, which are heterocyclic, sulphurous compounds with strong biocidal activity (Tang et al. 1987; Croes et al. 1989; Jacobs et al. 1994). In this study, the relative abundances of *R. solanacearum* in soils from the intercropping system were lower than that in the monocropping system at 50 d (decrease of 39.74%) and 100 d (decrease of 51.85%) post-transplantation, indicating that thiophenes excreted from marigolds might have had negative effects on colonization of *R. solanacearum.* Moreover, thiophenes may counteract autotoxic substances such as cinnamic and myristic, and then the damage of organic acid on tobacco roots was reduced, which needs further study.

Among the soil chemical properties (pH, OM, HN, AP, AK, Ca^{2+} and Mg^{2+}), pH and the content of Ca^{2+} and OM were significantly higher in the intercropping system. The results of redundancy analysis (RDA) analysis indicated that the two factors of pH and Ca^{2+} possibly had different effects on soil microbial communities in the two system. As reported, soil acidification is suitable for the occurrence of bacterial wilt (Wang et al. 2017). Indeed, in the tobacco monocropping system, the soil pH decreased by 0.32 (from 6.36 at pre-transplantation to 6.04 at 100 d post-transplantation), while in the intercropping system, the soil pH

remained at approximately 6.6 from pre-transplantation to 100 d post-transplantation. It is postulated that the phenomenon of soil acidification inhibited in the intercropping system might be one reason that assisted in mitigating TBW. Exchangeable calcium (Ca^{2+}) is found to be important in disease suppression and increasing Ca^{2+} concentrations could reduce the severity of bacterial wilt (Jiang et al. 2013; He et al. 2014). In this study, compared with tobacco monocropping system, the Ca^{2+} content in the tobacco-marigold intercropping system was increased by 20.26% at 100 d post-transplantation. This result implied that loss of Ca^{2+} may be restrained in the intercropping system, leading to a reduction in the severity of bacterial wilt. Therefore, soil pH and exchange Ca^{2+} were the main environmental factors which seemed to influence the bacterial and fungal community.

Conclusions

In this study, we demonstrated that the occurrence of TBW could be effectively mitigated under field conditions by intercropping with marigold. At 100 d post-transplantation, the incidence (*I*) and disease index (*DI*) for the tobacco-marigold intercropping system were 30.12% and 58.25% lower than that for tobacco monocropping system ($P < 0.01$), respectively. The soil chemical properties, as well as soil microbial composition and diversity, were distinct between the tobacco-marigold intercropping system and tobacco monocropping system. At 100 d post-transplantation, Sobs, Shannon and Chao 1 index of soil bacterial communities in the intercropping system were 10.34%, 1.41% and 5.13% higher than that in the monocropping system, respectively, showing a higher bacterial microbial richness and diversity in the intercropping system. The relative abundance of some beneficial microorganisms for mitigating plant diseases and promoting plant growth, such as *Actinomycetes, Gemmatimonadetes, Ascomycota* at phyla level and *Lysobacter*, *Burkholderia, Trichoderma*, *Mortierella*, *Chaetomium*, *Penicillium* at genus level, might have had a beneficial effect since the respective genera/phyla diversified under the intercropping system. The results from RDA analysis implied that in the intercropping system, loss of Ca^{2+} and soil acidification restrained might be one reason that assisted in mitigating TBW. Therefore, the results suggested that marigold may be one of important plants for controlling tobacco bacterial wilt and keeping soil healthy.

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