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 Ca²⁺ 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 (Mansfield 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
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 m² 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 m² in size. Each block was divided into two plots of 132 m², 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 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 phosphorus (AP), available potassium (AK), exchangeable calcium (Ca\textsuperscript{2+}) and exchangeable magnesium (Mg\textsuperscript{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′-GTGCCAGCMGCGGCGGTAA-3′) and 806 reverse (5′-GGACTACHVGGGTWTCTAAT-3′), and the ITS1 region of ITS rRNA gene was amplified using primers ITS5-1737 forward (5′-GGGACGTAAGAGTCAGGTAACAGG-3′) and ITS2-2043 reverse (5′-GCTGTGTTTCTTCTATCGATGC-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 (Thermo Scientific) 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](https://example.com/table.png)

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

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 (Mg²⁺) 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 (Ca²⁺) 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 Ca²⁺ 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 Ca²⁺ may inhibit in the intercropping system. Therefore, maintenance of soil pH, Ca²⁺ 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 and Chao 1 index of bacterial 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, Gemmatimonadetes and Chloroflexi in all samples was all higher than 5% for each phylum 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% and 26.83%, respectively (Supplementary Table 1). There was no significant difference of Sobs, Shannon and Chao1 index between the soil bacterial communities of I_50 and C_50 were separated from others at PC2 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.
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, Pseudoperotium and Entoloma were 4.40, 2.78, 1.67, 3.35, 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 51.85% than that in C_0, 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.
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 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.
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...
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. 3 The relative abundance of R. solanacearum in soils from tobacco monocropping system (C) and tobacco-marigold intercropping system (I)

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.
(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 $10^5$–$10^7$ 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).

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.
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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**References**

Avidano L, Gamalero E, Cossa GP, Carraro E (2005) Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. Appl Soil Ecol 30:21–33

Badri DV, Weir TL, van der Leie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. Curr Opin Biotechnol 20:642–650

Baraka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, Clément C, Ohoudhuy Y, van Wezel GP (2016) Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol Mol Biol Rev 80:1–43

Berg G, Opelt K, Zachow C, Lottmann J, Gotz M, Costa R, Smalla K (2006) The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. FEMS Microbiol Ecol 56:250–261

Boudreau MA (2013) Diseases in intercropping systems. Annu Rev Phytopathol 49:499–519

Costa R, Gotz M, Nicole M, Lottmann J, Berg G, Smalla K (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol Ecol 56:236–249

Cros AE, van den Berg AJR, Bosveld M, Breterel H, Wullems JG (1989) Thiophene accumulation in relation to morphology in roots of *Tagetes patula*. Planta 179:43–50

Denny T (2006) Plant pathogenic *Ralstonia* species. In: Gnanamaniycam SS (ed) Plant-associated Bacteria. Springer Netherlands, Dordrecht, pp 537–644

DiLegge MJ, Manter DK, Vivanco JM (2019) A novel approach to determine generalist nematophagous microbes reveals *Mortierella globalpina* as a new biocontrol agent against *Meloidogyne* spp. nematodes. Sci Rep 9:7521–7529

Dong Y, Dong K, Yang ZX, Zhang Y, Tang L (2016) Microbial and physiological mechanisms for alleviating fusarium wilt of faba bean in intercropping system. Chin J Appl Ecol 27(6):1984–1992 (in Chinese)

El-Hamawi MH, Youssef MMA, Jawahar HS (2004) Management of *Meloidogyne* incognita, the root-knot nematode, on soybean as affected by marigold and sea ambrosia (*damsisa*) plants. J Pest Sci 77:95–98

Elphinstone JG (2005) The current bacterial wilt situation: a global view. In: Allen C, Hayward PP, A. C. (eds) Bacterial wilt disease and the *Ralstonia solanacearum* species complex. APS Press, Saint Paul, pp 9–28

Flombaum P, Sala OE (2008) Higher effect of plant species diversity on productivity in natural than artificial ecosystems. PNAS 105: 6087–6090

Folman LB, Postma J, van Veen JA (2003) Characterization of *Lysobacter* enzymogenes (Christensen and Cook, 1978) strain

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**Corresponding Author:**

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacouard C, Klenk HP, Clément C, Ohoudhuy Y, van Wezel GP (2016) Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol Mol Biol Rev 80:1–43
3.1T8, a powerful antagonist of fungal disease of cucumber. Microbiol Res 158:107–115
Gao X, Wu M, Xu R, Wang X, Pan R, Kim HJ, Liao H (2014) Root interactions in a maize/soybean intercropping system control soybean soil-borne disease, red crown rot. PLoS One 9(5):e95031
Gómez RO, Zavaleta M, González H, Livera M, Cárdenas S (2003) Allelopathy and microclimatic modification of intercropping with marigold on tomato early blight disease development. Field Crop Res 83:27–34
Guo H, Mao Z, Jiang H, Liu P, Zhou B, Bao Z, Sui J, Zhou X, Liu X (2014) Community analysis of plant growth promoting rhizobacteria for apple trees. Crop Prot 62:1–9
Hao W, Ren L, Ran W, Shen Q (2010) Allelopathic effects of root exudates from watermelon and rice plants on Fusarium oxysporum f. sp. niveum. Plant Soil 336:485–497
He K, Yang S, Li H, Wang H, Li Z (2014) Effects of calcium carbonate on the survival of L. edaphicus var. aren in soil and control of tobacco bacterial wilt. Eur J Plant Pathol 140:665–675
Hiddink GA, Termorshuizen AJ, Van Bruggen AHC (2009) Mixed cropping and suppression of soil-borne disease. In: Lichtfouse E. (ed.). Genetic engineering, biofertilisation, soil quality and organic farming, Sustainable Agriculture Reviews, pp. 119-146
Hill TJC, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. EEMS Microbiol Ecol 43:1–11
Hooks CRR, Wang K, Ploeg A, McSorley R (2010) Review: using marigold (Tagetes spp.) as a cover crop to protect crops from plant-parasitic nematodes. Appl Soil Ecol 46:307–320
Islam MT, Hashidoko Y, Deora A, Ito T, Tahara S (2005) Suppression of damping-off disease in host plants by the rhizobacteria Lysobacter sp. strain sb-k88 is linked to plant colonization and antibiosis against soilborne pepperonisporomyces. Appl Environ Microbiol 71:3786–3796
Jacobs JJMR, Engelberts A, Croes AF, Wullems GJ (1994) Thiophene oxidation and antibiotic production by a novel Pseudomonas sp. strain T8, a powerful antagonist of fungal disease of cucumber. Microbiol Res 158:107–115
Kobayashi DY, Yuen GY (2005) The role of clp-regulated factors in the slow growth of mutant strains of Salmonella typhimurium. Microbiology 57:3
Kumar NUS, Krishnappa K, Reddy BMR, Ravichandra NG, Karuna K (2005) Intercropping for the management of root-knot nematode, Meloidogyne incognita in vegetable-based cropping systems. Indian J Nematol 35:46–49
Kobayashi DY, Yuen GY (2005) The role of clp-regulated factors in antagonism against Magnaporthe poae and biological control of summer patch disease of Kentucky bluegrass by Lysobacter enzymogenes C3. Can J Microbiol 51:719–723
Kumar NUS, Krishnappa K, Reddy BMR, Ravichandra NG, Karuna K (2005) Intercropping for the management of root-knot nematode, Meloidogyne incognita in vegetable-based cropping systems. Indian J Nematol 35:46–49
Larena I, Sabuquillo P, Melgarjeo P, De Cal A (2003) Biocontrol of Fusarium and Verticillium wilt of tomato by Penicilium oxalicum under greenhouse and field conditions. J Phytopathol 151:507–512
Larkin RP, Honeycutt CW, Griffin TS, Olanya OM, Halloran JM, He Z (2011) Effects of different potato cropping system approaches and water management on soilborne diseases and soil microbial communities. Phytopathology 101:58–67
Li S, Wu F (2018) Diversity and co-occurrence patterns of soil bacterial and fungal communities in seven intercropping systems. Front Microbiol 9:1521–1533
Li XG, Wang XX, Dai CC, Zhang TL, Xie XG, Ding CF, Wang HW (2014) Effects of intercropping with Atractylodes lancea and application of bio-organic fertilizer on soil invertebrates, disease control and peanut productivity in continuous peanut cropping field in subtropical China. Agrofor Syst 88:41–52
Li Y, Han M, Lin F, Ten Y, Lin J, Zhu D, Guo P, Weng Y, Chen L (2015) Soil chemical properties, ’Guansimiyou’ pummelo leaf mineral nutrient status and fruit quality in the southern region of Fujian province, China. J Soil Sci Plant Nut 15:615–628
Li X, Sun ML, Zhang HH, Xu N, Sun GY (2016a) Use of mulberry-soybean intercropping in salt-alkali soil impacts the diversity of the soil bacterial community. Microbiol Biotechnol 9:293–304
Li YY, Feng J, Liu HL, Wang L, Hsiang T, Li KH, Huang JB (2016b) Genetic diversity and pathogenicity of Ralstonia solanacearum causing tobacco bacterial wilt in China. Plant Dis 100:1288–1296
Li X, Xu C, Wang J, Guo B, Yang L, Chen J, Ding W (2017) Cinnamnic, myristic and fumeric acids in tobacco root exudates induce the infection of plants by Ralstonia solanacearum. Plant Soil 412:381–395
Lian TX, Mu HY, Ma QB, Cheng YB, Gao R, Cai ZD, Jiang B, Nian H (2018) Use of sugarcane-soybean intercropping in acid soil impacts the structure of the soil fungal community. Sci Rep 8:14488
Liu Y, Kanda A, Yano K, Kiba A, Hikichi Y, Aino M, Kagawuchi A, Mizoguchi S, Nakako K, Shiomi H, Takikawa Y, Ohnishi K (2009) Molecular typing of Japanese strains of Ralstonia solanacearum in relation to the ability to induce a hypersensitive reaction in tobacco. J Gen Plant Pathol 75:369–380
Liu YY, Shi JX, Feng YG, Yang XM, Li X, Shen QR (2013) Tobacco bacterial wilt can be biologically controlled by the application of antagonistic strains in combination with organic fertilizer. Biol Fertil Soils 49:447–464
Liu X, Zhang J, Gu T, Zhang W, Shen Q, Yin S, Qiu H (2014) Microbial community diversities and taxa abundances in soils along a seven-year gradient of potato monoculture using high throughput pyrosequencing approach. PLoS One 9:e86610
Mansfield J, Genin S, Magori S, Citovsky V, Siroyanum M, Ronald P, Dow M, Verdier V, Beer SV, Machado MA, Toth I, Salmond G, Foster G (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13:614–629
Meng QG (2009) Infection process and the influence of endophytic Chaetomium globosum ND35 on host plant and its molecular detection. Master’s thesis, Shandong University. (in Chinese)
Michel VV, Wang JF, Midmore DJ, Hartman GL (1997) Effects of intercropping and soil amendment with urea and calcium oxide on the incidence of bacterial wilt of tomato and survival of soil-borne Pseudomonas solanacearum in Taiwan. Plant Pathol 46:600–610
Newton AC, Begg G, Swanston JS (2009) Deployment of diversity for enhanced crop function. Ann Appl Biol 154:309–322
Niu J, Chao J, Xiao Y, Chen W, Zhang C, Liu X, Rang Z, Yin H, Dai L (2017) Insight into the effects of different cropping systems on soil bacterial community and tobacco bacterial wilt rate. J Basic Microbiol 57:3–11
Parahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F (2016) Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Mol Ecol 25:4059–4074
Ramatadass A, Fernandes P, Avelino J, Habib R (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron Sustain Dev 32:273–303
Ren L, Su S, Yang X, Xu Y, Huang Q, Shen Q (2008) Intercropping with aerobic rice suppressed Fusarium wilt in watermelon. Soil Biol Biochem 40:834–844
Shi WC, Li MC, Wei GS, Tian RM, Li CP, Wang B, Lin RS, Shi CY, Chi XL, Zhou B, Gao Z (2019) The occurrence of potato common scab correlates with the community composition and function of the geocaulosphere soil microbiome. Microbiome 7:14
Shiomi Y, Nishiyama M, Onizuka T (1999) Comparison of bacterial community structures in the rhizoplane of tomato plants grown in...
soils suppressive and conducive towards bacterial wilt. Appl Environ Microbiol 65:3996–4001
Silva RN, Monteiro VN, Gomes EV, Noronha EF, Ulhoa CJ (2019) Trichoderma/pathogen/plant interaction in pre-harvest food security. Fungal Biol. https://doi.org/10.1016/j.funbio.2019.06.010
Song Y, Zhang F, Marschner P, Fan F, Gao H, Bao X, Sun J, Li L (2007) Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (Triticum aestivum L.), maize (Zea mays L.), and faba bean (Vicia faba L.). Biol Fertil Soils 43:565–574
Su S, Ren L, Huo Z, Yang X, Huang Q, Xu Y, Zhou J, Shen Q (2008) Effects of intercropping watermelon with rain fed rice on Fusarium wilt and the microflora in the rhizosphere soil. Sci Agric Sin 41:704–712
Tang CS, Wat CK, Towers GHN (1987) Thiophenes and benzofurans in the undisturbed rhizosphere of Tagetes patula L. Plant Soil 98:93–97
Terblanche J (2007) Biological control of bacterial wilt in tobacco caused by Ralstonia solanacearum. Master’s thesis, University of the Free State, Bloemfontein, South Africa
Tian X, Wang C, Bao X, Wang P, Li X, Yang S, Ding G, Christie P, Li L (2019) Crop diversity facilitates soil aggregation in relation to soil microbial community composition driven by intercropping. Plant Soil 1:1–20
Unterseher M, Peršoh D, Schnittler M (2013) Leaf-inhabiting endophytic fungi of European beech (Fagus sylvatica L.) co-occur in leaf litter but are rare on decaying wood of the same host. Fungal Divers 60:43–54
Wang R, Zhang H, Sun L, Qi GF, Chen S, Zhao XY (2017) Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak. Sci Rep 7:343
Wieland G, Neumann R, Backhaus H (2001) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Appl Environ Microbiol 67:5849–5854
Wu K, Yuan S, Xun G, Shi W, Pan B, Guan H, Shen B, Shen Q (2014) Root exudates from two tobacco cultivars affect colonization of Ralstonia solanacearum and the disease index. Eur J Plant Pathol 141:667–677
Wu J, Jiao Z, Zhou J, Zhang W, Xu S, Guo F (2018) Effects of intercropping on rhizosphere soil bacterial communities in Amorphophallus konjac. Open J Soil Sci 8:225–239
Xie G, Cui H, Dong Y, Wang X, Li X, Deng R, Wang Y, Xie Y (2017) Crop rotation and intercropping with marigold are effective for root-knot nematode (Meloidogyne sp.) control in angelica (Angelica sinensis) cultivation. Can J Plant Sci 97:26–31
Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y (1995) Transfer of two Burkholderia and an Alcaligenes species to Ralstonia. Microbiol Immunol 39(11):897–904
Yang J, Ruegger PM, McKenry MV, Becker JO, Bormeman J (2012) Correlations between root-associated microorganisms and peach replant disease symptoms in a California soil. PLoS One 7:e46420
Yu J (1999) Allelopathic suppression of Pseudomonas solanacearum infection of tomato (Lycopersicon esculentum) in a tomato-chinese chive (Allium tuberosum) intercropping system. J Chem Ecol 25:2409–2417
Yuliar, Nion YA, Toyota K (2015) Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ 30:1–11
Zhang W, Long X, Huo X, Chen Y, Lou K (2013) 16s rRNA-based PCR-DGGE analysis of actinomycete communities in fields with continuous cotton cropping in Xinjiang, China. Microb Ecol 66:385–393
Zhang M, Wang N, Hu Y, Sun G (2018) Changes in soil physicochemical properties and soil bacterial community in mulberry (Morus alba L.)/alfalfa (Medicago sativa L.) intercropping system. Microbiol Open 7:e555
Zhou X, Wu F (2012) Dynamics of the diversity of fungal and fusarium communities during continuous cropping of cucumber in the greenhouse. FEMS Microbiol Ecol 80:469–478

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