DOI: http://dx.doi.org/10.1590/1678-992X-2019-0005

ISSN 1678-992X

SCIENTIA AGRICOLA

Research Article

Intercropping of wheat changed cucumber rhizosphere bacterial community composition and inhibited cucumber Fusarium wilt disease

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Edited by: Fernando Dini Andreote

Received January 08, 2019
Accepted April 23, 2019

ABSTRACT: Enhancing the plant rhizosphere microbial community function by increasing plant diversity in the field is a promising strategy for enhancing agricultural sustainability. This study evaluated the effectiveness of the intercropping of wheat in controlling cucumber Fusarium wilt disease. Bacterial community diversity and abundance in cucumber rhizosphere were estimated by high-throughput amplicon sequencing and quantitative polymerase chain reaction (PCR). Results showed that the intercropping of wheat inhibited the severity of cucumber seedling Fusarium wilt disease, increased the alpha diversity and altered the composition of the bacterial community in cucumber rhizosphere. Compared with monocropped cucumber, intercropped cucumber had higher relative abundances of Anaerolineae, Deltaproteobacteria, Phycisphaerae and Planctomycetacia, and lower Alphaproteobacteria and Cyanobacteria. Moreover, the intercropping of wheat promoted bacterial genera with plant-beneficial potential (e.g. *Pseudomonas*, *Haliangium* and *Archangium* spp.) in cucumber rhizosphere. Quantitative PCR confirmed that *Pseudomonas* spp. abundance was higher in intercropped cucumber rhizosphere than in monocropped cucumber rhizosphere. Overall, the intercropping of wheat decreased the severity of Fusarium wilt of cucumber and promoted potential plant-beneficial microbes in cucumber rhizosphere.

Keywords: *Cucumis sativus* L., *Triticum aestivum* L., *Fusarium oxysporum* f.sp. *cucumerinum*, *Pseudomonas* spp., bacterial diversity

Introduction

Crop disease is one of the greatest challenges to agricultural production (Chakraborty and Newton, 2011). Soil-borne diseases are difficult to control by conventional strategies, such as the use of synthetic fumigants and resistant host cultivars (Bonanomi et al., 2007). In agriculture, continuous monocropping can decrease crop performance, a phenomenon known as 'soil sickness' (Zhou et al., 2017). The accumulation of soil-borne pathogens is a possible factor contributing to soil sickness (Sun et al., 2018; Zhou et al., 2017). Diversified cropping (e.g., crop rotation, cover cropping and intercropping), that increases plant diversity in the field, can inhibit soil-borne diseases and increase crop productivity (Bennett et al., 2012; Couedel et al., 2018; Jin et al., 2019a, b; Liu et al., 2018a; Zhou et al., 2017).

Crop rhizosphere microorganisms are important to crop fitness and productivity and are sensitive to agricultural practices, such as fertilization, cropping system, irrigation and tillage (Ferreira et al., 2018; Liu et al., 2018b; Shi et al., 2019; Moronta-Barrios et al., 2018). Plant-beneficial microbes (e.g., some *Pseudomonas* species) can effectively decrease crop disease severity (Pieterse et al., 2014). Diversified cropping can change soil microbial diversity and composition (Borrell et al., 2017; Inderbitzin et al., 2018; Lv et al., 2018; Zhou et al., 2011). Increasing plant diversity can promote indigenous soil plant-beneficial microbes (Inderbitzin et al., 2018; Latz et al., 2012; 2015). Thus, engineering crop rhizosphere microbial communities, through increases in plant diversity to suppress crop diseases and enhance agricultural sustainability, is a promising strategy (Hartman et al., 2018).

Cucumber (*Cucumis sativus*), a vegetable crop commonly monocropped in the greenhouse, is vulnerable to soil sickness (Jia et al., 2018; Zhang et al., 2018a, b). Fusarium wilt, a serious vascular wilt disease in cucumber production, is caused by *Fusarium oxysporum* f.sp. *cucumerinum* (FOC) (Zhou et al., 2017). Wheat (*Triticum aestivum*) root exudates and leachates of decomposing wheat straws have been shown to suppress Fusarium wilt pathogens of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and cucumber (Wang et al., 2010; Xu et al., 2015). However, whether or not the intercropping of wheat is capable of controling Fusarium wilt in cucumber is not clear. In this study, the effects of the intercropping of wheat on cucumber growth and Fusarium wilt disease severity were evaluated. Next, the abundance and composition of bacterial community in cucumber rhizosphere were estimated by quantitative PCR and Illumine MiSeq sequencing, respectively.

Materials and Methods

Greenhouse experiment

Soil used in this study was collected from the upper soil layer (0-15 cm) of a greenhouse cultivated with cucumber in Harbin, China (45°41′ N, 126°37′ E). The soil was a sandy loam, containing organic matter, 35.12 mg kg^{-1}; organic N 146.60 mg kg^{-1}; available P, 284.20 mg kg^{-1}; available K, 341.80 mg kg^{-1}; EC (1:2.5, w:v), 0.43 mS cm^{-1}; and pH (1:2.5, w:v), 7.64.

Plastic pots (20 cm in diameter, 17 cm in height) were filled with fresh soil (after sieving through 2 mm mesh). Each pot contained 2.5 kg of fresh soil. Three cucumber seedlings (cv. Jinyan 4, susceptible to FOC) with two cotyledons were transplanted to each pot, and were thinned to one seedling per pot three days later. Ten days later, wheat seeds (cv. PZ25) were directly seeded into pots on one side of the cucumber seedlings. There were 20 wheat seeds in each pot and the distance between wheat and cucumber seedlings was about 7 cm. Five days after seeding wheat, the wheat was thinned to 15 seedlings per pot. There were two treatments in this study, cucumber monocropping and cucumber intercropped with wheat. There were three replicates for each treatment and 20 pots for each replicate. For these 20 pots per treatment in each replicate, 15 were used for inoculating FOC and measuring Fusarium wilt disease severity. The other five pots were used for measuring cucumber seedling dry biomass and sampling soil from the rhizosphere. All pots were randomly placed in a greenhouse (32 °C day/22 °C night, relative humidity of 60-80 %, 16 h light/8 h dark). Soil water content was maintained at approximately 65 % of its water holding capacity.

Inoculating FOC and measuring Fusarium wilt disease severity

Twenty days after transplanting, cucumber seedlings were inoculated with FOC as previously described (Zhou et al., 2018a). After being carefully removed from the soil, the roots of the cucumber seedlings were rinsed with sterilized H_2O_2 and their tips gently cut. After being soaked in a 2×10^8 conidia mL^{-1} suspension of FOC for ten min, these seedlings were planted back in the pots they had been grown in. Fusarium disease severity of cucumber seedlings was recorded 15 days after inoculating FOC as described by Zhou et al. (2018a).

Measuring cucumber seedling biomass and sampling rhizosphere soil

Cucumber seedlings without inoculating FOC at the five-leaf stage were harvested 30 days after transplanting and the plant dry biomass measured. Meanwhile, cucumber seedling rhizosphere soils were collected (Zhou et al., 2018b). Briefly, cucumber seedling roots were carefully removed from the soil and shaken by hand to remove loosely attached soil. Next, rhizosphere soils were removed from the root surface with a sterile brush. To limit the influence of soil heterogeneity, preparation of composite soil samples for DNA extraction is recommended (Vestergaard et al., 2017). Therefore, a composite sample was prepared from samples from five cucumber seedlings in each replicate of the individual treatment. This resulted in three composite cucumber rhizosphere soil samples for each treatment. After sieving (2 mm mesh), these fresh soil samples were stored at -80 °C for DNA extraction.

Soil DNA extraction and quantitative PCR analysis

Soil DNA was extracted from a 0.25 g soil sample using the PowerSoil DNA Isolation Kit. Each rhizosphere soil sample was extracted in triplicate and the extracted DNA solutions were pooled. Thus, three composite DNA solution samples were prepared for each treatment.

Cucumber rhizosphere bacteria and *Pseudomonas* spp. abundances were estimated by SYBR Green quantitative PCR assays with primers 338F/518R (Muyzer et al., 1993) and PsF/PsR, targeting the partial 16S rRNA gene (Garbeva et al., 2004), respectively, as previously described (Zhou et al., 2017; 2018c; Zhou and Wu, 2018). Quantitative PCR was performed on an IQ5 real-time PCR system. Copy numbers of the 16S rRNA genes of bacteria or *Pseudomonas* spp. of each DNA sample were calculated by comparing the threshold cycle values with standard curves, which were generated with 10-fold dilution series of plasmids containing the 16S rRNA genes of bacteria and *Pseudomonas* spp. from the soil samples. Sterile water, instead of soil DNA, served as a negative control. Each DNA sample was amplified in triplicate.

Illumine MiSeq sequencing and data processing

The cucumber rhizosphere bacterial community composition was analyzed with the primer F515/R907, which targets the V4-V5 regions of the bacterial 16S rRNA gene, as previously described (Zhou et al., 2018a). To distinguish each sample, both the forward and reverse primers also had a six-bp barcode. Each composite soil DNA was amplified in triplicate and the amplicons were pooled as suggested by Schöler et al. (2017). The composite amplicons were purified and quantified. Equimolar amounts of the amplicons were mixed together accordingly and then commercially sequenced on an Illumina Miseq platform (2 × 300).

The raw sequence data was de-multiplexed, quality-filtered, and processed with FLASH (Magoc and Salzberg, 2011) as previously described (Zhou et al., 2017). Operational taxonomic units (OTUs) were generated at the 97 % sequence similarity threshold through the UPARSE pipeline. Next, the taxonomic information of a representative sequence of each OTU was determined using the Ribosomal Database Project database (Wang et al., 2007). Chimeric sequences were identified and removed using USEARCH 6.1 in QIIME (Caporaso et al., 2010). The raw sequences were deposited in the NCBI-Sequence Read Archive under the Accession Number PRJNA506808.

Statistical analysis

To avoid potential bias caused by sequencing depth, randomly subsampled 20,900 16S rRNA gene sequences per sample were used for subsequent community analysis. Bacterial community alpha diversity indices, including the number of observed OTUs, the Shannon index and the inverse Simpson index were calculated. The defined OTUs were used to calculate taxon accumulation curves. To identify microbial taxa that were higher in each treat-

ment, linear discriminant effect size (LEfSe) analysis was performed with an alpha value of 0.05 for the Kruskal-Wallis test and a threshold of 2.0 for logarithmic linear discriminant analysis (LDA) scores (Segata et al., 2011). Principal coordinates analysis (PCoA) was used to analyze the beta-diversity of bacterial community on the basis of Bray-Curtis distance. Taxon accumulation curves, PCoA and ANOSIM analyses, alpha diversity indices were calculated using the 'vegan' package in the 'R' environment (version 3.3.1).

All data were checked for normality Shapiro-Wilk's test and homogeneity of variances by Levene's test. Data from quantitative PCR analysis were logarithmically transformed and analyzed using one-way analysis of variance and mean comparison with Welch's *t* test at the 0.05 probability level. Data analyses of relative abundances of bacterial taxa were performed with 'STAMP' (Parks et al., 2014). Other data analyses were conducted with the 'car' package in the 'R' environment (version 3.3.1).

Results

Cucumber seedling biomass, disease severity and bacteria abundances

Monocropped and intercropped cucumber seedlings had similar dry biomass (Table 1). However, intercropped cucumber seedlings had lower Fusarium wilt disease severity than monocropped cucumber seedlings ($p < 0.05$).

Compared with monocropped cucumber seedlings, intercropped cucumber seedlings had more bacteria and *Pseudomonas* spp. abundances ($p < 0.05$) in the rhizosphere (Table 1).

Summary of sequencing data

In total, we obtained 160,058 quality bacterial 16S rRNA gene sequences with 20,900-33,328 quality sequences per samples. The average length of all quality sequences was 397 bp. A total of 2,030 OTUs were classified at the 97 % sequence similarity level across all samples. The rarefaction curves of all soil samples tended to achieve the saturation curve at the OTU level (Figure 1A) and Good's coverage was in excess of 98 % for each sample. Therefore, the number of sequences was sufficient to assess the diversity of cucumber rhizosphere bacterial communities.

Bacterial community diversity in cucumber rhizosphere

For alpha diversity, number of observed OTUs, Shannon and Inverse Simpson indices of bacterial community were higher in the rhizosphere of intercropped cucumber seedlings than monocropped cucumber seedlings ($p < 0.05$) (Table 1).

For beta diversity, PCoA analysis revealed that the bacterial community composition differed between monocropped and intercropped cucumber rhizosphere soil samples (Figure 1B), and the difference between the two treatments was significant (ANOSIM, R = 0.79, $p < 0.05$).

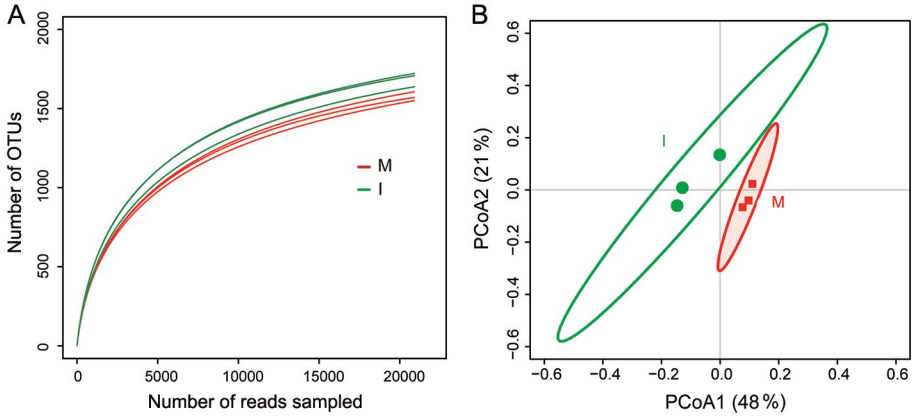

Figure 1 – Rarefaction curves of the number of OTUs (A) and beta diversity (B) of cucumber rhizosphere bacterial communities. M and I represent the treatments of cucumber monocropping and cucumber intercropped with wheat, respectively. Ellipses indicate 95 % confidence interval for replicates.

Table 1 – Cucumber seedling dry biomass, Fusarium wilt disease index, rhizosphere bacteria and *Pseudomonas* spp. abundances, and rhizosphere bacterial community alpha diversity in monocropping and intercropping systems.

	Plant dry weight	Fusarium wilt Disease index	Bacteria abundance	*Pseudomonas* spp. Abundance	No. of OTUs	Shannon index	Inverse Simpson index
	g per plant	%	10^{11} copies g^{-1} soil	10^{8} copies g^{-1} soil			
M	8.06 ± 0.32 a	63.82 ± 1.32 a	3.11 ± 0.25 b	5.65 ± 0.32 b	1575 ± 16 b	6.15 ± 0.02 b	172.42 ± 9.21 b
I	7.78 ± 0.22 a	52.78 ± 1.84 b	5.66 ± 0.35 a	15.53 ± 0.97 a	1689 ± 26 a	6.39 ± 0.08 a	265.51 ± 41.71 a

M and I represent the treatments of cucumber monocropping and cucumber intercropped with wheat, respectively. Different letters indicate statistically significant differences between treatments (Welch's *t* test, $p < 0.05$).

Bacterial community composition in cucumber rhizosphere

In total, 29 phyla were found and 2 % sequences cannot be classified into any known phylum. The dominant phyla with average relative abundances higher than 10 % across all samples were Actinobacteria, Firmicutes and Proteobacteria. Compared with monocropped cucumber, intercropped cucumber had higher relative abundances of Chloroflexi and Planctomycetes, but lower relative abundance of Cyanobacteria ($p < 0.05$) (Figures 2 and 3).

In total, 78 classes were found at the class level. The dominant classes with average relative abundance higher than 5 % across all samples were Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Clostridia and Gammaproteobacteria. Compared to monocropped cucumber, intercropped cucumber had higher relative abundances of Anaerolineae, Deltaproteobacteria, Phycisphaerae and Planctomycetacia, but lower Alphaproteobacteria and Cyanobacteria ($p < 0.05$) (Figures 2 and 4).

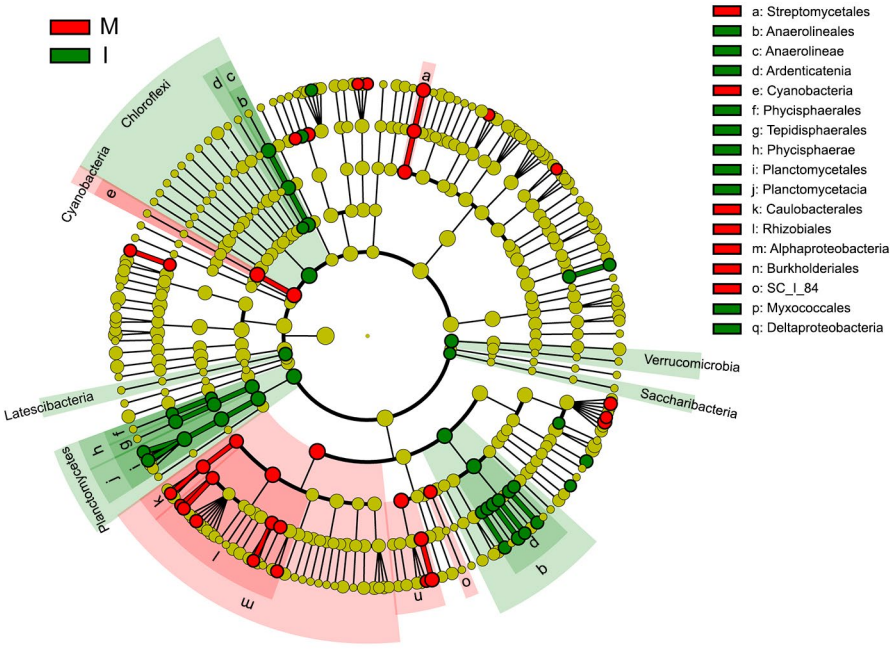

Figure 2 – Cladograms, generated from LEfSe analysis, represent the polygenetic distribution of bacterial taxa. Significantly different taxon nodes are colored: red for M (cucumber monocropping), green for I (cucumber intercropped with wheat). Yellow circles represent non-significant changed taxon. Each circle's diameter is proportional to the taxon's abundance. Labels are shown in the phylum to order levels.

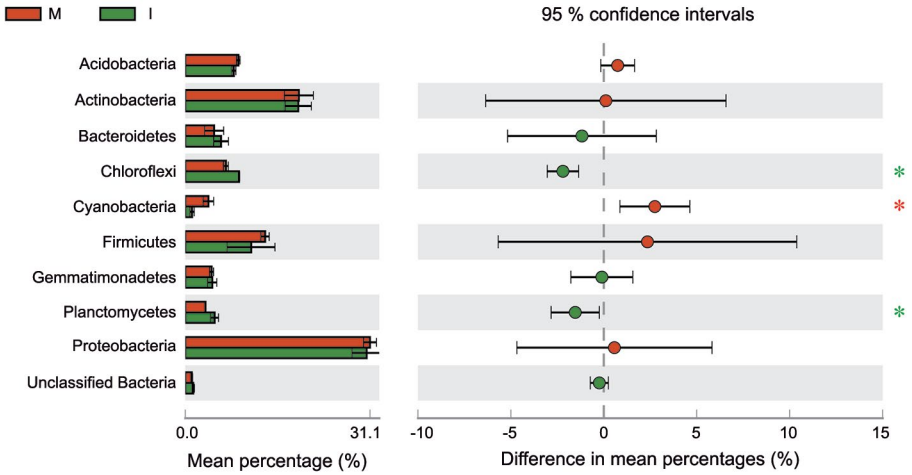

Figure 3 – Relative abundances (%) of main bacterial phyla in cucumber rhizosphere. M and I represent the treatments of cucumber monocropping and cucumber intercropped with wheat, respectively. Different letters indicate statistically significant differences between treatments (Welch's *t* test, $p < 0.05$).

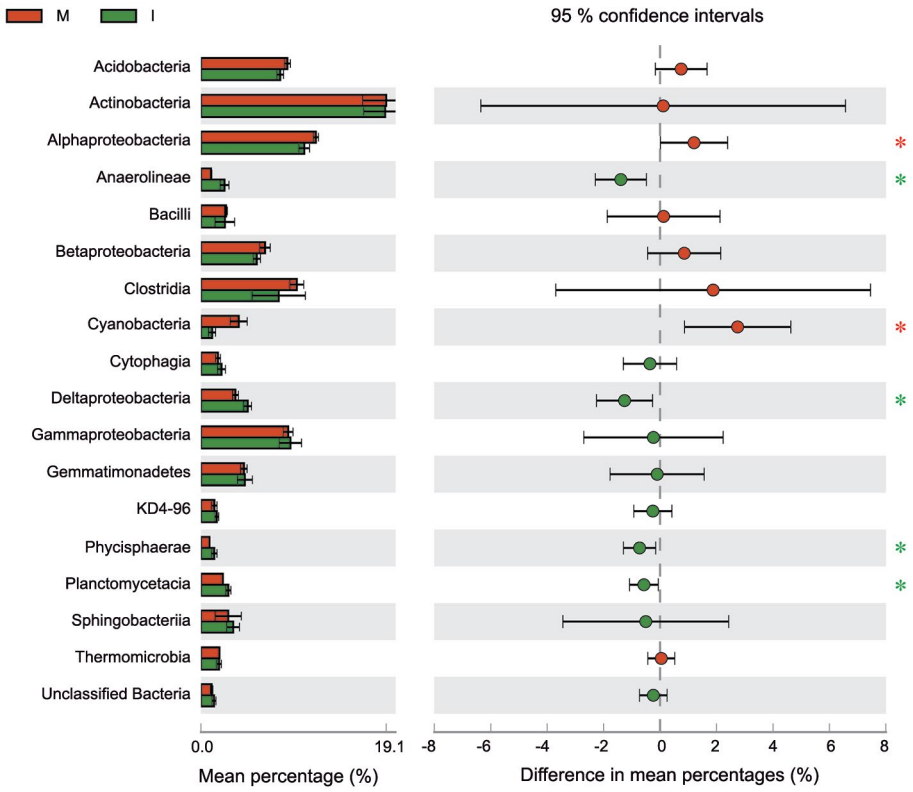

Figure 4 – Relative abundances (%) of main bacterial classes in cucumber rhizosphere. M and I represent the treatments of cucumber monocropping and cucumber intercropped with wheat, respectively. Different letters indicate statistically significant differences between treatments (Welch's *t* test, $p < 0.05$).

At the genus level, more than 540 bacterial genera were found. Compared with monocropped cucumber, intercropped cucumber had higher relative abundances of *Haliangium*, *Pirellula*, *Archangium*, *Gemmata*, *Ilumatobacter*, *Pseudomonas* and *Sorangium* spp., but lower relative abundances of *Lysobacter*, *Pseudoduganella*, *Rhodanobacter*, *Streptomyces*, *Bradyrhizobium*, *Hyphomicrobium*, *Tumebacillus* and *Variibacter* spp. ($p < 0.05$) (Table 2).

Shared and unique OTUs

Treatments of monocropped cucumber and intercropped cucumber shared 1,858 OTUs, which accounted for 92 % of total OTUs (Figure 5). The number of OTUs unique to the treatment of intercropped cucumber was higher than the number unique to the treatment of monocropped cucumber. OTUs unique to the treatment of monocropped cucumber were dominated by sequences affiliated to the classes Cyanobacteria, Planctomycetacia, Actinobacteria and Alphaproteobacteria. OTUs unique to the treatment of monocropped cucumber were dominated by sequences affiliated to classes Deltaproteobacteria, Gammaproteobacteria, Cytophagia and Alphaproteobacteria.

Discussion

Soil-borne diseases can cause considerable losses in crop production and are difficult to control by conventional strategies (Bonanomi et al., 2007; Boudreau, 2013). In this study, we found that intercropping wheat decreased cucumber Fusarium wilt severity, validating the idea that intercropping with appropriate plants can effectively suppress crop soil-borne diseases. For example, the intercropping of wheat or rice (*Oryza sativa*) can suppress Fusarium wilt disease of watermelon (Lv et al., 2018; Ren et al., 2008; Xu et al., 2015). The intercropping of Chinese chive (*Allium Tuberosum* Rottler) was able to control Panama disease in bananas (Zhang et al., 2013). The intercropping of crown daisy (*Chrysanthemum coronarium*) protected tomato (*Solanum lycopersicum*) from root-knot nematode (Dong et al., 2014). The intercropping of maize (*Zea mays*) reduced Phytophthora blight severity in pepper (*Capsicum annuum*) (Yang et al., 2014).

Our Illumina Miseq sequencing results showed that the intercropping of wheat increased the abundance and diversity of bacterial community in cucumber rhizosphere. These were in line with the notion that plant diversity can promote the diversity and abundance of

Table 2 – Relative abundances (%) of main classified bacterial genera in cucumber rhizosphere.

	M	I		M	I		M	I
Clostridium sensu stricto 1	5.99 ± 0.28	4.72 ± 1.00	Solirubrobacter	0.52 ± 0.08	0.55 ± 0.14	**Archangium**	0.27 ± 0.05	0.41 ± 0.02
Gaiella	2.11 ± 0.30	2.30 ± 0.35	**Rhodanobacter**	0.70 ± 0.04	0.37 ± 0.07	Devosia	0.37 ± 0.05	0.29 ± 0.02
Acidibacter	1.48 ± 0.16	1.79 ± 0.16	Phenylobacterium	0.52 ± 0.02	0.51 ± 0.02	**Variibacter**	0.37 ± 0.00	0.29 ± 0.01
Terrisporobacter	1.69 ± 0.15	1.44 ± 0.40	**Pirellula**	0.42 ± 0.00	0.60 ± 0.09	Pseudonocardia	0.32 ± 0.03	0.33 ± 0.07
Steroidobacter	1.37 ± 0.14	1.40 ± 0.11	Pir4 lineage	0.45 ± 0.03	0.57 ± 0.06	Mycobacterium	0.36 ± 0.05	0.30 ± 0.06
Streptomyces	1.62 ± 0.27	0.87 ± 0.04	Chryseolinea	0.43 ± 0.03	0.52 ± 0.05	**Sorangium**	0.28 ± 0.00	0.37 ± 0.03
Lysobacter	1.40 ± 0.10	1.00 ± 0.09	Sporosarcina	0.54 ± 0.01	0.40 ± 0.10	**Bradyrhizobium**	0.36 ± 0.03	0.27 ± 0.01
Pseudoduganella	1.89 ± 0.18	0.47 ± 0.03	Opitutus	0.31 ± 0.03	0.59 ± 0.13	Iamia	0.29 ± 0.04	0.31 ± 0.02
Nocardioides	1.04 ± 0.09	1.29 ± 0.13	Pedomicrobium	0.49 ± 0.03	0.47 ± 0.05	Hamadaea	0.24 ± 0.02	0.36 ± 0.08
Gemmatimonas	1.01 ± 0.11	1.00 ± 0.19	**Hyphomicrobium**	0.48 ± 0.00	0.44 ± 0.02	**Gemmata**	0.23 ± 0.01	0.37 ± 0.02
Actinoplanes	1.00 ± 0.09	0.97 ± 0.03	Planctomyces	0.44 ± 0.02	0.45 ± 0.06	Microvirga	0.26 ± 0.02	0.33 ± 0.03
Bacillus	0.64 ± 0.09	1.21 ± 0.41	Roseiflexus	0.42 ± 0.02	0.45 ± 0.06	Ensifer	0.31 ± 0.02	0.27 ± 0.00
Haliangium	0.69 ± 0.02	1.04 ± 0.13	Altererythrobacter	0.40 ± 0.02	0.41 ± 0.06	Rubrobacter	0.25 ± 0.07	0.32 ± 0.06
Nitrospira	0.89 ± 0.03	0.83 ± 0.03	Marmoricola	0.39 ± 0.01	0.41 ± 0.05	**Ilumatobacter**	0.23 ± 0.02	0.32 ± 0.02
Turicibacter	0.88 ± 0.11	0.56 ± 0.18	RB41	0.44 ± 0.06	0.35 ± 0.03	Nitrosospira	0.32 ± 0.04	0.23 ± 0.06
Microlunatus	0.65 ± 0.01	0.59 ± 0.07	Blastococcus	0.36 ± 0.03	0.42 ± 0.07	Ohtaekwangia	0.23 ± 0.05	0.31 ± 0.06
Bryobacter	0.64 ± 0.03	0.53 ± 0.02	Ramlibacter	0.38 ± 0.06	0.35 ± 0.02	Arenimonas	0.21 ± 0.01	0.32 ± 0.05
H16	0.58 ± 0.05	0.57 ± 0.02	Rhizocola	0.31 ± 0.01	0.41 ± 0.10	**Tumebacillus**	0.30 ± 0.00	0.14 ± 0.03
Novosphingobium	0.66 ± 0.07	0.45 ± 0.08	**Pseudomonas**	0.28 ± 0.04	0.43 ± 0.08	Flavobacterium	0.10 ± 0.01	0.31 ± 0.13
Agromyces	0.58 ± 0.04	0.52 ± 0.04	Sphingomonas	0.36 ± 0.09	0.33 ± 0.02	Aeromicrobium	0.26 ± 0.03	0.30 ± 0.02

M and I represent the treatments of cucumber monocropping and cucumber intercropped with wheat, respectively. Bacterial genus in bold indicates its relative abundance was significantly different between treatments (Welch's *t* test, $p < 0.05$).

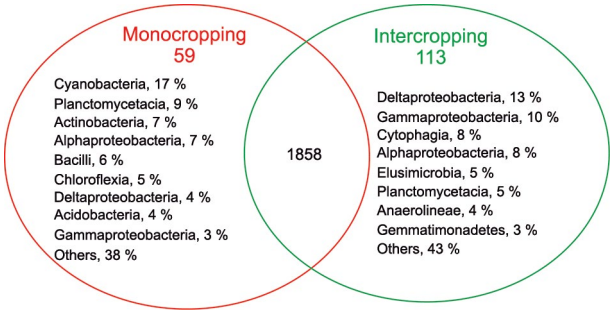

Figure 5 – Venn diagrams demonstrating the numbers of shared and unique observed OTUs between treatments of cucumber monocropping and cucumber intercropped with wheat. Frequencies of OTUs unique to each treatment at the bacterial class level are shown.

soil biota by increasing the habitat heterogeneity and resource diversity for soil biota (Eisenhauer et al., 2010). Increasing microbial community diversity and abundance can enhance the community's ability to resist invasion of pathogens (van Elsas et al., 2012). Therefore, the increased abundance and diversity of the rhizosphere's bacterial community may contribute to the lower Fusarium wilt severity observed in intercropped cucumber.

PCoA analysis showed that intercropping of wheat changed the composition of the cucumber rhizosphere's bacterial community. Moreover, several bacterial taxa sensitive to intercropping of wheat were identified. Plant-beneficial microbes can decrease plant disease severity by inhibiting the plant growth pathogens and inducing systemic resistance in plants (Pieterse et al., 2014). Illumina Miseq sequencing demonstrated that the relative abundances of several bacterial genera that contained potential plant-beneficial microbes, such as *Pseudomonas* (Pieterse et al., 2014), *Haliangium* (Fudou et al., 2001), *Archangium* (Leibold et al., 2004) and *Sorangium* (Niggemann et al., 2005) spp. were promoted by the intercropping of wheat. Quantitative PCR validated the higher abundance of *Pseudomonas* spp. in intercropped cucumber rhizospheres. Similarly, Bini et al. (2018) found that intercropping *Acacia mangium* stimulated arbuscular mycorrhizal fungi colonization in *Eucalyptus grandis*. Therefore, it was possible that the decreased cucumber Fusarium wilt severity was associated with the increased abundance of plant-beneficial microbes when intercropped with wheat.

In this study, Fusarium wilt disease was not observed when cucumber seedlings were grown in soils taken from the greenhouse, indicating the abundance of FOC was relatively low in the soil used. Therefore, cucumber seedlings were inoculated with FOC to assess the effects of intercropping of wheat on Fusarium wilt of cucumber. Bacterial communities in the rhizosphere of cucumber without inoculating FOC were analyzed. It has been shown that, in the presence of a pathogen, plants can adjust their root microbiome and recruit specific rhizosphere communities (such as plant-beneficial microbes) (Berendsen et al., 2018). Therefore, the response of cucumber rhizosphere bacterial communities to the intercropping of wheat might be different under conditions of inoculating and non-inoculating of FOC, an observation which should be stressed in the future.

The accumulation of autotoxic compounds is an important factor contributing to soil sickness, as autotoxic compounds can promote the propagation of soil-borne pathogens (Bever et al., 2012; Zhou et al., 2017). It has been shown that interspecific plant interactions are able to change plant secondary metabolism (Broz et al., 2010; Li et al., 2016; Liu et al., 2017). Lv et al. (2018) found that the intercropping of wheat inhibited watermelon from exudating phenolic autotoxins. Previous studies have shown that phenolic autotoxins changed cucumber rhizosphere microbial compositions and, especially, decreased the abundance of plant-beneficial microbes, including *Haliangium* and *Pseudomonas* spp. (Jia et al., 2018; Wang et al., 2018; Zhang et al., 2018a, b; Zhou et al., 2018b, c). In the present study, we found that *Pseudomonas* and *Haliangium* spp. in cucumber rhizospheres were stimulated by the intercropping of wheat. These suggested that the intercropping of wheat might decrease the amount of phenolic autotoxin in cucumber rhizospheres and, thus, alleviate the adverse effects of phenolic autotoxins on these plant-beneficial microbes. However, future research should be undertaken to verify this by measuring phenolic autotoxins in cucumber rhizospheres.

Overall, our results showed that the intercropping of wheat stimulated the alpha diversity and altered the composition of bacterial community in cucumber seedling rhizospheres. Moreover, the intercropping of wheat inhibited Fusarium wilt disease in cucumber seedlings and promoted an abundance of potential plant-beneficial microbes in the rhizosphere. Our results provided cues for new investigations about the inhibition of *Fusarium* infection in cucumber. This study stressed the viewpoint that engineering the plant rhizosphere microbial community through increasing plant diversity in the field is a promising strategy for enhancing agricultural sustainability. However, further field studies are needed to evaluate if wheat is an appropriate intercropping crop for cucumber in terms of cucumber yield, soil nutrient cycling and leaching, and incidences of cucumber diseases other than Fusarium wilt.

Acknowledgments

This work was supported by the National Key Research and Development Program (2018YFD1000800), National Natural Science Foundation of China (31772361), 'Academic Backbone' Project of Northeast Agricultural University (17XG05) and China Agricultural Research System (CARS-23-B-10).

Authors' Contributions

Conceptualization: Zhou, X.; Wu, F. Data acquisition: Jin, X.; Zhou, X.; Shi, Y.; Pan, K. Data analysis: Zhou, X.; Jin, X. Design of methodology: Zhou, X. Writing and editing: Zhou, X.; Jin, X.

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