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REGULAR ARTICLE

Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth

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Abstract
Background and aims Loss of biodiversity caused by intensive agriculture is a major worldwide concern. Crop rotation can enhance crop productivity and increase soil microbial diversity. However, the functional significances of changes in soil microbial communities are poorly understood.
Methods Soil microbial communities from a cucumber monoculture system and the cucumber season of a two-year rotation system (tomato-celery-cucumber-Chinese cabbage) were analyzed by quantitative PCR and high throughput amplicon sequencing. Then, feedback effects of soil biota on cucumber seedlings were evaluated through soil sterilization, addition of soil inoculum, and application of bactericide and fungicide.
Results Crop rotation increased cucumber yield and bacterial diversity, but decreased fungal diversity and abundance. The bacterial and fungal community compositions also differed between the two cropping systems. Abundances of potential plant pathogens and antagonistic microorganisms were lower while potential plant-growth-promoting microorganisms were higher in the rotation system. The overall effect of soil biota was positive on cucumber seedling growth, and was stronger in the rotation system.
Conclusions Increasing temporal plant diversity can change soil microbial communities and enhance crop productivity through positive plant-soil feedbacks mediated by soil biota.

Keywords Crop diversification · *Cucumis sativus* L. · Plant-soil feedback · Soil microbial communities

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Introduction

The loss of biodiversity can have clearly negative impacts on ecosystem functioning related to biomass production, nutrient cycling and decomposition (Cardinale et al. 2012). Modern agricultural practices are often characterized by monoculture, which led to the simplification of the components of agricultural systems (Cook 2006). Alternative management practices, such as crop rotation and intercropping, which can increase spatial and temporal plant diversity, have the potential to maintain yields while minimize the need for external inputs (Cook 2006; Zhou et al. 2011; Tiemann et al. 2015). Therefore, developing effective diversified cropping systems and understanding the underlying mechanism involved have important implications for sustainable agriculture. Empirical works suggest that the positive relationship between

biodiversity and plant biomass (overyielding) is due mainly to the sampling effect and complementarity between species (Cardinale et al. 2012).

Aboveground and belowground organisms dynamically interact with each other, and plant diversity is an important driving agent of belowground processes (Wardle 2006; Eisenhauer et al. 2010; van der Putten et al. 2013). Recently, plant-soil feedback has been suggested to play an important role in diversity-productivity relationships (van der Putten et al. 2013). Plant-soil feedbacks occur when plants change the biotic and abiotic soil environments in ways that alter subsequent plant performance (Brinkman et al. 2010; van der Putten et al. 2013). In grasslands ecosystem, the overyielding in species-rich communities was found to be due to the lower abundances of soil pathogens in mixtures compared to monocultures (Maron et al. 2011; Schnitzer et al. 2011). In agriculture, the yield decline of crops in monoculture can be viewed as a phenomenon of negative plant-soil feedback (Cook 2006; van der Putten et al. 2013). However, previous plant-soil feedback experiments mainly focused on natural ecosystems so far, little is known about the exact mechanisms of plant-soil feedback in agricultural ecosystems.

Soil microorganisms have profound effects on the growth, nutrition and health of plants in natural and agricultural ecosystems (Garbeva et al. 2004; Almario et al. 2013). Soil microbial communities were shown to be different between monoculture and diversified cropping systems. Commonly, soil microbial biomass, activities and diversity were lower in monoculture systems than in diversified cropping systems (van der Heijden and Wagg 2013; Tiemann et al. 2015). Evidences also suggest that increasing soil microbial diversity can increase plant productivity (Maherali and Klironomos 2007; Wagg et al. 2014). However, empirical studies from agricultural ecosystems found that higher soil microbial diversity from tree based intercropping systems (Bainard et al. 2013) and organically managed fields (Verbruggen et al. 2012) did not directly benefit crop growth. Thus, testing the functional significance of soil microbial communities in agricultural ecosystems is an important area for future research (van der Heijden and Wagg 2013).

The build-up of species-specific soil pathogens is an important factor contributing to the yield penalty in monoculture systems (Cook 2006; van der Putten et al. 2013). Specific antagonistic microbial communities (e.g., *Pseudomonas fluorescens*) have the potential to suppress soil-borne plant diseases such as Fusarium wilt. Production of secondary metabolites with antifungal activities, such as 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA) and pyrrolnitrin (PRN) is one of the mechanisms responsible for soil fungal pathogen suppression (Mazurier et al. 2009; Almario et al. 2013). Recently, it is demonstrated that increasing land use intensity can decrease these antagonistic microbial communities while increasing plant diversity can promote them (Garbeva et al. 2004; Latz et al. 2012). However, there are also contrasting evidences showing that plant monocultures produced more antagonistic soil microorganisms than diverse plant communities (Bakker et al. 2013; Schlatter et al. 2015). Thus, the relationship between plant diversity and antagonistic microorganisms deserves further attention.

Cucumber (*Cucumis sativus* L.), a vegetable crop of high economic importance in many countries, is commonly monocultured under greenhouse conditions. Fusarium wilt, caused by soil-borne necrotrophic fungus *Fusarium oxysporum* f.sp. *cucumerinum* Owen (FOC), is a yield-limiting factor in cucumber production (Zhou and Wu 2012). Our previous studies showed that cucumber growth and yield were negatively affected by continuous monoculture, while crop rotation could improve cucumber yield and increase soil bacterial diversity (Wu et al. 2011; Zhou et al. 2011, 2012). In this study, we first investigated the differences in soil microbial communities in a cucumber monoculture system and a tomato (*Solanum lycopersicum* L.)-celery (*Apium graveolens* L.)-cucumber-Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) rotation system. The diversities and compositions of soil bacterial and fungal communities were analyzed by amplicon sequencing of 16S rRNA gene fragment and rRNA internal transcribed spacer (respectively) using Illumina MiSeq platform, the abundances of bacteria, fungi, FOC, *Pseudomonas* communities, and genes encoding DAPG, PCA and PRN syntheses were determined with quantitative PCR. Then, we evaluated the feedback effects of these soil microbial communities on cucumber seedling growth. We hypothesized that (1) the rotation system had higher soil microbial community diversities and abundances than the monoculture system; and (2) FOC abundance was lower while antagonistic microbial abundances were higher in the rotation system; thus, (3) relative to the monoculture system, soil microbial communities from the rotation system can facilitate cucumber growth.

Materials and methods

Greenhouse experiment

The greenhouse experiment was initiated in 2006 in the Experimental Station of Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E). Before the greenhouse was constructed, the open was cultivated with maize (*Zea mays* L.) for more than 20 years. A cucumber monoculture system and a two-year rotation system (tomato-celery-cucumber-Chinese cabbage) were included. All cropping systems were cropped twice a year in spring (April to July) and autumn (July to October). In spring, cucumber or tomato were transplanted in late April and harvested in middle July. In autumn, celery or Chinese cabbage for the rotation system and cucumber for the monoculture system were transplanted in late July and harvested in middle October.

There were three replicate plots (6-m long, 2.4-m wide) for each cropping system, arranged in a randomized block design. Each plot contained four rows, while the center two rows were used for cucumber yield recording and soil sampling. Raised beds were formed about five days before plant seedlings were transplanted. In the spring of each year, 45,000 kg ha^{-1} decomposed manure (containing 0.5% N, 0.5% P and 0.4% K) and 200 kg ha^{-1} diammonium hydrogen phosphate were applied as basal fertilizer. Urea was applied as topdressing at the rate of 150 kg ha^{-1} on 45 days after transplanting for tomato and cucumber, and 80 kg ha^{-1} on 20 days after transplanting for celery and Chinese cabbage. The plant density was 6 plants per m^2 for cucumber and tomato, 30 plants per m^2 for celery, 8 plants per m^2 for Chinese cabbage. Flooding irrigation with groundwater was performed when necessary. Weeds were manually removed. After harvest, plant aboveground debris and main roots were cleared from the greenhouse. The soil was a black soil (Mollisol) with a sandy loam texture, and contained sand, 326 g kg^{-1}; silt, 384 g kg^{-1}; clay, 270 g kg^{-1}; organic matter, 3.51%; available N (NH_4^+-N and NO_3^--N), 146.60 mg kg^{-1}; Olsen 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.

Soil sampling

In May 2013, seven years after the establishment of the greenhouse experiment, bulk soils were sampled from the cucumber monoculture system and the cucumber season of the fourth round of the rotation system on 30 days after cucumber transplanting. Thirty six soil cores (8 cm diameter, 20 cm deep) were randomly collected from the inner two rows of cucumber in each plot and each of these twelve soil cores were mixed to make a composite sample. Thus, there were three composite soil samples in each plot for every cropping system. Soils were sieved (2 mm), and large stone and plant derbies were removed. One part of these fresh sampled soils was air-dried (<30 °C) for soil chemical property analysis; another part was stored at −70 °C for DNA extraction; the other part was used for plant-soil feedback experiment.

Soil samples were also collected from the upper soil layer (0–20 cm) of an open field near by the greenhouse, where the cucumber monoculture and rotation systems were performed. The open field was cultivated with one cropping of maize each year since 1992. In the plant-soil feedback experiment, these soils were sterilized to eliminate soil biota and were inoculated with fresh soils from cucumber monoculture and rotation systems. These soils from the open field were termed as 'background soil' in this experiment.

Soil chemical analysis

Soil pH was determined with 10 g soil in water suspensions at a soil/water ratio of 1:2.5 with a glass electrode. For soil inorganic N (NH_4^+-N and NO_3^--N), 10 g soil was extracted with 2 M potassium chloride and analyzed with a continuous flow analyzer (San++, SKALAR, Netherlands). Soil organic carbon was determined by digesting 0.5 g soil with potassium dichromate and sulphuric acid, and titrating the residual potassium dichromate with ammonia ferrous sulphate. Soil phenolics were extracted from 25 g soil with 2 M NaOH and measured by the Folin-Ciocalteau method (Dalton et al. 1987; Zhou et al. 2012). A calibration curve was constructed with different concentrations of ferulic acid. The unit of soil phenolics was expressed as μg of ferulic acid equivalents per gram of soil dry-weight. Each soil sample was measured in triplicate.

DNA extraction and quantitative PCR

Total soil DNA was extracted from 0.25 g soil with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) according to the manufacturer's instructions. Each composite soil sample was extracted in triplicate and the extracted DNA solutions were pooled.

Soil microbial abundances were estimated by quantitative PCR assays in an IQ5 real-time PCR system (Bio-Rad Lab, LA, USA). For total bacteria and *Pseudomonas* spp., primer sets of 338F/518R (Muyzer et al. 1993) and PsF/PsR (Garbeva et al. 2004) were used to amplify the partial bacterial 16S rRNA genes. For fungal community, primer set of ITS1F/ITS4 (Gardes and Bruns 1993) was used to amplify the fungal internal transcribed spacer (ITS) regions of the rRNA gene. A FOC-specific SCAR primer set FocF8/FocR2 (Scarlett et al. 2013) was used for FOC. For antibiotic producers, genes *phlD*, *phzC*, and *prnD* responsible for the production of DAPG, PCA and PRN synthesis were amplified with primer sets of B2BF/B2BR3 (Almario et al. 2013), PHZJR1/PHZJR2 (Mazurier et al. 2009) and PRND1/PRND2 (de Souza and Raaijmakers 2003), respectively. These primers used in this study had been validated for qPCR analysis of soil microbes. The amplicon sizes for total bacteria, fungi, *Pseudomonas* spp., FOC, and *phlD*, *phzC* and *prnD* genes were 230, 750, 960, 108, 319, 522 and 786 bp, respectively. All amplifications were performed in triplicate for each composite soil sample. The specificity of the products was confirmed by melting curve analysis and agarose gel electrophoresis. Standard curves were created with 10-fold dilution series of plasmids containing the target genes from soil samples. The R^2 of standard curves ranged from 0.95–0.99. The threshold cycle (Ct) values obtained for each sample were compared with the standard curve to determine the initial copy number of the target gene. The detection limits for antibiotic producers were 10^3 copies g^{-1} soil, FOC was 5×10^2 copies g^{-1} soil.

Illumina Miseq sequencing and data processing

Soil bacterial and fungal community compositions were analyzed with Illumina MiSeq sequencing. Primer sets of F338/R806 and ITS1F/ITS2 were used to amplify V3-V4 regions of the bacterial 16S rRNA gene and the ITS1 regions of the fungal rRNA gene as described before (Crowther et al. 2014; Derakhshani et al. 2016), respectively. Both the forward and reverse primers also had a 6-bp barcode unique to each sample, which were used to permit multiplexing of samples. Each composite soil sample was independently amplified, the products of the triplicate PCR reactions were pooled and purified using the Agarose Gel DNA purification kit (TaKaRa). Then, purified amplicons were quantified by a TBS-380 μ fluorometer with Picogreen reagent (Invitrogen, USA), and mixed accordingly to achieve the equal concentration in the final mixture. The mixture was then paired-end sequenced (2 × 300) on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Raw sequence reads were de-multiplexed, quality-filtered, and processed using FLASH (Magoc and Salzberg 2011) with the following criteria: (i) The 300 bp reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) exact barcode matching. 2 nucleotide mismatch in primer matching, reads containing ambiguous characters were removed. (iii) only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded. Sequences were trimmed to 220 bp using -fastq_filter in UPARSE (Edgar 2013). Operational taxonomic units (OTUs) were delineated at 97% sequence similarity with UPARSE using an agglomerative clustering algorithm (Edgar 2013). Then, a representative sequence of each OTU was taxonomically classified through BLAST in Ribosomal Database Project (RDP) (Wang et al. 2007) (bacteria) and Unite (Kõljalg et al. 2013) (fungi) databases. Chimeric sequences were identified and removed using USEARCH 6.1 in QIIME (Caporaso et al. 2010). After filtering reads by basal quality control and removing singleton OTUs, 24,693 quality bacterial sequences and 65,869 quality fungal sequences were obtained per sample on average. The average read lengths were 438 bp and 252 bp for the 16S rRNA genes and ITS regions, respectively.

Growth responses of cucumber seedlings to soil biota from cucumber monoculture and rotation systems

Soil and cucumber seedling preparation

Soils from the field and part of soils from cucumber monoculture and rotation systems were sterilized with three cycles of autoclaving (121 °C, 30 min) and cooling to room temperature. In the following pot experiment, these sterilized soils were referred as sterile soils, while unsterilized soils were referred as intact fresh soils.

Cucumber seeds (cv. Jinyou 1) were sterilized with 2.5% NaClO solution for 10 min and washed thoroughly with distilled water, and then germinated in the dark at 28 °C. Fourteen hours later, germinated cucumber seeds were transferred to sterilized

sands. Seven days later, cucumber seedlings with two cotyledons were used to transplant.

Experiment 1: Effects of soil sterilization on cucumber seedling growth

Pot experiment was performed to test the effects of soil sterilization (non-sterile vs. sterile soil) on cucumber seedling growth. There were four treatments: (1) intact fresh monoculture soils, (2) sterile monoculture soils, (3) intact fresh rotation soils, and (4) sterile rotation soils.

Experiment 2: Effects of total soil biota inoculation on cucumber seedling growth

The elimination of soil biota by autoclaving may also change soil chemistry, especially can induce nutrient flushes (Brinkman et al. 2010). To avoid this potential confounding effect, a second pot experiment was performed by adding soil biota inoculum to sterile soils as described before (Brinkman et al. 2010).

Fresh soils from cucumber monoculture and rotation systems were used as inocula to test the effects of total soil biota on cucumber seedling growth. Plastic pots were filled with 658 g sterile field soil (background soil) and 42 g inoculum soil. The ratio of inoculum-to-background soil was 6% (mass/mass). There were four treatments: sterile field soils inoculated with (1) intact fresh monoculture soils, (2) sterile monoculture soils, (3) intact fresh rotation soils, and (4) sterile rotation soils. Each mixture was thoroughly mixed in sterilized 1000 ml conical flasks by shaking.

Experiment 3: Effects of bactericide and fungicide on cucumber seedling growth

Soil fungi and bacteria were suppressed by fungicide and bactericide to test the effects of soil fungi and bacteria on cucumber seedling growth, respectively. There were six treatments: (1) intact fresh monoculture soils, (2) monoculture soils treated with fungicide, (3) monoculture soils treated with bactericide, (4) intact fresh rotation soils, (5) rotation soils treated with fungicide, (6) rotation soils treated with bactericide. Fungicide benomyl and bactericide streptomycin sulphate were applied at the rate of 50 $mg \cdot kg^{-1}$ as a soil drench as described before (Nijjer et al. 2007). Benomyl fungicide is expected to suppress a wide range of soil fungi including ascomycete and basidiomycete fungi, as well as arbuscular mycorrhizal fungi; streptomycin sulphate is a broad-spectrum antibiotic that can inhibit both Gram-positive and Gram-negative bacteria. Both benomyl and streptomycin sulphate were shown to have minimal direct effects on plants (Bashan and de-Bashan 2002; Callaway et al. 2004).

Cucumber seedlings with two cotyledons were transferred into plastic pots (14 cm diameter, 16 cm height) contained 700 g different soils prepared in Experiment 1, 2 and 3 in May 2013. There was one cucumber seedling per pot. The experiment was set up following a randomized block design with three replicate blocks. For each treatment in each block, there were ten pots prepared with soils from the same plot of the greenhouse monoculture or rotation system. The seedlings were maintained in a greenhouse (28 °C day/18 °C night, relative humidity of 75%, 12 h light/12 h dark). The position of blocks was randomized every three days and the pots with in plot were randomized per day. Soil water content (about 60% of its water holding capacity) was adjusted every two days with distilled water to maintain a constant weight of pots. Ten days later, cucumber seedlings were harvested. Plant dry weight was measured after oven drying at 70 °C to constant weight.

Statistical analysis

For the Illumina MiSeq sequencing data, read counts were not rarefied to equal sampling depths because this unnecessarily discards data (McMurdie and Holmes 2014). Hill's series of diversity were calculated with the square root transformed read counts (Bálint et al. 2015) with the 'vegan' package in 'R' (Oksanen et al. 2014). The series consists of three numbers: N0 is the number of species in a sample; N1 is the antilogarithm of the Shannon diversity (representing the abundant species in a sample); and N2 is the inverse Simpson diversity (representing the very abundant species in a sample) (Hill 1973). For beta diversity analysis, read counts were centered log-ratio (CLR) transformed (Fernandes et al. 2014) with the 'rgr' package in 'R' (Oksanen et al. 2014). Microbial community composition was analyzed using principal component analysis based on a Euclidean distance matrix. Analysis of similarities (ANOSIM) was carried out to test for significant differences in microbial communities. The principal component analysis and ANOSIM analysis were performed with the 'vegan' package in 'R' (Oksanen et al. 2014). 'ALDEx2' package in 'R' (Fernandes et al. 2014) was used to compare the

relative abundances of phyla, proteobacterial classes and genera between treatments. Significance was based on the Benjamini-Hochberg corrected *P* value of the Wilcoxon test ($P < 0.05$).

The data of cucumber yield, soil chemical properties, microbial abundances determined by quantitative PCR and diversity indices, and cucumber seedling biomass were checked for normality (Shapiro-Wilk's test) and homogeneity of variances (Levene's test). Data of soil microbial abundances determined by quantitative PCR were logarithmically transformed. Then, these data were analyzed with one-way analysis of variance (ANOVA). Mean comparison between treatments was performed based on the Tukey's honestly significant difference (HSD) test at the 0.05 probability level.

Results

Cucumber yield and soil chemical properties

In 2013, cucumber yield was significantly higher in the tomato-celery-cucumber-Chinese cabbage rotation system than in the cucumber monoculture system ($P < 0.05$) (Table 1). Compared with the monoculture system, cucumber yield had an increase of 9.9% in the rotation system.

Soil pH and organic carbon were higher, while inorganic N and phenolics were lower in the rotation system than in the monoculture system ($P < 0.05$) (Table 1).

Soil microbial abundances

Abundances of soil bacteria and *Pseudomonas* spp. were similar in the cucumber monoculture and rotation systems (Table 2). Soil total fungi, FOC, DAPG and PRN producer abundances were significantly lower in the cucumber rotation system than in the monoculture system ($P < 0.05$). Soil total fungi and FOC abundances of the monoculture system were 2.5 and 6.6 times that of the rotation system, respectively. Soil DAPG and PRN producer of the monoculture system were 1.96 and 3.46 times that of the rotation system, respectively. Soil PCA producer was not detected in both cropping systems (the detection limit was 10^3 copies g^{-1} soil).

Soil bacterial community composition and structure

For bacterial community, 27 phyla were found across all samples with Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Chloroflexi and Acidobacteria as the dominant phyla, which accounted for more than 94% of the bacterial sequences (Fig. 1a). However, there were variations in relative abundances of these groups. Compared with the monoculture system, the rotation system had higher relative abundances of Acidobacteria and Verrucomicrobia, and lower relative abundances of Gammaproteobacteria and Actinobacteria ($P < 0.05$). At the genus level, the relative abundances of *Actinomadura*, *Steroidobacter*, *Rhodanobacter*, *Streptomyces*, *Chitinophaga*, *Dokdonella*, and *Saccharothrix* spp. were lower while *Flavobacterium*, and *Ohtaekwangia* spp. were higher in the rotation system ($P < 0.05$) (Fig. 2a).

The richness and diversity indices of the bacterial community were significantly higher in the rotation system than in the monoculture system ($P < 0.05$) (Fig. 3a). The principal component analysis applied to visualize the differences samples showed a clear separation between monoculture and rotation systems (Fig. 4a). ANOSIM also confirmed that the bacterial communities were significantly different between the cropping systems ($R = 0.85$, $P = 0.001$).

Soil fungal community composition and structure

The dominant fungal phyla were Ascomycota and Zygomycota, accounting for more than 77% of the

Table 1 Cucumber yield and soil chemical properties in cucumber monoculture (M) and rotation (R) systems

	Cucumber yield (kg plant^{-1} FW)	Soil pH	Inorganic N (mg kg^{-1})	Soil organic carbon (g kg^{-1})	Soil phenolics (μg ferulic acid equivalents g^{-1} soil)
M	1.72 ± 0.06 b	6.41 ± 0.10 b	172.42 ± 11.62 a	20.68 ± 0.48 b	231.61 ± 22.70
R	1.89 ± 0.03 a	6.70 ± 0.09 a	136.69 ± 13.86 b	21.74 ± 0.33a	97.38 ± 13.40

Values (mean ± standard error) in the same column followed by different letters are significantly different ($P < 0.05$, Tukey's HSD test)

Table 2 Soil microbial abundances in cucumber monoculture (M) and rotation (R) systems

	Bacteria (10^{11} copies g^{-1} soil)	Fungi (10^{9} copies g^{-1} soil)	*Pseudomonas* spp. (10^{9} copies g^{-1} soil)	FOC (10^{3} copies g^{-1} soil)	DAPG producer (10^{4} copies g^{-1} soil)	PRN producer (10^{4} copies g^{-1} soil)
M	1.68 ± 0.01 a	3.07 ± 0.12 a	1.19 ± 0.03 a	6.71 ± 0.50 a	5.00 ± 0.21 a	4.22 ± 0.61 a
R	1.82 ± 0.18 a	1.22 ± 0.05 b	1.20 ± 0.02 a	1.02 ± 0.08 b	2.55 ± 0.45 b	1.22 ± 0.09 b

Values (mean ± standard error) in the same column followed by different letters are significantly different at the 0.05 probability level according to Tukey's HSD test based on logarithmically transformed values

fungal sequences. Ciliophora, Basidiomycota and Chytridiomycota were found at relatively low abundances (Fig. 1b). The relative abundance of Chytridiomycota was higher in the rotation system ($P < 0.05$). At the genus level, both cropping systems were dominated by *Mortierella* and *Cephaliophora* spp. Relative to the monoculture system, the rotation system had higher relative abundances of *Monographella* and *Operculomyces* spp. and lower relative abundances of *Fusarium*, *Acremonium*, *Pseudaleuria*, *Cladosporium*, *Davidiella*, *Microsporum*, *Antarctomyces*, and *Lecanicillium* spp. ($P < 0.05$) (Fig. 2b).

The richness and diversity indices of the fungal community were significantly lower in the cucumber rotation system than in the monoculture system ($P < 0.05$) (Fig. 3b). Principal component analysis revealed that samples from monoculture and rotation systems were separated from each other (Fig. 4b), and the difference was statistically significant (ANOSIM, $R = 0.83$, $P = 0.001$).

Effects of soil sterilization on cucumber seedling growth

Cucumber seedlings grown in intact fresh rotation soils had significantly higher root dry biomass than in intact fresh monoculture soils ($P < 0.05$) (Fig. 5a). Sterilization reduced cucumber seedling root and shoot dry biomasses, and significant reductions were observed for the rotation system ($P < 0.05$). Cucumber seedlings had similar growth parameters in sterile soils from monoculture and rotation systems.

Effects of total soil biota on cucumber seedling growth

Cucumber seedlings root and shoot dry biomasses did not differ between treatments inoculated with sterile monoculture or rotation soils (Fig. 5b). Generally, sterile field soils inoculated with intact fresh soils had higher cucumber seedling biomass than inoculated with sterile soils ($P < 0.05$) (Fig. 5b). The treatment inoculated with intact fresh rotation

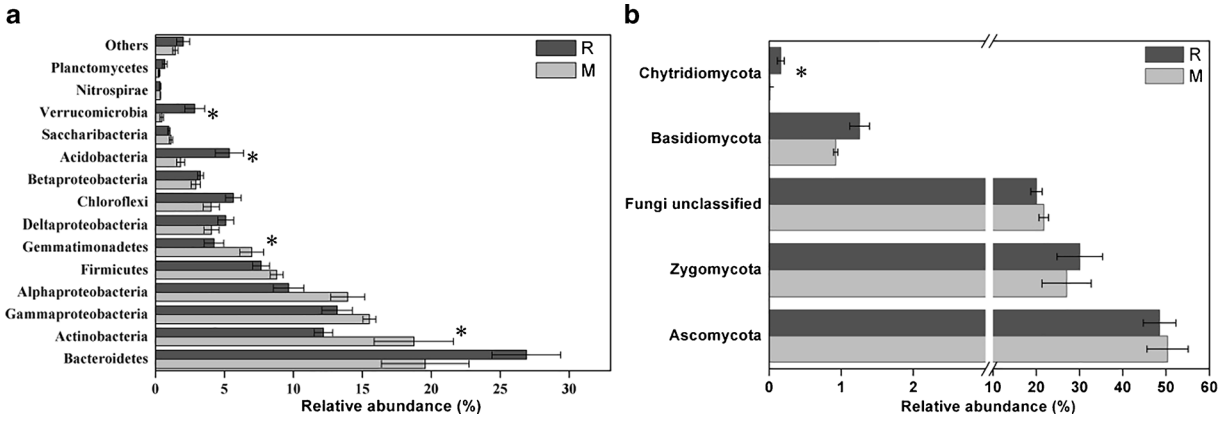

Fig. 1 Relative abundances of main bacterial phyla and proteobacterial classes (**a**) and fungal phyla (**b**) in the cucumber monoculture (M) and rotation (R) systems. Values are expressed as mean ± standard error. The centered log-ratio transformed data were used for comparison between cropping systems. Asterisks indicate significant difference based on Benjamini-Hochberg corrected Wilcoxon test ($P < 0.05$)

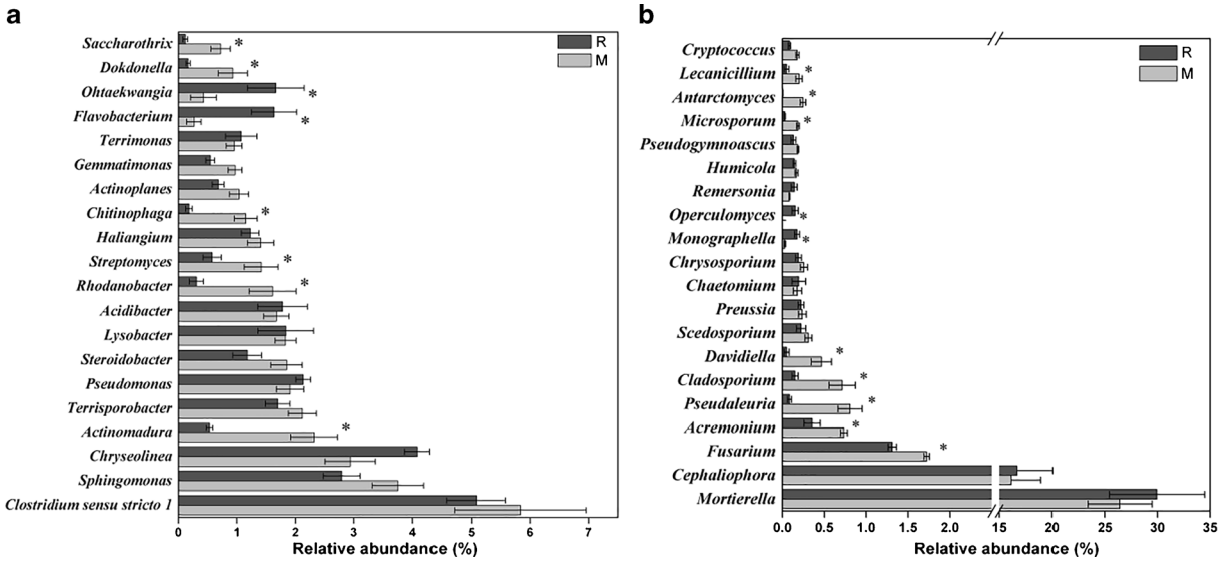

Fig. 2 The relative abundance of the top 20 classified bacteria genera (**a**) and fungal genera (**b**) in the cucumber monoculture (M) and rotation (R) systems. Values are expressed as mean ± standard error. The centered log-ratio transformed data were used for comparison between cropping systems. Asterisks indicate significant difference based on Benjamini-Hochberg corrected Wilcoxon test ($P < 0.05$)

soils had higher shoot dry biomass than the treatment inoculated with intact fresh monoculture soils. Compared with treatments inoculated with sterile soils, the increase in shoot biomass was larger in the treatment inoculated with intact fresh rotation soils (71% and 69%) than in the treatment inoculated with intact fresh monoculture soils (40% and 41%).

Effects of fungicide and bactericide on cucumber seedling growth

Fungicide and bactericide treatments had no significant effects on cucumber seedling biomass in monoculture soils (Fig. 5c), but significantly reduced cucumber seedling biomass in rotation soils

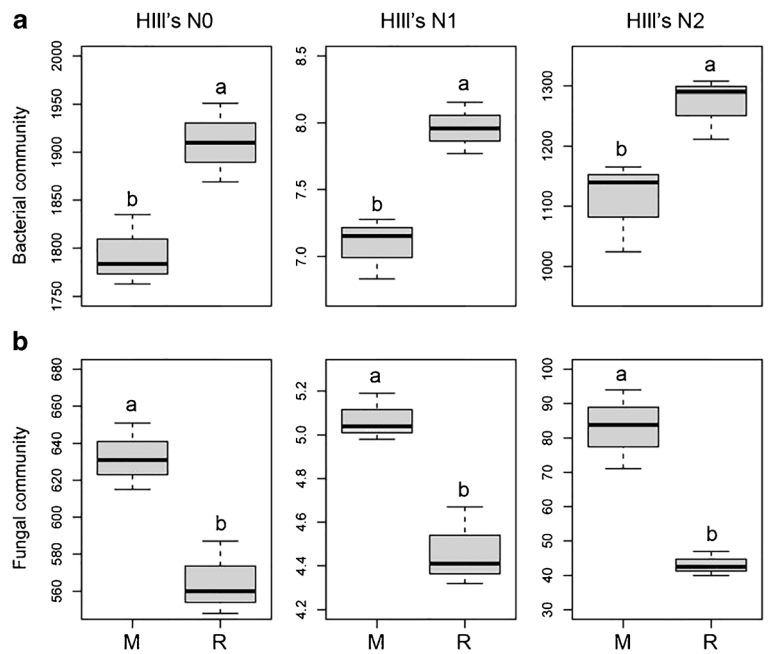

Fig. 3 Diversity and richness indices based on Illumina MiSeq sequencing of bacterial (**a**) and fungal (**b**) communities in cucumber monoculture (M) and rotation (R) systems. Operational taxonomic units (OTUs) were delineated at 97% sequence similarity. Hill's series of diversity were calculated with the square root transformed read counts. Hill's N0, OTU richness; Hill's N1, antilogarithm of Shannon's diversity; Hill's N2, inverse of Simpson's diversity. Different letters indicate significant difference based on Tukey's HSD test ($P < 0.05$)

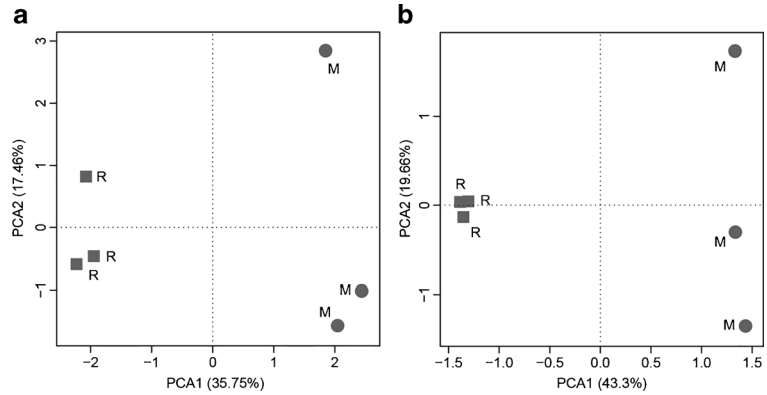

Fig. 4 Principal component analyses of bacterial (**a**) and fungal (**b**) communities in the cucumber monoculture (M) and rotation (R) systems based on Euclidean distance at the OTU level

($P < 0.05$). For rotation soils, cucumber seedling root dry biomass was similar in fungicide and bactericide treatments, cucumber seedling shoot dry biomass was significant lower in fungicide treatment than in bactericide treatment ($P < 0.05$).

Discussion

Compared with monoculture system, the rotation system increased cucumber yield in 2013, which was consistent with results in 2007 (Wu et al. 2011). The magnitude of

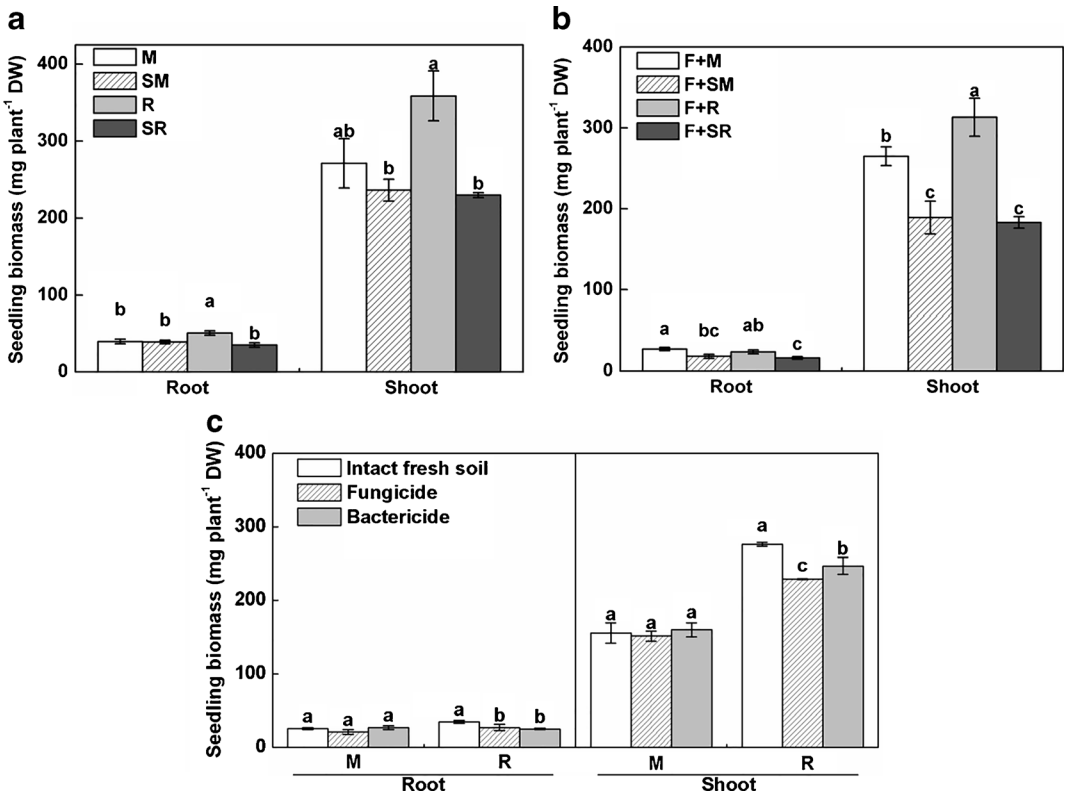

Fig. 5 Growth responses of cucumber seedlings to soil sterilization (**a**), soil biota inoculation (**b**), bactericide and fungicide treatments (**c**). M: intact fresh monoculture soils, SM; sterile monoculture soils, R: intact fresh rotation soils, SR: sterile rotation soils. F + M: sterile field soils inoculated with intact fresh monoculture soils, F + SM: sterile field soils inoculated with sterile monoculture soils, F + R: sterile field soils inoculated with intact fresh rotation soils, F + SR: sterile field soils inoculated with sterile rotation soils. Values are expressed as mean ± standard error. Different letters indicate significant difference based on Tukey's HSD test ($P < 0.05$)

increase in cucumber yield was comparable in 2007 and 2013 (11% vs 9%). Previous plant diversity experiments in grassland communities demonstrated that the net effects of biodiversity on ecosystem functions grow stronger as experiments run longer because the magnitude of resource complementarity increases over time (Cardinale et al. 2012). However, high rate of fertilizer was used in our experiment, which may make the resource complementarity a less important driver for the increased cucumber productivity in our rotation system.

In this study, soil microbial communities differed between cucumber monoculture and rotation systems. The rotation system had higher bacterial diversity but lower fungal diversity, indicating our first hypothesis was not fully validated. Though it is intuitive to speculate that higher plant diversity can increase soil biota diversity by increasing soil carbon resource diversity and habitat heterogeneity (Eisenhauer et al. 2010, 2011), studies focused on the effects of living plant and plant litter diversity on soil diversity revealed inconsistent results, ranging from negative, positive, neutral or species-specific relations (Wardle 2006; Schlatter et al. 2015). It was suggested that this disputed issue may be partially explained by the time-lag response of soil biota to plant diversity changes (Eisenhauer et al. 2010, 2011). For example, plant diversity effects on soil organisms only significantly occurred four-six years after the establishment of the experiments (Eisenhauer et al. 2011). In our study, soil microbial communities were analyzed seven years after the establishment of cucumber monoculture and rotation cropping systems. Thus, we may not substantially underestimate the effects of crop diversity on soil biota.

Although shifts in soil microbial communities induced by various cropping systems have been previously identified, their consequences for plant growth are still not clear (Verbruggen et al. 2012; Bainard et al. 2013; van der Heijden and Wagg 2013). In this study, we investigated the growth responses of cucumber seedlings to soil biota from cucumber monoculture and rotation systems. Results showed that soil sterilization reduced cucumber seedling growth for the rotation system, inoculation of total soil biota promoted cucumber seedling growth for monoculture and rotation systems, fungicide and bactericide treatments reduced cucumber seedling growth for the rotation system (Fig. 5). Thus, the overall effect of soil biota on cucumber seedling growth was positive, suggesting that the effects of beneficial soil microorganisms on plant outweighed those of pathogenic ones. In the soil sterilization experiment, the magnitude of decreases in cucumber seedling growth in soil sterilization treatments was larger for rotation system; while in the soil biota inoculation experiment, the magnitude of increases in cucumber seedling growth was larger when inoculated with soil biota from rotation system. These implied that the feedback of total soil biota was more positive in cucumber rotation system than in monoculture system, which supported our third hypothesis. However, it should be stressed that there was possibility that microorganisms from the environment may colonize the sterile soil and thus affect cucumber seedling growth. Therefore, the growth performances of cucumber seedlings in sterile soils may be not due to elimination of soil microorganisms, but to invasion by these microorganisms.

Soil microorganisms range from plant pathogens to potential mutualists, such as mycorrhizal fungi and nitrogen-fixing bacteria, to free-living ones (Maherali and Klironomos 2007; van der Putten et al. 2013). In this study, the abundance of FOC, a soil-borne fungal pathogen of cucumber, was significantly higher in the monoculture system, which supported the notation that plant species-specific soil pathogen is a driver of diversity-productivity relationships (Cook 2006; Maron et al. 2011; Schnitzer et al. 2011). Moreover, potential plant growth promoting microbes, such as *Flavobacterium* spp. (Singh et al. 2011), were enriched in the rotation system. Thus, the enhanced promoting effect of soil biota on cucumber growth in the rotation system was linked to decreases in the negative effects of plant pathogens and increases in the positive effects of beneficial microorganisms.

Plant diversity can increase plant resistance against soil pathogens by fostering antagonistic soil microorganisms (Latz et al. 2012, 2015). In this study, the abundance of *Pseudomonas* spp., which has species with pathogen-antagonistic potential, was similar between the two cropping systems. Moreover, abundances of soil DAPG, PRN producer, relative abundances of *Saccharothrix*, *Streptomyces*, *Actinomadura*, *Lecanicillium*, *Cladosporium* (teleomorph *Davidiella*), *Acremonium* and *Fusarium* spp. (Sanchez et al. 2012; Brakhage 2013) which have the potential to produce secondary metabolites and extracellular enzymes to inhibit other soil microorganisms were higher in the monoculture system. Therefore, our second hypothesis was not fully supported and indicated that the inhibition of FOC was not by fostering antagonistic

microorganisms in the rotation system. Our results supported the predication that low soil carbon resource diversity as a result of low plant diversity can cause more intense competition and favor antagonistic microorganisms (Bakker et al. 2013; Schlatter et al. 2015). In this study, PCA producer was not detected in our soils. Therefore, particular antibiotic producer was selected in the cucumber cultivation system, which may be due to the fact that agricultural practices can differentially affect these microorganisms (Mavrodi et al. 2012).

The cucumber rotation system had higher soil organic carbon than the monoculture system, which was in accordance with results from natural grass land and agricultural systems (Cardinale et al. 2012; Cong et al. 2015). Quantitative PCR analysis showed that soil bacterial abundance was largely insensitive while fungal abundance had negative response to crop rotation. This was contrary to the general observation that plant diversity had positive effects on soil microbial biomass, which was ascribed to the increases in soil carbon resources in diverse communities (Eisenhauer et al. 2010). Previous studies found that Gammaproteobacteria and Actinobacteria responded positively while Acidobacteria responded negatively to exogenously applied labile carbon resources (Fierer et al. 2007; Badri et al. 2013). However, in our study, the relative abundance of Acidobacteria were higher while Gammaproteobacteria and Actinobacteria were lower in the rotation system. Therefore, soil carbon resources availability may be not a key driver of soil microbial community changes in our cucumber cropping systems.

The release of toxic chemical compounds is suggested as one possible mechanism to explain negative plant-soil feedbacks (Cook 2006; van der Putten et al. 2013). Phenolics from cucumber, such as *p*-coumaric acid, can inhibit cucumber growth and change soil microbial communities (Zhou and Wu 2012). Specifically, *p*-coumaric acid decreased bacterial diversity while increased fungal diversity. Moreover, *p*-coumaric acid promoted the growth of FOC in the soil and increased the relative abundances of Firmicutes and Gammaproteobacteria, decreased that of Bacteroidetes, Deltaproteobacteria, Verrucomicrobia, Chloroflexi and Planctomycetes (Zhou and Wu 2012). Badri et al. (2013) also found that Actinobacteria was negatively affected by phenolics. These changes in soil microbial communities were broadly similar with the results observed in cucumber monoculture system of the present study, which had higher phenolic content. The relative abundance of *Acremonium* spp. (DeRito and Madsen 2009), which can degrade phenolics, increased in the monoculture system. This indicated that these phenolics degraders were stimulated by phenolics in the monoculture system. Therefore, the changes in microbial communities in our cucumber cropping systems are mediated in part by changes in soil phenolics content.

Chinese cabbage, a Brassicaceae crop that produce secondary metabolites glucosinolates, was included in our rotation system. Brassicaceae cover crops, glucosinolates and their hydrolysis products can inhibit soil-borne pathogens, including FOC (Matthiessen and Kirkegaard 2006). The relative abundance of *Mortierella* spp. increased in the rotation system, which was in agreement with the observation that hydrolysis products of glucosinolates can change soil microbial communities and stimulate *Mortierella* spp. (Hu et al. 2015). Therefore, the inclusion of a special plant species, Chinese cabbage, may play some role in shaping soil microbial communities and decreasing soil-borne pathogens in our cucumber rotation system.

Compared with the rotation system, the observed higher soil N content in the monoculture system may be due to (1) the higher N input in the monoculture system and (2) the bad cucumber growth performance, which absorbed less N and, thus, led more N accumulate in the soil. For the monoculture system, the higher N input and soil N content may contribute to the lower soil pH because most N-containing fertilizers tend to acidify soil (Belay et al. 2002). Soil pH was shown to be an important factor structuring soil microbial communities (Lauber et al. 2009). For example, the relative abundances of Actinobacteria and Bacteroidetes positively while Acidobacteria negatively correlated with soil pH (Lauber et al. 2009). In this study, the relative abundance of Actinobacteria was lower while that of Acidobacteria was higher in the cucumber rotation system, which had higher soil pH. Thus, our results were not fully consistent with previous observations and suggested that soil pH did not play a major role in shaping soil microbial communities in our cropping systems. One shortcoming of this experiment was that soil chemical properties were analyzed with air-dried samples. Studies found that air drying could increase NO_3^- concentration but decrease soil pH (Qian and Wolt 1990). Therefore, caution should be taken that soil pH and inorganic N reported here may be lower and higher than their actual values, respectively.

The higher relative abundance of Actinobacteria and lower relative abundances of Acidobacteria and

Verrucomicrobia in the monoculture system were in line with previous results observed under enriched N conditions (Ramirez et al. 2012), indicating soil N content is a potential driver of soil microbial community changes in our cucumber cropping systems. Moreover, the relative abundances of *Dokdonella* and *Rhodanobacter* that contained possible denitrifiers (Sanford et al. 2012; Tian et al. 2015) were higher in the monoculture system. These suggested that soil microbial communities in the monoculture system might increase concentration of atmospheric nitrous oxide, a potent greenhouse gas. Therefore, increasing crop diversity through crop rotation may have the potential to minimize the adverse effects of fertilization on the environment.

Conclusions

Taken together, our results provided empirical evidence that increasing temporal plant diversity increased crop productivity through soil biota-mediated plant-soil feedbacks in an agroecosystem. Crop rotation increased the diversity of soil bacterial communities but reduced that of the fungal communities. The enhanced promoting effect of total soil biota on cucumber growth in the rotation system was related to decreases in the negative effects of plant pathogens and increases in the positive effects of beneficial microorganisms. Changes in carbon resource quality, such as decreases in phenolics and glucosinolates produced by Chinese cabbage in the rotation system, may be associated with the changes in soil microbial communities and reduction in soil-borne pathogens in the rotation system. However, our experiment can not discern whether plant diversity per se or sampling effect, the inclusion of a specific crop species, play a larger role in determining soil microbial diversity and function, which need to be stressed in the future. For example, the inclusion of monocultures of crops other than cucumber, such as tomato and celery, can help to answer this question. Moreover, our findings require further validation from large-scale experiments for developing sustainable and environmentally compatible agricultural systems.

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