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Full Length Article

Effects of Different Summer Cover Crops and Residue Management on Plant Growth and Soil Microbial Community

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Abstract

The structure and function of soil microbial communities as affected by agricultural management practices play an vital role in plant production. We explored the effects of summer cover crops [wheat (*Triticum aestivum* L.), mustard (*Brassica campestris* L.), rye (*Secale cereal* Linn.), sage (*Salvia japonica* Thunb.), sundangrass (*Sorghum sundanense* (Piper) Stapf.) and basil (*Ocimum basilicum* L.)] on cucumber growth, soil phenolic content and soil microbial communities in three year continuously cropping cucumber black soil in summer fallow period. Results demonstrated that the fresh weight of cucumber plant and soil phenolic content were significantly higher in summer cover crops. High throughput sequence analysis showed that summer cover crops altered the structure and composition of soil bacterial and fungal community and the wheat cover crop treatment had a higher microbial diversity index than others. Taken altogether, summer cover crops especially wheat cover crop can increase the crop yield by changing the soil microbial community structure to improve the soil condition well. © 2017 Friends Science Publishers

Keyword: Summer cover crop; Soil microbial community; Cucumber growth; Soil phenolic content; Miseq-sequencing

Introduction

Plastic-greenhouse cultivation gradually expanded in China, and its artificial environment is usually more suitable for vegetable growth (Zhou *et al*., 2014). Cucumber (*Cucumis sativus* L.) as a considerable economic crop, is one of the crucial vegetables in the China greenhouse system (Huang *et al*., 2009). Whereas long-term continuous planting of the same kind of crops in the greenhouse can lead to a decline in soil quality, such as soil acidification, nutritional imbalance and soil salinization, resulting in crop reduction (Zhou *et al*., 2012b; Akmal *et al*., 2015). Therefore, taking effective measures to improve the condition of continuous cropping soil are essential for crop production.

The planting of cover crops is a potentially cheap strategy for restoring degraded soil (Shen *et al*., 2016). Application of cover crops on cultivated lands has been indicated to positively improve soil food web and also have an influence on a great many of soil physicochemical properties, such as SOM and soil bulk density (Treonis *et al*., 2010; Olson *et al*., 2014). Plentiful crops, Brassica crops (*R. sativus* L. variety oleiformis), fodder radish (*R. sativus cv* Brutus), sweet corn (*Zea mays* L.), have been chosen to improve soil environment. A previous study reported that Cereal rye (*Secale cereal* L.) and hairy vetch (*Vicia villosa* Roth.), as common cover crops, can improve soil structure and change soil microbial communities (Wu *et al*., 2006; Guo *et al*., 2008; Poffenbarger *et al*., 2015).

Soil microorganisms play an integral and unique role in ecosystem processes and sustainability. Numerous studies have reported the soil microbial communities are relevant to soil nutrient content, pH, SOC and other soil physicochemical properties (Guo *et al*., 2008; Tian *et al*., 2011a, b; Olson *et al*., 2014) less known about the relationship between soil microbial communities and different summer cover crops. The utilization of summer cover crops can ameliorate the degraded soils by changing soil microbial communities and improving soil microbial activity (Poffenbarger *et al*., 2015; Shen *et al*., 2016). Research had shown that sweet corn and Garland chrysanthemum planting in summer leisure time can increase microbial biomass considerably in the rhizosphere of cucumber (Tian *et al*., 2013). With the increase of growers using cover crops in the production systems, it becomes more crucial to better understand the effects of these strategies on soil microbes.

Here, we sequenced 16S rRNA gene as well as ITS gene to investigate diversity and structure of soil microbiota thriving at cucumber root-soil interface. Considering that summer cover crops have an effect on allelopathic compound and then shift soil microbial communities, and ultimately influence the cucumber growth. Our objectives therefore were to (1) Determine the dynamics of soil phenolic content in several cover crop modes (2) Illustrate the effects of summer cover crops on cucumber growth (3) Address how the types of crops in summer fallow seasons influence soil microbial abundance and diversity.

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Materials and Methods

Plant Materials and Soil Basic Properties

Wheat, mustard, rye, sage, sudangrass, and basil were selected as cover crops in the experiment the fallow period is the control, namely, W, M, R, Sa, Su, B and F (Table 1). The soil selected was a 3 years of continuous cropping cucumber black soil, collected from a plastic greenhouse in the experimental station of Northeast Agricultural University, Heilongjiang Province, China (45°41´N, 126°37´E) that contained organic matter, 27.8 g kg^{-1}; available N, 78 mg kg^{-1}; available P, 48.32 mg kg^{-1}; available K, 76.58 mg kg^{-1}; pH (1:2.5, w/v) 6.72. After sampling, soils were transplanted into the pot.

Experimental Description

The pot experiment started in June 2013. Six cover crop and residue management-related rotations were shown in Table 1. Each treatment was repeated 3 times, each containing 12 pots and randomly arranged. Plastic pots (17 cm × 20 cm) were equipped with 650 g soil, and all pots were sown with 30 seeds of cover crops. Growing for one month later, the aboveground part of cover crops were removed from the soil and then placed on top of the pot. After naturally drying the plants were mixed into the soil decomposition at room temperature for 30 days. The cucumber was conventional seedling the seedlings at 2-cotyledons stage were translated into each pot. We collected the cucumber rhizosphere soils after planting for 10, 20 and 30 days. All samples were sieved through 2 mm mesh collected in sterile plastic bags, and then transferred to the laboratory and stored at -80℃ for further analysis.

Soil Phenolics Extraction and Determination

The rhizosphere soil samples (Chen *et al*., 2015) were collected from the harvested and uprooted cucumber plants. The samples were sieved through 2 mm mesh sieve. The extraction of soil phenolics was performed as previously described (Chen *et al*., 2011; Zhou *et al*., 2012a). For HPLC analysis, the methanol solution of soil extracts was filtered through 0.22 μm filter membrane and used for soil phenolics analysis using HPLC system (Waters, Milford, MA). A mixture of 20% methanol and 80% water was used to the mobile phase. Using a UV detector to monitor at 280nm. The standard phenolics were purchased from Dalian China ittrich Co. Ltd.

DNA Extraction, PCR Amplification, High Throughput Sequencing

The Power Soil DNA Isolation Kit (MOBIO Laboratories Inc., Carisbad, CA, USA) was used to extract the total DNA from 0.25 g of the mixed samples, which were composed of three samples in the same treatment, following the manufacture's protocol. The total DNA was checked on 1% agarose gel and DNA concentration and quality (A260/A280) of the extracts were estimated visually using a NanoDrop ND-1000 UV-Vis spectrophotometer (ThermoScientific, Rockwood, TN, USA). Polymerase chain reaction (PCR) was used to amplify the V3-V4 hypervariable region of the bacterial 16S rRNA gene and the ITS1 hypervariable region of fungal gene. The primers used for bacterial 16S rRNA gene PCR were 338F/806R (Dennis *et al*., 2013) and the primers for fungal gene PCR were ITS1F/ITS2 (Gardes and Bruns, 1993). The products from the amplification were pooled and evaluated on 2% agarose gels (TBE buffer) and finally sequenced on an Illumina MiSeq PE300 platform at Majorbio Bio-pharm Technology CO., Ltd. (Shanghai, China). The raw reads of bacteria and fungi were placed in the NCBI Sequence Read Archive (SRA) database under accession number SRP076761 and SRP09176 respectively.

Statistical Analysis

Raw data generated from Illumina Miseq sequencing were using QIIME (1.9.0) with the below procedure; (i) discarding any low quality or ambiguous reads; (ii) clustered the operational units using RDP classifier at a 97% identity threshold; (iii) removed putative chimeras and singletons from OTU tables yielded from Illumina sequencing data; (iv) computed α-diversity indices to assess the internal complexity of individual microbial populations (Caporaso *et al*., 2010).

The data were calculated with dry weight, and statistical analysis was carried out with R (R Development Core Team, 2010) and the analysis of variance (ANOVA) and mean comparison between treatments were performed based on the Tukey's honestly significant difference (HSD) test at the 0.05 probability level with SAS 9.1 software.

Results

Dry Matter Weight and Fresh Weight

Summer cover crops had no significantly effects ($P > 0.05$) on fresh weight and dry matter weight in the 10d and 20 d (Fig. 1). The dry matter of Su and W, compared with other summer cover crops were evidently higher than F and the fresh weight of F was obviously lower than cover crops in the 30 d except M ($P < 0.05$).

Phenolics in Soil

Five phenolic compounds (ferulic acid, vanillic acid, *p*-hydroxybenzoic acid, vanillin, syringic acid) were monitored by HPLC in the different treatments, valillic acid was most abundant in both phenolic compounds detected (Table 2). The content of these phenolic compounds were significantly improved with the crop culture time increasing, what's more the content of phenolic compound in W compared to other cover crops, was more abundant than F in the 10 d, 20 d and 30 d ($P < 0.05$).

Table1: Experiment setup of the pot experiment demonstrating crop species

Treatment Codes	Winter-spring season (Apr to Jun)	Summer fallow season (Jun to Aug)	Autumn-winter season (Aug to Oct)	Catch crop residue management in July
W	Cucumber	Wheat	Cucumber	Removed above-ground from pot, airing, incorporated into the soil
R	Cucumber	Rye	Cucumber	Removed above-ground from pot, airing, incorporated into the soil
M	Cucumber	Mustard	Cucumber	Removed above-ground from pot, airing, incorporated into the soil
Sa	Cucumber	Sage	Cucumber	Removed above-ground from pot, airing, incorporated into the soil
Su	Cucumber	Sudangrass	Cucumber	Removed above-ground from pot, airing, incorporated into the soil
B	Cucumber	Basil	Cucumber	Removed above-ground from pot, airing and incorporated into the soil
F	Cucumber	Fallow	Cucumber	Removed root and rock

Table 2: Soil phenolic content in different cover crop systems

Different crop time	treatment	Soil phenols and their contents (μg·g^{-1} soil DW)				
		p-hydroxybenzoic acid	vanillic acid	vanillin	syringic acid	ferulic acid
10 d	F	5.74±0.26c	6.56±1.21c	1.26±1.23a	5.87±0.26c	4.65±0.59c
	Su	7.63±0.44a	8.42±0.67a	1.76±0.68a	7.65±0.14a	7.28±0.26a
	B	7.25±0.14b	7.89±0.89b	1.52±0.26a	6.59±0.24b	6.85±0.08b
	M	7.74±0.34b	7.37±0.26a	1.35±0.58a	7.65±0.45a	5.89±0.59c
	W	7.68±0.02a	8.26±0.40a	1.75±0.04a	7.85±0.08a	7.12±0.16a
	R	7.86±0.32a	8.76±0.19a	1.95±0.59a	7.94±0.58a	7.76±0.46a
	Sa	7.28±0.12b	7.48±0.84b	1.65±0.10a	7.54±1.64a	6.65±0.17b
20 d	F	5.32±0.26c	7.62±1.23c	1.22±1.02c	7.56±1.03c	5.62±0.26d
	Su	8.65±0.26c	9.32±0.64a	1.93±0.56c	8.65±1.32b	8.56±0.03b
	B	8.08±0.39b	8.26±0.85ab	1.84±0.26b	9.12±0.26b	7.52±0.85c
	M	7.36±0.36b	8.68±0.2ab	1.66±0.39b	8.56±0.40b	6.92±0.67c
	W	8.95±0.16a	9.85±0.38a	2.13±0.15a	9.15±1.14a	9.25±0.15a
	R	8.26±0.01a	9.66±0.48a	1.78±0.12b	8.80±0.85b	8.45±0.13b
	Sa	7.32±0.25b	8.78±1.06b	1.86±0.25b	8.56±0.95b	7.96±0.65c
30 d	F	6.52±0.46c	8.29±0.89d	1.89±0.24c	8.62±1.65d	6.52±0.80c
	Su	9.26±0.26a	10.42±1.02b	2.62±0.70a	10.62±0.84b	9.18±0.53a
	B	8.79±0.16b	9.46±0.65c	2.26±0.13a	9.26±0.75c	9.87±0.68a
	M	8.56±0.16b	9.37±0.63c	2.12±0.13b	9.56±1.23c	8.16±0.87b
	W	9.05±0.14a	11.42±1.30a	3.02±0.26a	11.78±0.65a	9.85±0.23a
	R	9.26±0.26a	10.13±0.16b	2.82±0.58a	11.82±0.52a	9.95±0.25a
	Sa	8.65±0.26b	9.63±0.85c	2.23±1.87b	8.12±0.83d	8.16±0.59b

Note: Values with different letters are significantly different between treatments ($P< 0.05$, Tukey'sHSD test)

In the 30 d the content of phenolic compounds in the cover crops were significantly improved and the R and W had a higher content than other cover crops in all phenolic compounds ($P < 0.05$).

Soil Microbial Community Diversity and Structure

There are totally 366398 high quality bacterial sequences and 405526 valid sequences of ITS gene generated from 7 treatments through miseq sequencing analysis. Subsequent analyses were accomplished using standardized data and the number of bacteria and fungi sequences in all treatments ranged from 36709 to 70867 and 47294 to 73873 respectively (Table 3). In the bacterial community the Shannon index for the cover crop treatments were higher than the control and the Simpson index was the opposite. The Chao1 index was only B treatment higher than the control. However, in the fungal community the Shannon index in addition to Sa treatment were higher than the control the Chao1 index were all lower than the control. Remarkably, the diversity index of W treatment was higher than that of control (Table 3). The rarefaction curves demonstrated that high throughput sequencing data covered a large proportion of soil bacterial and fungal community composition (Fig. 2). Principle component analysis (PCA) illustrated that there were differences between soil microbial communities in different samples (Fig. 3). The total percentage variance of species explained in the soil bacterial and fungal community was 90.19% and 94.78% respectively. The W treatment had a apparent distinction with the control relative to other cover crops (Fig. 3a and 3b).

Soil Microbial Community Composition

The abundances of soil microbial community presented were different in all soil samples (Fig. 4 and 5). At the level of soil microbial community of less than 1% of the population is classified as others. There are 18 identified phyla observed in the soil bacterial community, and Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Chloroflexi, Acidobacteria and Gemmatimonadetes

Table 3: Diverse parameters of soil microbial species in different treatments

Species	Samples	Raw reads	Number of OTUs	Shannon index	Chao1	Simpson index
Bacteria	M	36709	9145	7.16	33530	0.0100
	R	45186	9423	7.12	33067	0.0093
	B	48464	11963	7.46	50233	0.0061
	Su	49758	10290	7.40	33887	0.0053
	Sa	60737	11834	7.48	38818	0.0044
	W	70867	12199	7.56	39225	0.0028
	F	54677	10901	6.98	40491	0.0153
Fungi	M	47294	382	2.99	495	0.1033
	R	59537	379	3.07	480	0.0980
	B	48246	429	3.12	512	0.0950
	Su	59703	452	3.58	489	0.0636
	Sa	63012	387	2.76	514	0.1431
	W	53861	434	3.60	461	0.0590
	F	73873	447	2.80	581	0.1154

were the dominant phyla, which obligated to more than 97% of soil microbial abundance. Proteobacteria was 32.10% of the soil bacterial community in F, and enriched in R, B, Sa and M, while Su and W had a lower abundance of Proteobacteria compared with F. Firmicutes, another prominent phylum of soil bacterial community, was 26.87% in F and the percentage of Firmicutes in cover crops (R, B, Su, Sa, W and M) were 21.07%, 16.28%, 15.28%, 12.30%, 10.59% and 20.47%, which were lower than F in per cover crop samples. The relative abundance of Actinobacteria was enriched in W and Su which had a higher relative abundance than F (Fig. 4a). Further analysis illustrated that Kaistobacter, SMB53, Pseudomonas, Steroidonbacter, Rhodoplans, Bacillus, Streptomyces were the dominant genus, and Kaistobacter and SMB53 had a higher abundance in all samples (Fig. 4b).

Fungal community detected 5 phyla, Ascomycota, Zygomycota and Basidimycota were the dominant phyla, but Chytridiomycota only appeared in Su treatment (Fig. 5a). Ascomycota as the richest phlum was enriched in Su, M, R and W treatments, and the Su treatment had a relatively higher abundance across all samples. Zygomycota, another dorment phylum, was enriched in R, W and B treatments. Compared to F, Cryptococcus, Preussia, Rhizopus, Cladosporium, Pseudogymnoascus, Cochliobolus, Penicillium, Zopfiella and Huminicola were also increased in all cover crop samples. However, only the percentage of Ascobolus was decreased in all cover mode and Waitea, the lower percentage of genus, was only in the wheat cover crops (0.57%).

The heatmap analysis of soil microbial community was used to monitor the differences of soil microbial community structures (Fig. 6). The color code indicates relative abundance, ranging from the black (low abundance) to the red (high abundance), most of these OTUs (≤ 1%) were not generally found in the control. Additionally, various patterns of OTUs presence were observed for every sample category in whole heatmap. The W had a significant differences with other treatments in soil bacterial and fungal community structures (Fig. 6a, b).

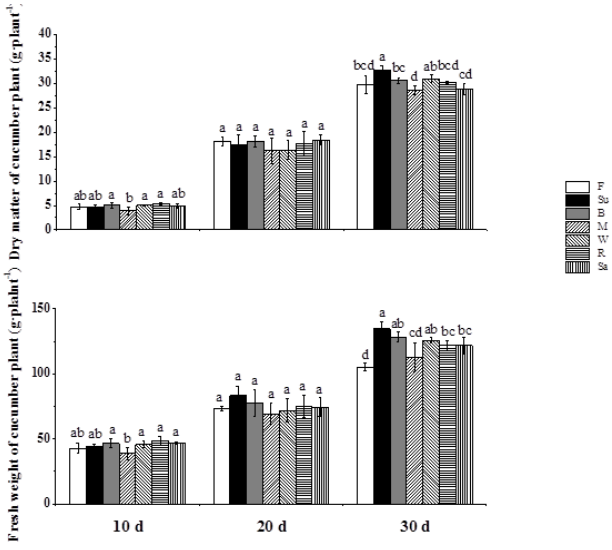

Fig. 1: Effects of cover crops on fresh weight and dry matter production. Bars indicate the standard errors of the means from three replicates. Columns with different letters are statistically different between treatments (P<0.05, Tukey's HSD test)

Discussion

Although no significantly differences (P>0.05) in the dry matte of the cucumber between F and summer cover crops except Su summer cover crops evidently increased (P<0.05) fresh weight of cucumber (Fig. 1). That is summer cover crops can improve the cucumber growth, which might be also caused by the interaction between the soil microbe and root, root exudates, phenolics.

In this study phenolic content was accumulated with the time increased and had significantly differences (P<0.05) between summer cover crops and the control (Table 2). Phenolics had negative effects on plant growth by inhibiting soil structure and function (Inderjit and Duke, 2003). Though the W and R had higher phenolic content than others, their cucumber

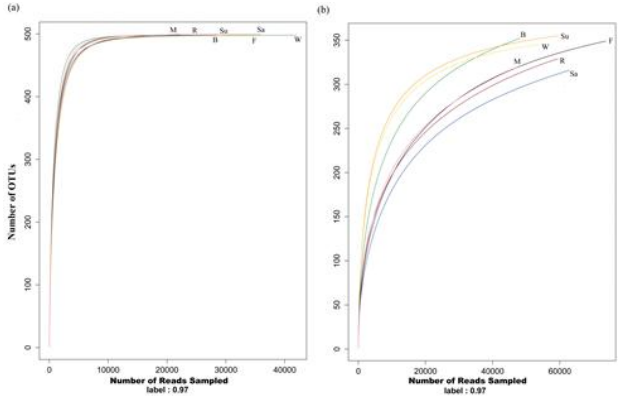

Fig. 2: Rarefaction curves of soil bacterial (a) and fungal (b) communities in different treatments

F: Fallow; M: Mustard cover crop; W: wheat cover crop; R: rye cover crop; Su: Sudangrass cover crop; Sa: Sage cover crop; B: Basil cover crop

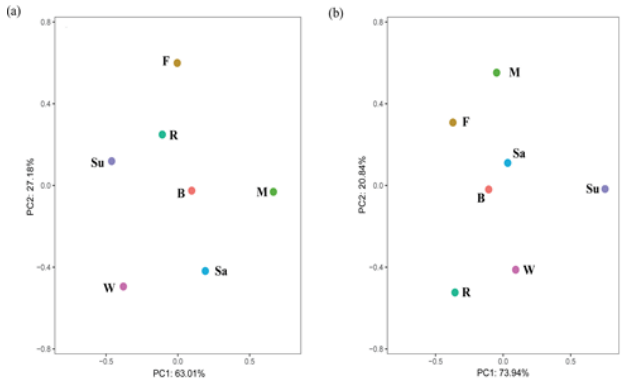

Fig. 3: Multiple samples PCA analysis on the 16S RNA (a) and ITS (b)

F: Fallow; M: Mustard cover crop; W: wheat cover crop; R: rye cover crop; Su: Sudangrass cover crop; Sa: Sage cover crop; B: Basil cover crop

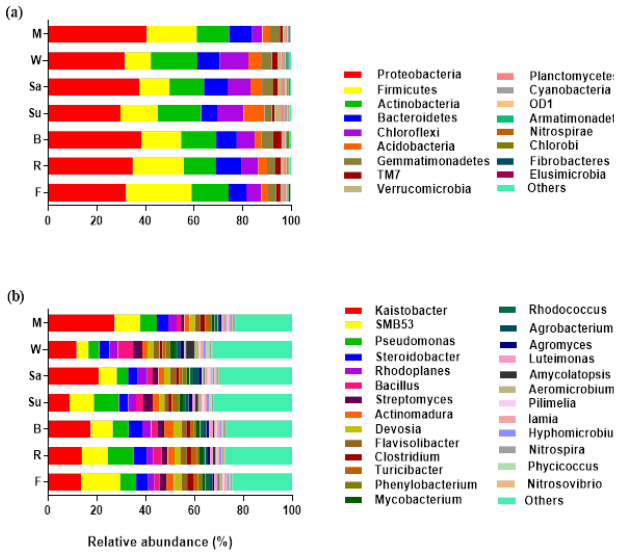

Fig. 4: Relative abundances of chief soil bacterial phylum (a) and genus (b) in several treatments

F: Fallow; M: Mustard cover crop; W: wheat cover crop; R: rye cover crop; Su: Sudangrass cover crop; Sa: Sage cover crop; B: Basil cover crop

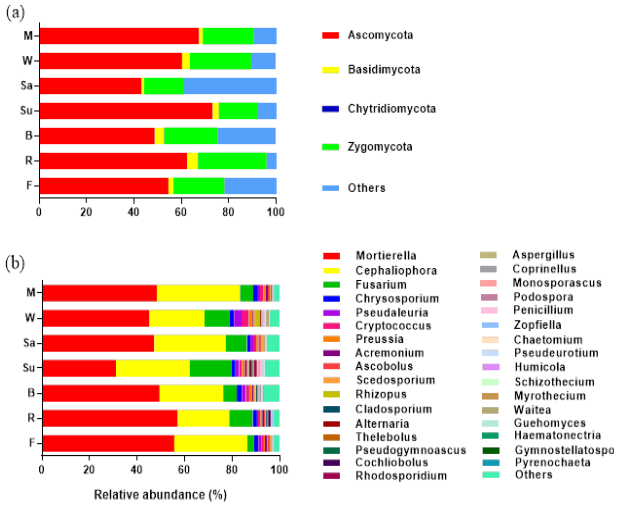

Fig. 5: Relative abundances of chief soil fungal phylum (a) and genus (b) in several treatments

F: Fallow; M: Mustard cover crop; W: wheat cover crop; R: rye cover crop; Su: Sudangrass cover crop; Sa: Sage cover crop; B: Basil cover crop

growth was definitely better in the last time (Fig. 1). This means that cucumber growth in two treatments was not affected by phenolic content. Many processes, such as transport, retention and transformation, and direct effects of phenolics on soil microbes need to explore due to the interaction with other compounds and the complexity of soil food.

The potential of microbial diversity as an indicator for soil quality is inhibited owing to the difficulties in measuring. In our study sequencing results demonstrated that summer cover crops could influence soil microbial community structure and diversity. Specifically, the abundance index of soil microbial community is an indicator reflected the change of soil microbial diversity (Zhu *et al.*, 2013). The numbers of species diversity index illustrated that summer cover crops can affect soil microbial community richness and diversity (Table 3). Results of rarefaction curves of soil microbial community demonstrated that soil microbial communities were displayed well when they tended to relatively smooth with the number of sequences increasing (Fig. 2). PCA results displayed an obvious separation of soil microbial communities in all samples, illustrated that soil microbial communities were mainly influenced by cover crops (Fig. 3). Wu *et al.* (2006) showed that Garland chrysanthemum as cover crops in summer could increase cucumber rhizosphere soil microbial biomass and population. Steenwerth and Belina (Steenwerth and Belina, 2008) reported that cover crops could enhance the soil's capacity for supporting

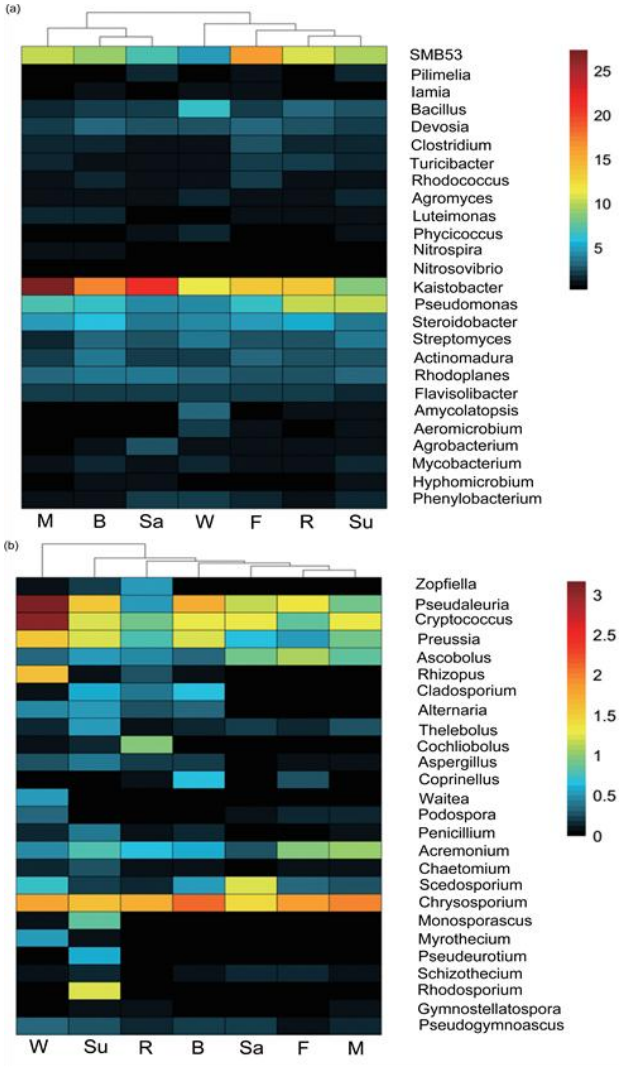

Fig. 6: Soil bacterial (a) and fungal (b) community heatmap analysis

F: Fallow; M: Mustard cover crop; W: wheat cover crop; R: rye cover crop; Su: Sudangrass cover crop; Sa: Sage cover crop; B: Basil cover crop

greater MBN and improve microbiological activity. Our results showed that cover crops increase soil bacterial and fungal community.

Several papers suggested that agricultural soils are dominated by bacteria, this might be less true in organically managed systems (Six *et al*., 2006). It could not be ignored the importance of the fungal community. In our study, summer cover crops could also influence soil fungal community structure and diversity. Summer cover crops increased the percentage of Fusarium compared to the control, however, maybe it could not affect cucumber growth due to the intricacy of plant-soil interaction systems. Soil microbial community is very complex, and also influenced by environmental factors. Soil physicochemical properties significantly influenced to soil microbial community structure and diversity (Uroz *et al*., 2010), such as DOC, pH and other nutrient contents (Robin *et al.*, 2006; Vink *et al.*, 2014; Aslam *et al.*, 2016). Therefore, it is essential to think about the effect of external environment factor in future studies of soil microbial community.

Conclusion

In sum, summer cover crops had higher soil microbial diversity and improved cucumber growth compared to traditional fallow systems. Thereinto the wheat cover crop increased the content of phenolic compounds it had a distinguished effect on soil microbial community diversity and plant growth in both samples. Moreover, high throughput sequencing analysis of soil microbial community also verified the vital role of soil microbial biomass and activity. Sequencing results associated with phenolic content as well as cucumber growth give a deep understanding of the potential relative mechanism between soil microbial community and plant diversity.

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