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Impact of Agro-Farming Activities on Microbial Diversity of Acidic Red Soils in a *Camellia Oleifera* Forest

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ABSTRACT: The production of *Camellia oleifera* (oil tea), typically planted in acidic red soils in southern China, is limited by low soil fertility. Agro-farming is one way to promote soil fertility by increasing organic matter and microbial communities. To understand the impact of agro-farming activity on soil fertility, three types of agro-farming, namely, raising laying hens under forest (RLH), cultivating *Lolium perenne* grass under forest (LPG), and maintenance of native grass (MNG), were employed in an oil tea farm with acidic red soil in Changsha, China. Soil samples were collected from the farm to estimate microbial communities, pH, and total organic carbon (TOC) in different seasons. The results indicated that TOC and temperature were the dominant factors influencing the variations of bacterial communities, while temperature and pH affected the fungal communities in the soil. The most abundant bacterial phyla were *Acidobacteria*, *Proteobacteria*, *Actinobacteria,* and *Chloroflexi*, while the most abundant fungal phyla were *Ascomycota*, *Basidiomycota,* and *Zygomycota*. Regardless of treatment, the bacterial richness and diversity were both low in spring, and the fungal richness and diversity in summer and autumn were higher than in spring and winter. The TOC content and pH in LPG were significantly higher than in other treatments. Microbial communities in LPG and MNG were more stable than in RLH. In summary, cultivating grass under forest treatment was the best way to improve the microenvironment with the highest TOC content and fewer pathogenic microorganisms.

Keywords: oil tea, season, soil microbial community, high throughput sequencing.

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INTRODUCTION

Camellia oleifera Abel. (oil tea) is an important woody oilseed plant which is mainly cultivated in Hunan, Jiangxi, and Guangxi provinces and is endemic to hilly areas of southern China with acidic red soil, which is classified as Oxisols in Soil Taxonomy or as Lixisols in World Reference Base for Soil Resources (Ma et al., 2011; Hu et al., 2016; Liu et al., 2017a). The low yield is the primary factor limiting the expansion and development of *C. oleifera* (Liu et al., 2017, 2018). One critical reason for low yield is infertile soil. Most regions of *C. oleifera* plantations have acidic red soils with low levels of pH, microorganisms, organic carbon, and phosphorus compounds (He et al., 2011). Increasing soil nutrients and improving soil microbial communities are necessary to improve soil physical and biochemical properties. The conventional approaches to improving soil are through application of fertilizers and plowing (Balasubramanian et al., 1999; Xu and Liang, 2006; Nayak et al., 2009). However, in recent years, excessive use of chemical fertilizers and farming activities have caused soil compaction and loss of organic matter which can lead to further degradation of the soil and reductions of microbial activity in croplands (Liang et al., 2010; Motounu, 2010; Hegde et al., 2015; Wang et al., 2017).

Microorganism plays a significant role in the decomposition process of soil organic matter, as well as litter and wood residues (Progar et al., 2000; Hamilton III and Frank, 2001; Wood et al., 2012). Microbial community structure reflects the condition of soil nutrients and health. Some bacteria phyla only proliferate in copiotrophic environments, including *Alphaproteobacteria*, *Betaproteobacteria,* and *Bacteroidetes*, while others in oligotrophic environments, such as *Acidobacteria* (Martin and Macleod, 1984; Fierer et al., 2007; Nemergut et al., 2010). Some fungi are interdependent with the plant rhizosphere, like arbuscular mycorrhizal fungi (AMF), while others can cause plant disease, like *Venturia inaequalis* (Smith and Read, 1997; Islam et al., 2014; Masny, 2017). Microorganisms are sensitive to many factors, such as fertilization, cultivation, irrigation, soil coverage, and cropping patterns (Carpenter-Boggs et al., 2003; Rabary et al., 2008; Singh et al., 2008; Meriles et al., 2009; Heidari et al., 2016; Nivelle et al., 2016). These factors can further affect the soil microenvironment and vegetation growth. Thus, environmentally friendly and sustainable farming practices, which may improve microbial community structure and soil nutrient condition, have been attempted over croplands and forest lands (Montanaro et al., 2007; Larkin et al., 2011; Liu et al., 2016b; Wang et al., 2016a, 2016b, 2016c, 2017). For instance, raising chickens improved soil structure and increased organic matter (Wang et al., 2016b); sowing grass increased soil coverage and reduced soil erosion (Tebrügge and Düring, 1999). All of these activities not only reduced plowing time and application rate of chemical fertilizers but also increased microbial diversity and abundance (Hodge et al., 2001; Leff et al., 2015). However, there are little researches on the investigation of new biological farming activities for oil tea plantation. Also, there are no established guidelines regarding fertilization and management to maintain soil health and crop productivity for farmers (Liu et al., 2018).

Understanding the microbial community structure is essential to make scientifically sound recommendations to cropping systems in red soil regions for farmers. This study aimed to investigate the impact of three agro-farming practices on the microbial diversity in an oil-tea-tree forest with: (a) raising laying hens (RLH); (b) cultivating of *Lolium perenne* grass (LPG); and (c) maintenance of native grass (MNG). We assumed that (1) the RLH treatment could bring more abundant and various microorganisms; (2) the MNG treatment could stabilize soil environment and microbial community structure.

MATERIALS AND METHODS

Study site and sample treatment

The experimental field is located at Wangcheng, Changsha, China (112° 03’ E, 20° 58’ N) with 15-year old *C. oleifera* ‘Xianglin210’. The climate is subtropical monsoon with mean

annual total rainfall of 1,370 mm. The mean annual temperature is 17 °C. The average lowest and highest temperatures are 4.4 °C in January and 30 °C in July, respectively. The soil at the experimental site is a Quaternary red clay with a pH of 5.3.

Three different farming managements were employed in plots of oil-tea woodlands with an area of 0.67 ha each: raising 500 laying hens under the forest (RLH); cultivating *Lolium perenne* grass under the forest (LPG) at a density of 85 kg seeds per hectare in October 2013 and cultivating again in June 2014 and February 2015; and control field plots consisting of the maintenance of native grass (MNG) by removing the most troublesome weeds (*Amaranthus retroflexus*, *Chenopodium glaucum*, *Datura stramonium*, and *Cirsium setosum*) and keeping the benignant weeds (*Digitaria sanguinalis*, *Setaria viridis*, *Chloris virgata*, *Eleusine indica*). The height of the grasses in MNG was kept below 0.10 m by mowing.

For each treatment, three sampling plots with a dimension of 10 × 10 m were selected on December 29, 2014, May 14, 2015, September 9, 2015, and October 20, 2015, respectively. In each plot, soils were sampled four times at five different points between 0.00 and 0.10 m depth from December 2014 to October 2015 (Table 1). After removing debris and roots, soil samples were well mixed, ground, and sieved (<2 mm). Approximately 200 g of each soil was placed into a sterile bag and stored at -80 °C for microbial analyses (Gao et al., 2015).

Determination of temperature and soil physical-chemical index

Soil temperatures at each sampling time were recorded by a mercurial thermometer. The total organic carbon (TOC) of soil samples was determined using an elemental analyzer (Vario EL III, Germany). Soil pH (soil:distilled water = 1:2.5) of each sample was determined by a PHS-3C pH meter (INESA Scientific Instrument Co. Ltd).

Bacterial and fungal community assessment

Total DNA (0.5 g wet weight) was extracted using an E.Z.N.A Soil DNA kit (OMEGA, USA) according to the manufacturer's instruction. The extracted DNA was diluted in

Table 1. Temperature, total organic content (TOC), and pH of soil samples subjected to the following treatments: raising laying hens under forest (RLH), cultivating *Lolium perenne* grass under forest (LPG), and maintenance of native grass under forest (MNG) collected at four times during the year

Sample time	Seasons	Average temperature	Treatments	TOC	pH(H_2O)
		°C		g kg^{-1}	
2015/05/14	Spring	24	RLH1	11.14±1.52 Cb	4.8±0.2 Ab
			LPG1	22.30±2.11 Ba	5.2±0.1 ABa
			MNG1	5.80±2.10 Dc	4.5±0.2 Bc
2015/09/09	Summer	31	RLH2	12.05±3.11 Cb	4.8±0.1 Ab
			LPG2	23.20±1.52 Ba	5.0±0.2 Ca
			MNG2	13.06±2.00 Cb	4.6±0.3 ABb
2015/10/20	Autumn	20	RLH3	16.45±0.42 Bb	4.6±0.2 Bc
			LPG3	21.00±2.62 Ba	5.3±0.2 Aa
			MNG3	15.22±1.80 Bb	4.8±0.2 Abc
2014/12/29	Winter	4	RLH4	18.23±2.51 Ab	4.7±0.3 BCb
			LPG4	31.02±1.11 Aa	5.4±0.2 Aa
			MNG4	17.85±1.02 Ab	4.5±0.1 Bc

TOC of soil samples was determined using an elemental analyzer (Vario EL III, Germany). pH(H_2O) at a soil:water ratio of 1:2.5 v/v. Data presented are mean ± SD. Different capital letters in the same column indicate significant difference among different seasons within same treatments at p≤0.05 (Duncan's multiple range tests); different lowercase letters in the same column indicate significant difference among different treatments within same seasons at p≤0.05. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

a TE buffer (Tris-HCl 10 mmol L^{-1}, EDTA 1 mmol L^{-1}, pH 8.0) and stored at -20 °C until analysis (Liu et al., 2014).

An aliquot of the extracted DNA from each sample was used as a template for amplification. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Peng et al., 2017). The hypervariable regions of the fungal ITS1 gene were amplified with primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (White, 1990; Gardes et al., 1993). The following thermal program was used for amplification: at an initial denaturation at 98 °C for 30 s, followed by 10 cycles of 98 °C for 10 s, 65 °C for 30 s, and 72 °C for 30 s, followed by an extension at 72 °C for 5 min. Each sample was amplified in triplicate, and the PCR products were pooled and purified using the Agarose Gel DNA purification kit (TaKaRa). An equal amount of the PCR product from each sample was combined in a single tube to be run on an Illumina MiSeq PE300 platform at Biomarker Technologies Co., Beijing, China. After sequencing, paired-end reads were assembled with a minimum overlap of 10 bp using FLASH (version 1.2.7). Sequences with an average quality score <20 over a 50-bp sliding window were truncated using Trimmomatic (version 0.33). Chimeras were identified and removed using UCHIME (version 4.2).

Statistical and bioinformatics analysis

Effective sequences were clustered into operational taxonomic units (OTUs), with a similarity cutoff of 97 % using QIIME (version 1.8.0). Finally, the taxonomy of bacterial and fungal sequences were annotated by Ribosomal Database Project RDP classifier (version 2.2) based on Silva (Release 119) and Unite (Release 7.0) databases, respectively. Alpha diversity indexes, including OTUs and abundance-based coverage estimator (ACE), which both mean the microbial richness, and Shannon diversity, which means the microbial diversity, were estimated by MOTHUR (version 1.30). Classification heatmaps, Venn diagrams, and redundancy analysis of environmental factors and microorganisms were drawn by the R programming language platform. The variance analysis of environmental factors was performed by Duncan's multiple range tests using SPSS for Windows (version 17.0)

RESULTS

Physicochemical properties of soil

The TOC content increased gradually over time in all treatments during 2015 (Table 1). The TOC content of all treatments was significantly higher in winter than in any other seasons. The highest TOC content was always detected in LPG in every season. The TOC content in RLH, LPG, and MNG treatments ranged from 11-19 %, 20-32 %, and 5-32 %, respectively. The pH ranged from 4.5 to 5.4. The highest pH was detected in LPG across all seasons (Table 1).

Microbial diversity and richness

In the RLH and LPG treatments, the highest bacterial richness and diversity were both observed in autumn (Table 2). However, in the MNG treatment, the highest bacterial richness was in winter and the highest bacterial diversity was in summer. According to table 3, in the RLH and LPG treatments, the fungal richness in summer and autumn were both higher than in spring and winter. In the MNG treatment, the highest fungal richness and diversity were both in summer and the lowest both in winter.

In spring, the rank of bacterial richness and diversity was LPG>MNG>RLH and MNG>LPG>RLH, respectively; in summer, the ranks of OTUs and Shannon were both

Table 2. Bacterial diversity (Shannon) and richness, number of operational taxonomic units (OTUs), and abundance-based coverage estimator (ACE) of soil samples subjected to the following treatments: RLH, LPG, and MNG

Treatments	Effective reads	OTUs	ACE	Shannon
RLH1	168177	1255	1388.7	5.55
RLH2	193436	1591	1661.2	5.89
RLH3	188959	1613	1671.9	5.93
RLH4	201415	1328	1412.0	5.03
LPG1	92925	1416	1480.6	5.56
LPG2	81368	1433	1490.1	5.62
LPG3	173387	1535	1580.2	5.77
LPG4	197364	1477	1540.9	5.76
MNG1	118779	1340	1468.5	5.61
MNG2	119741	1526	1597.1	5.78
MNG3	114777	1510	1552.6	5.73
MNG4	84372	1609	1696.1	5.78

RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

Table 3. Fungal diversity (Shannon) and richness (OTUs, ACE) of soil samples subjected to the following treatments: RLH, LPG, and MNG

Treatments	Effective reads	OTUs	ACE	Shannon
RLH1	263867	451	544.42	3.059
RLH2	227738	753	763.77	4.473
RLH3	270666	685	693.93	4.213
RLH4	270622	461	492.43	3.207
LPG1	121577	594	631.44	4.110
LPG2	103082	635	657.78	3.871
LPG3	271820	772	819.98	4.328
LPG4	288909	594	628.89	3.939
MNG1	96504	727	748.37	4.883
MNG2	118700	775	780.61	5.224
MNG3	113592	744	745.95	5.084
MNG4	133504	703	730.66	4.485

RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

RLH>LPG>MNG; in autumn, the ranks of bacterial richness and diversity were both RLH>LPG>MNG, but they were MNG>LPG>RLH in winter (Table 2). Except in autumn, the highest fungal richness and diversity were observed in the MNG treatment in other three seasons; in autumn, the lowest fungal richness and diversity was detected in the RLH treatment (Table 3).

Distribution of microorganisms

Distribution of bacteria

Acidobacteria, *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* were the dominant phyla in every sample (Figure 1). At the class level, *Acidobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Actinobacteria* were dominant in all treatments and seasons.

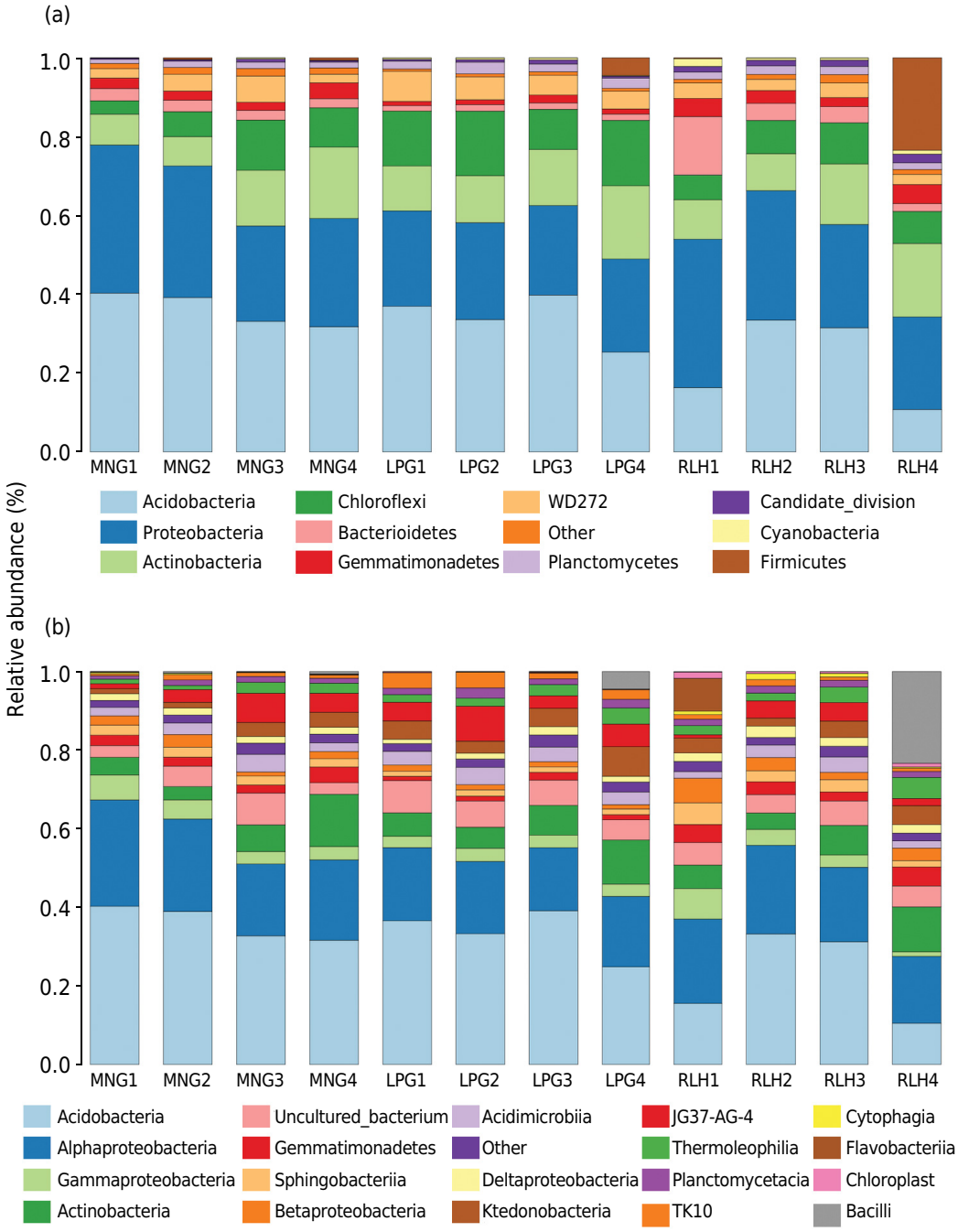

Figure 1. Relative abundance at the bacterial taxonomic levels of different treatments. Note: Bacteria phylum (a); Bacteria class (b). RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

However, the abundance of phyla and classes of the bacterial community were significantly more diverse among treatments and seasons.

At the phylum level, *Actinobacteria* gradually increased with time and the annual dynamics in MNG had a similar pattern to that in LPG and RLH (Figure 1a). The distribution of *Proteobacteria* showed similar relative abundance in every sample. Annual dynamics of *Chloroflexi*, which gradually increased with time and then decreased in autumn in MNG, was significantly different from that in LPG and RLH which were distributed evenly in each season.

The major classes (*Acidobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria*) decreased gradually with time which was contrary to *Actinobacteria* (Figure 1b). Most

of the classes in LPG remained relatively stable in spring and autumn; yet the minor classes, like *Actinobacteria* and *Bacilli*, increased significantly in summer and winter while *Acidobacteria* decreased significantly. In RLH, there were significant differences between the four seasons. *Acidobacteria* in spring was much lower than in summer and winter that lead to a more homogenous bacterial community. It is noteworthy that *Alphaproteobacteria* always remained in relatively stable abundance ranging from 15 to 25 % in all samples.

Distribution of fungus

At the phylum level (Figure 2a), *Ascomycota* were higher than other phyla in most samples. In MNG, *Ascomycota* and *Basidiomycota* in summer and autumn were higher than in spring and winter whereas *Zygomycota* was lower in summer and autumn than in spring and winter; *Ascomycota* remained in relatively stable abundance in four quarters. The variation of phyla, except *Ascomycota*, in LPG was similar to that in MNG, but the ranges of variation were greater in LPG than in MNG. In LPG treatment, *Ascomycota* in

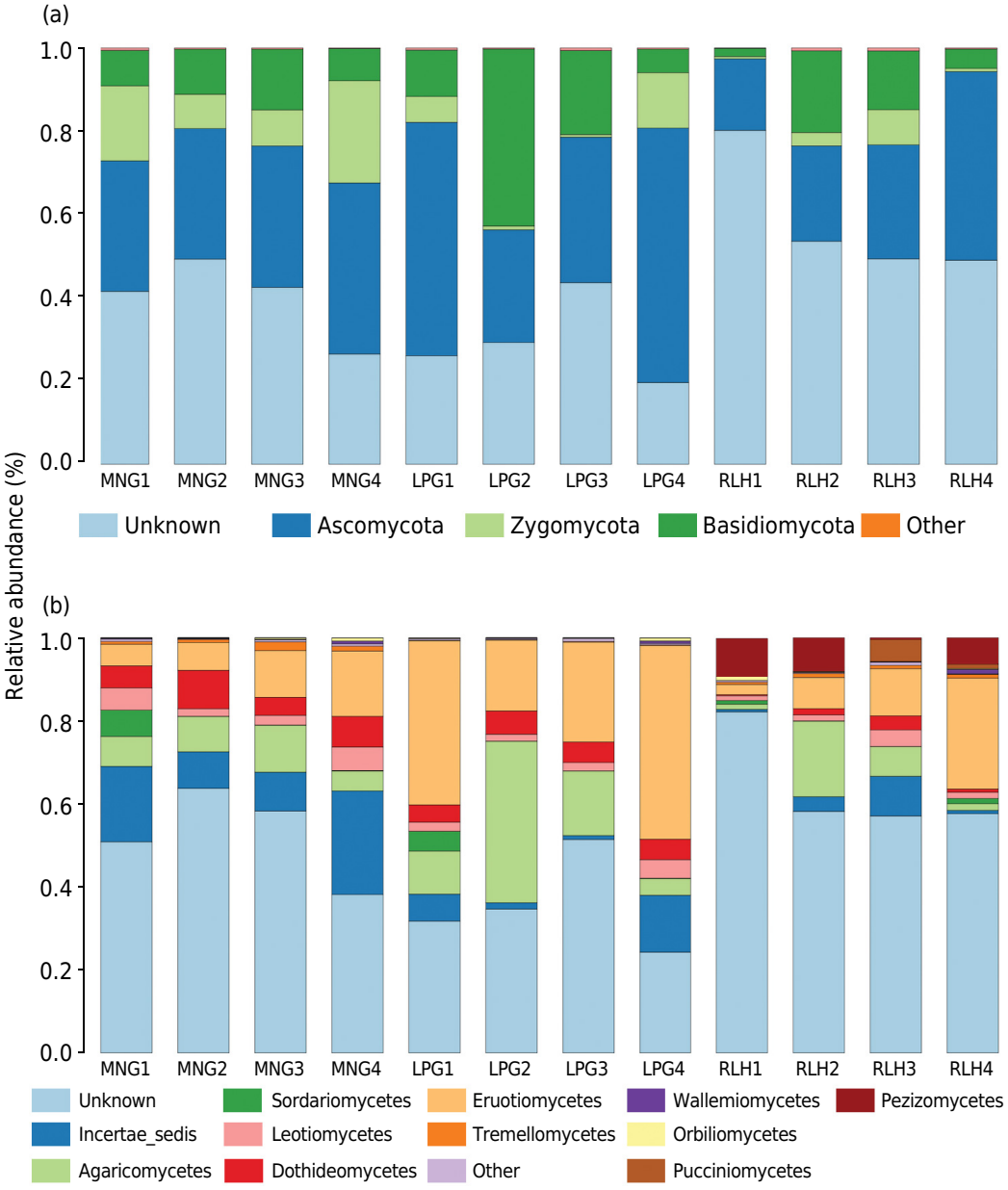

Figure 2. Relative abundance at the microbial taxonomic levels of different treatments. Note: a: fungus phylum; b: fungus class. RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

spring and winter was much higher than other phyla and lower in summer and autumn. In RLH, the *Ascomycota* gradually increased with time; *Basidiomycota* in summer was much higher than that in any other seasons and *Zygomycota* was highest in autumn.

At the fungal class level (Figure 2b), in MNG, *Leotiomycetes* were higher in summer and winter than in spring and winter, and *Eurotiomycetes* increased along with time. *Agaricomycetes* and *Dothideomycetes* in LPG were both significantly higher than that in MNG and RLH in every season. During the whole year, variation of *Agaricomycetes* in LPG was the same as in RLH. *Eurotiomycetes* in RLH increased along with time which was the same as that in MNG. In MNG, LPG, and RLH treatment, there were still many unknown fungi, which were between 30-50, 18-40, and 45-80 %, respectively at phylum level as well as between 37-65, 25-50, and 56-83 %, respectively at class level.

Relationships between microbial communities

Venn diagrams were used to show shared and unique communities in various seasons (Figure 3). For bacterial communities (Figure 3a), four seasons shared 1230, 984, and 1174 OTUs, accounting for 73.35, 55.06, and 67.43 % of each total reads in LPG, RLH, and MNG, respectively. The variation of bacterial communities in LPG was more stable than in RLH and MNG because of the high ratio of shared OTUs and the lower difference in unique communities.

For fungal communities (Figure 3b), four seasons shared 397, 240, and 426 OTUs, accounting for 42.78, 24.19, and 45.61 % of each total reads, in LPG, RLH, and MNG, respectively. The variation of fungal communities in MNG was more stable than in LPG and RLH because of the high ratio of sharing OTUs and the less difference of unique communities.

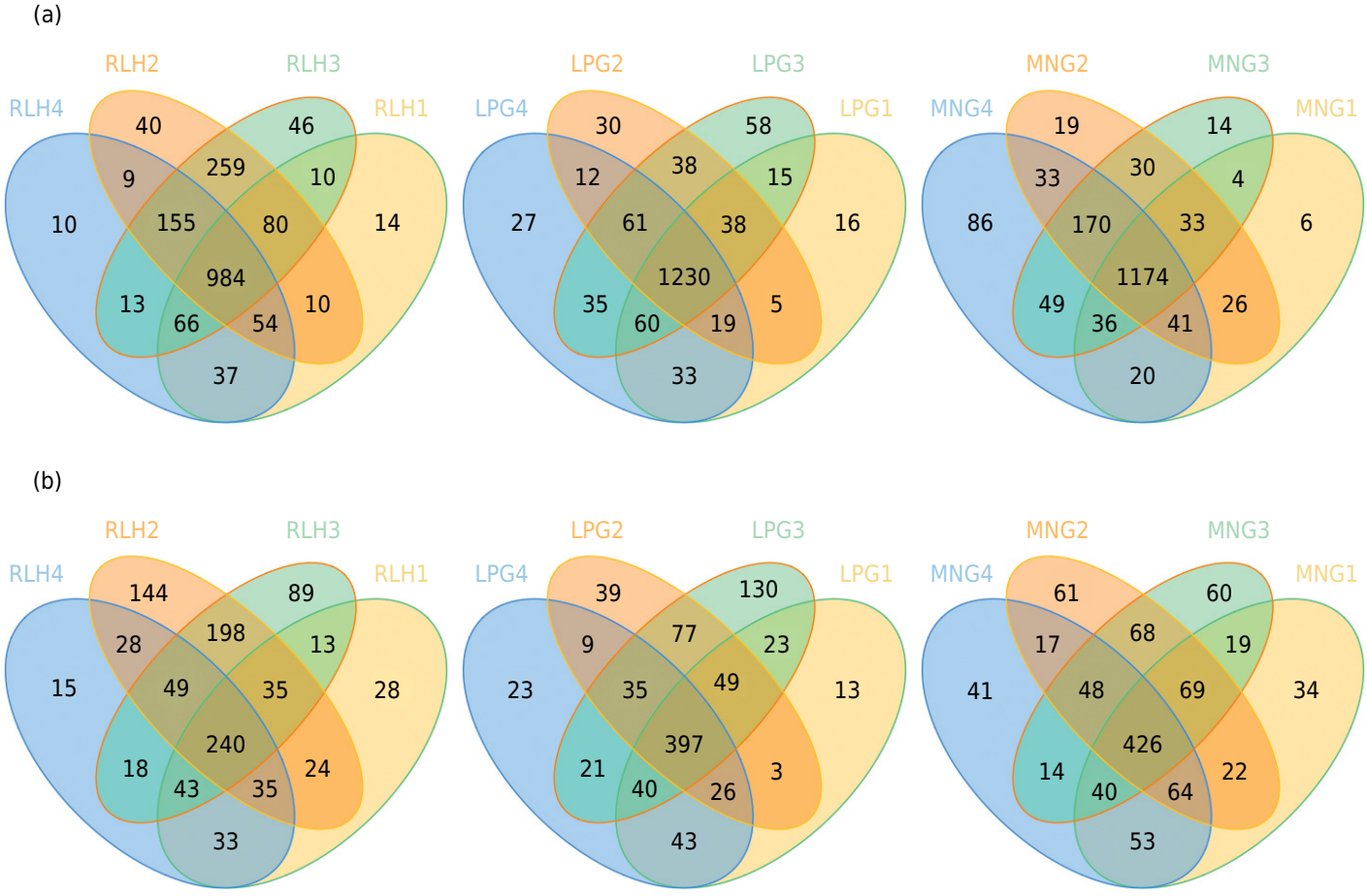

Figure 3. Bacterial (a) and fungal Venn (b) diagrams of OTUs within same methods in different seasons. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

To get an overall view of the identified connections among the samples, hierarchically clustered heatmaps were generated. The closer the color was to the red, the more dominant microorganism was. There were differences among every treatment and every season.

According to the heatmaps, the fluctuation of bacterial communities in RLH was greater than in LPG and MNG (Figure 4 and Figure 5). At the family level, MNG1 and MNG2, LPG1 and LPG2 clustered together, respectively. Clustering of MNG3 and LPG3 at family and order level showed that there were similar bacterial communities between MNG and LPG

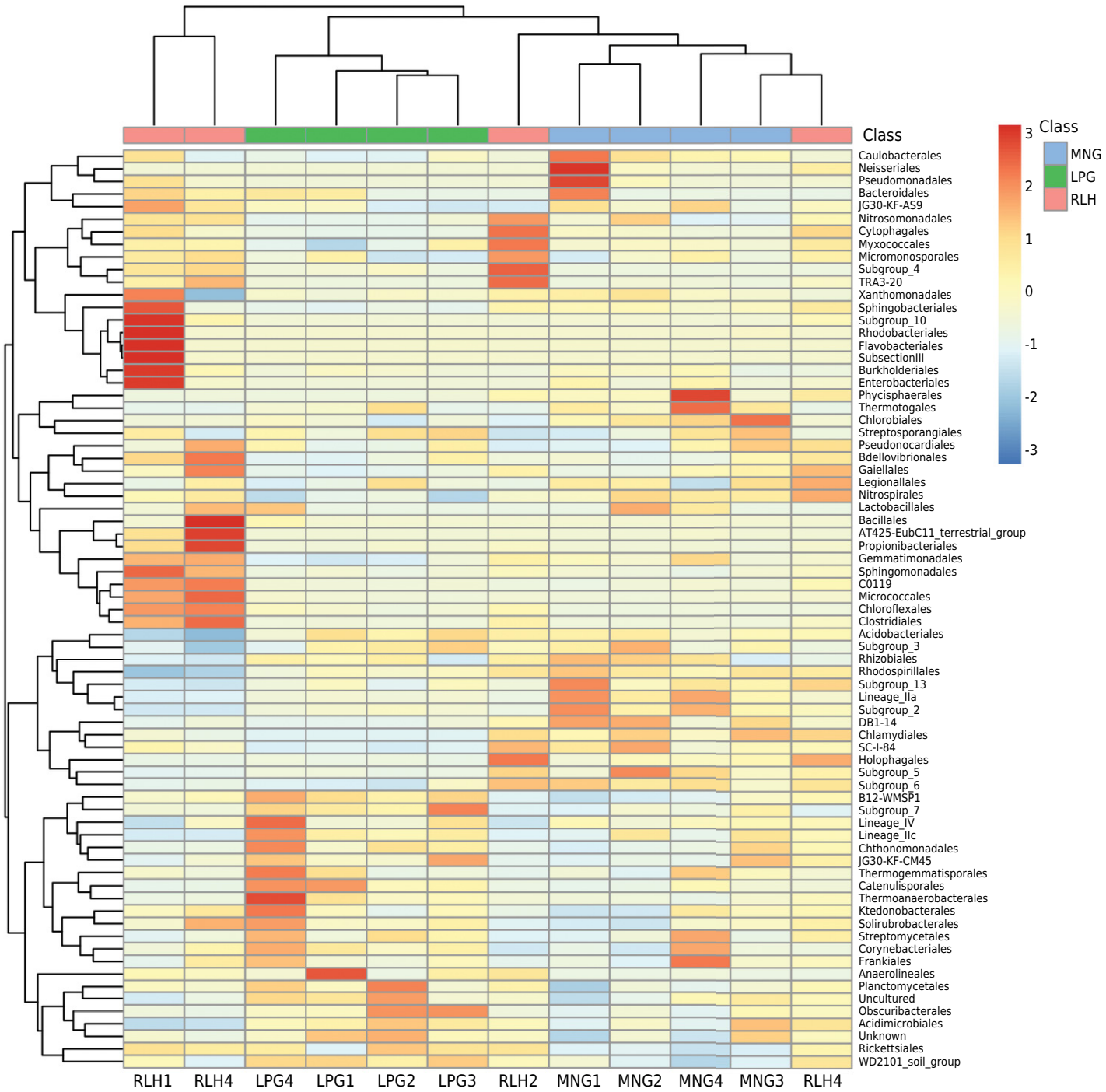

Figure 4. Heatmap analysis of the bacterial community of soil samples subjected to RLH, LPG, and MNG treatments collected at four times during the year and multiple samples similarity tree at the order level. The connecting lines on top describe the bacterial communities clustering of each sample. The connecting lines on the left side describe the clustering of each bacterium. The closer the color to red was, the more dominant microorganism was. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

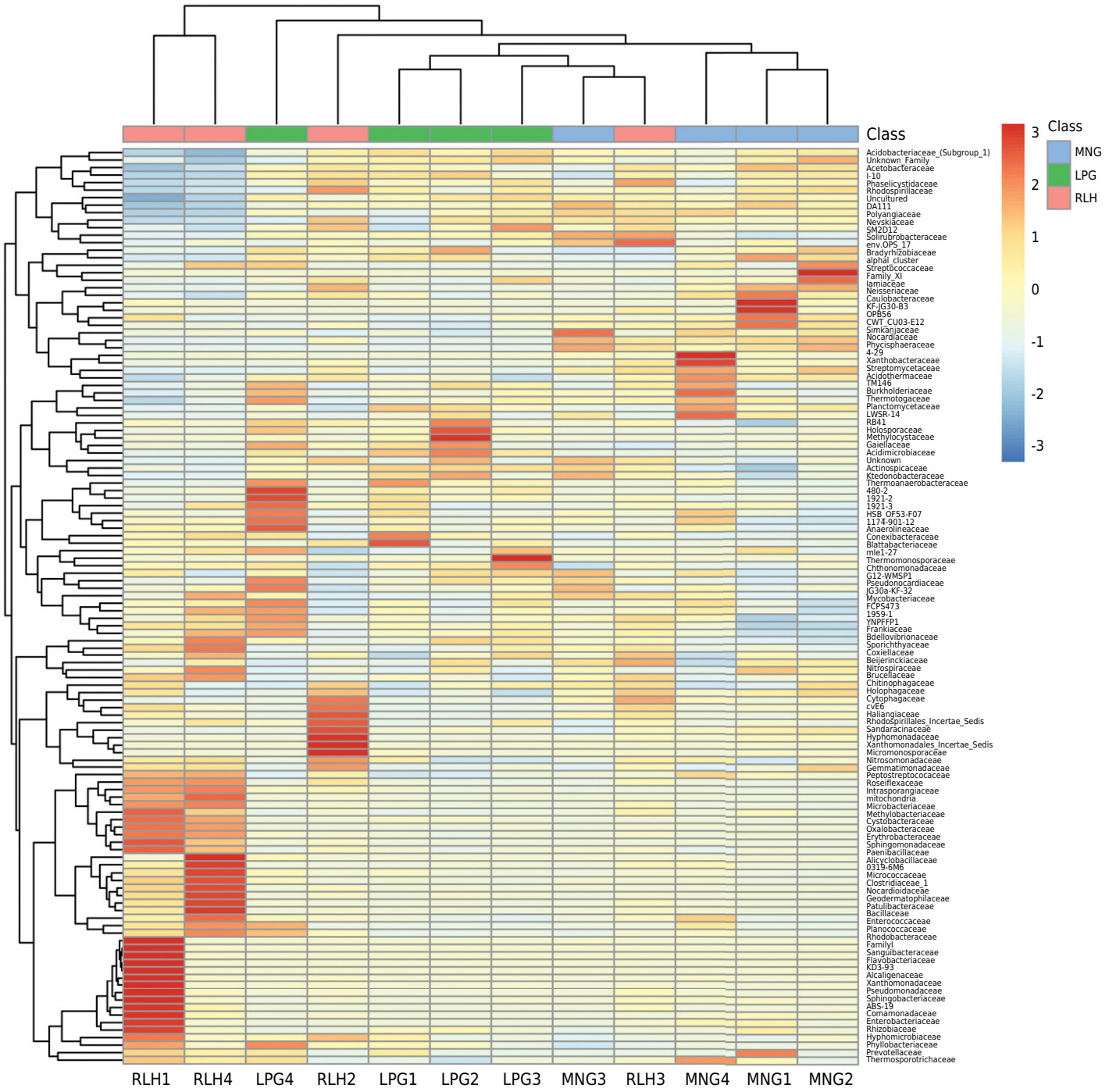

Figure 5. Heatmap analysis of the bacterial community of soil samples subjected to RLH, LPG, and MNG treatments collected at four times during the year and multiple samples similarity tree at the family level. The connecting lines on top describe the bacterial communities clustering of each sample. The connecting lines on the left side describe the clustering of each bacterium. The closer the color to red was, the more dominant microorganism was. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

in autumn. In autumn, the activity of bacteria was the lowest during the whole year, and there were very little dominant bacteria at order and family level.

According to heatmaps of fungal communities (Figure 6 and Figure 7), the fungal communities in LPG were more stable than in other treatments during the whole year. At the order level, the clustering of LPG1 and LPG4 assembled with the clustering of LPG2 and LPG3. The LPG1 and LPG4, MNG1 and MNG4, RLH1 and RLH4 clustered together respectively at the family level.

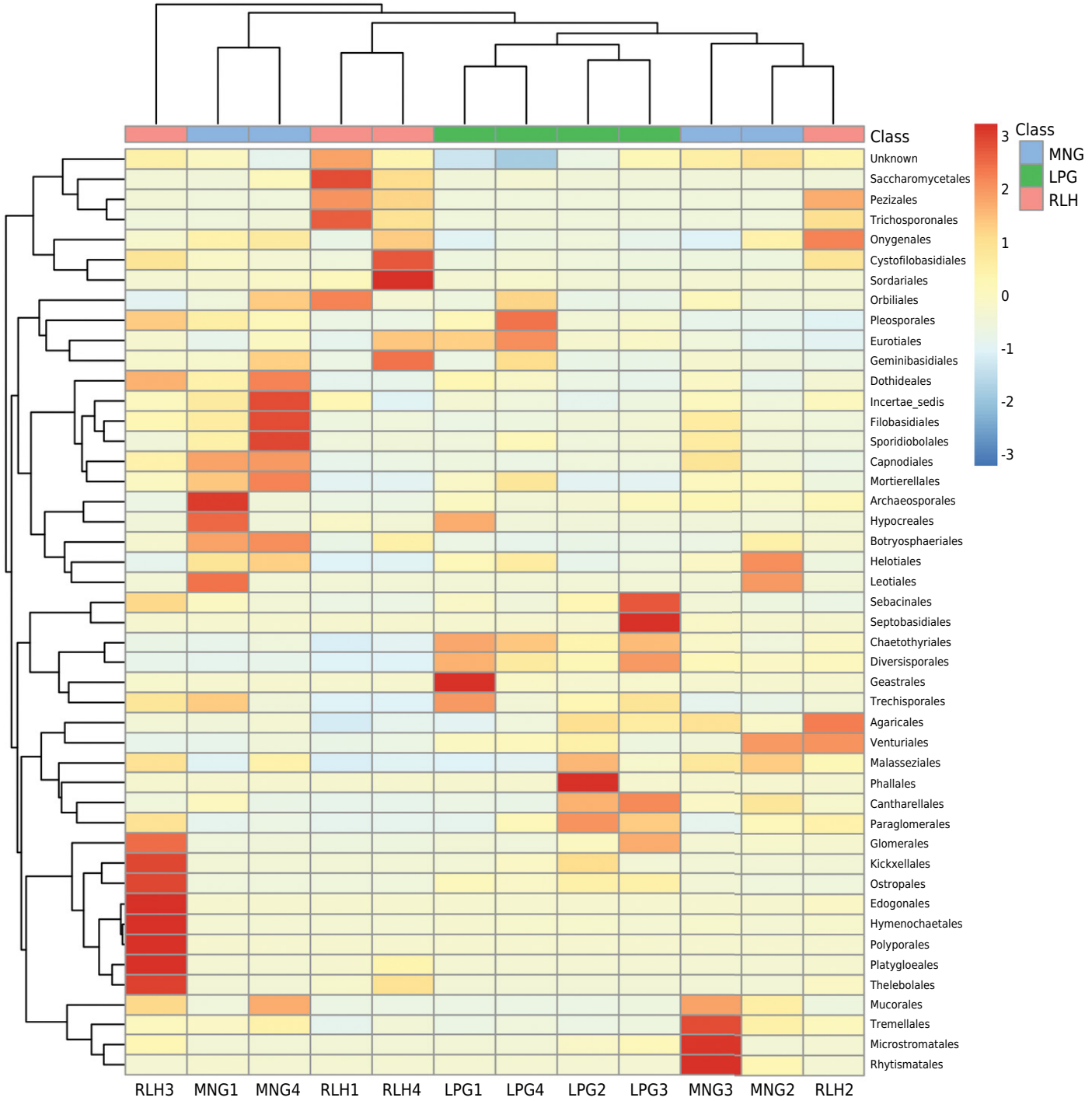

Figure 6. Heatmap analysis of the fungal community of soil samples subjected to RLH, LPG, and MNG treatments collected at four times during the year and multiple samples similarity tree at the order level. The connecting lines on top describe the fungal communities clustering of each sample. The connecting lines on the left side describe the clustering of each fungus. The closer the color close to red was, the more dominant microorganism was. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

The influence factor on microbial communities

Redundancy analysis (RDA) indicated that temperature and TOC were the dominant factors for explaining the most variations in bacterial and fungal communities of four seasons and three treatments (Figure 7 and Figure 9).

According to figure 8, temperature positively influenced *Bryobacter*, *Candidatus_ Solibacter*, and *Bradyrhizobium* while negatively influenced *Acidothermus*, *Paenibacillus*,

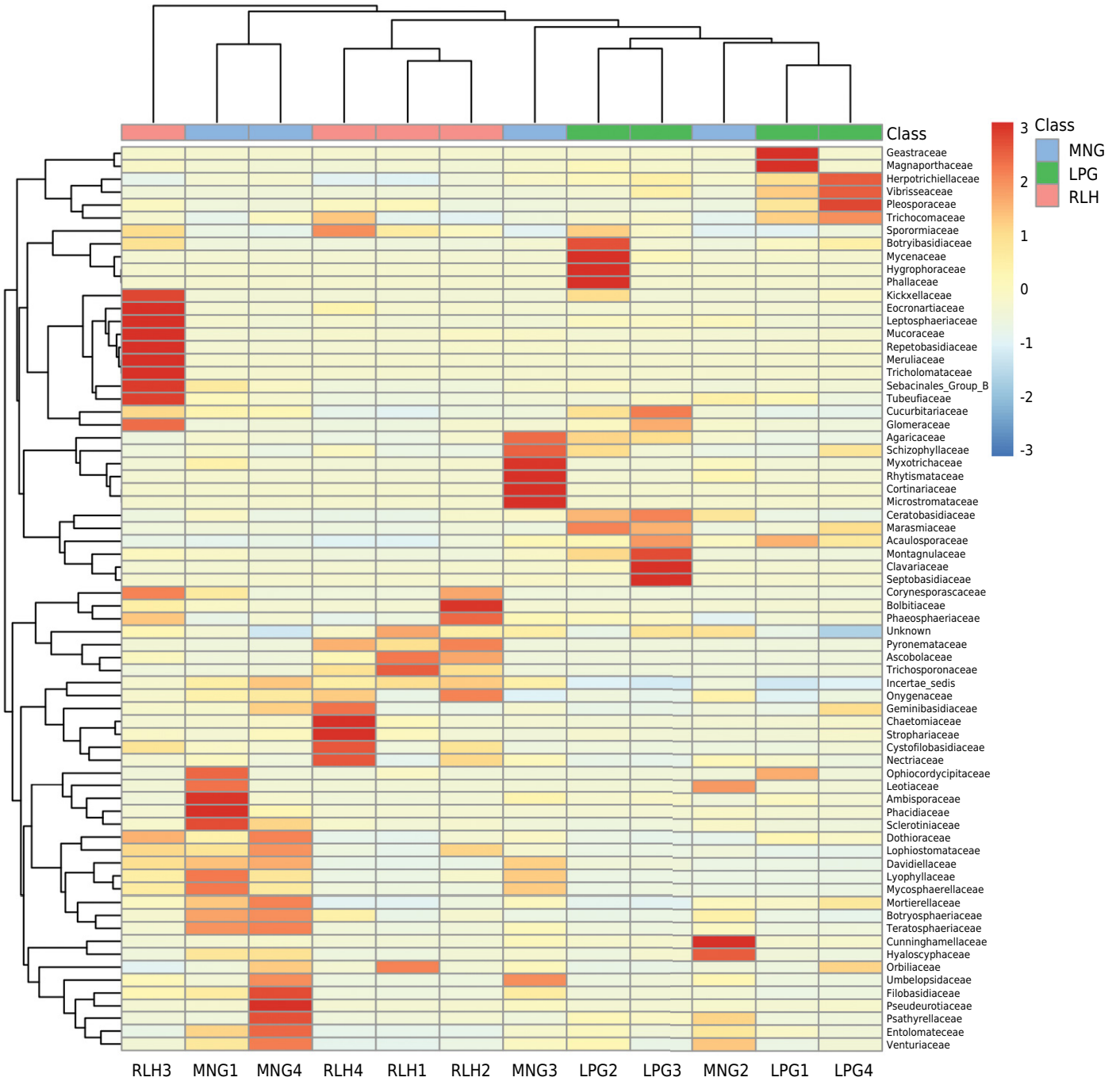

Figure 7. Heatmap analysis of the fungal community of soil samples subjected to RLH, LPG, and MNG treatments collected at four times during the year and multiple samples similarity tree at the family level. The connecting lines on top describe the fungal communities clustering of each sample. The connecting lines on the left side describe the clustering of each fungus. The closer the color close to red was, the more dominant microorganism was. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter.

Modestobacter, and *Crossiella*. The TOC and pH positively influenced *Burkholderia* while negatively influenced *Rhizomicrobium*, *Flavobacterium*, *Stenotrophomonas*, *Phenylobacterium*, *Brevundimonas*, *Sorangium*, and *Sphingomonas*.

According to figure 9, temperature positively influenced *Purpureocillim*, *Leucoagaricus*, and *Dictyophora* while negatively influenced *Gymnopilus*, *Penicillium*, and *Talaromyces*. There was a significant positive influence of pH on *Rasamsonia*, *Leucocoprinus*, *Gymnopus*, and *Hydropus* while there was negative influence on *Hyaloscypha*, *Scleroconidioma*, *Cephaliphora*, *Cladophialophora*, and *Cryptococcus*. The TOC appeared to positively

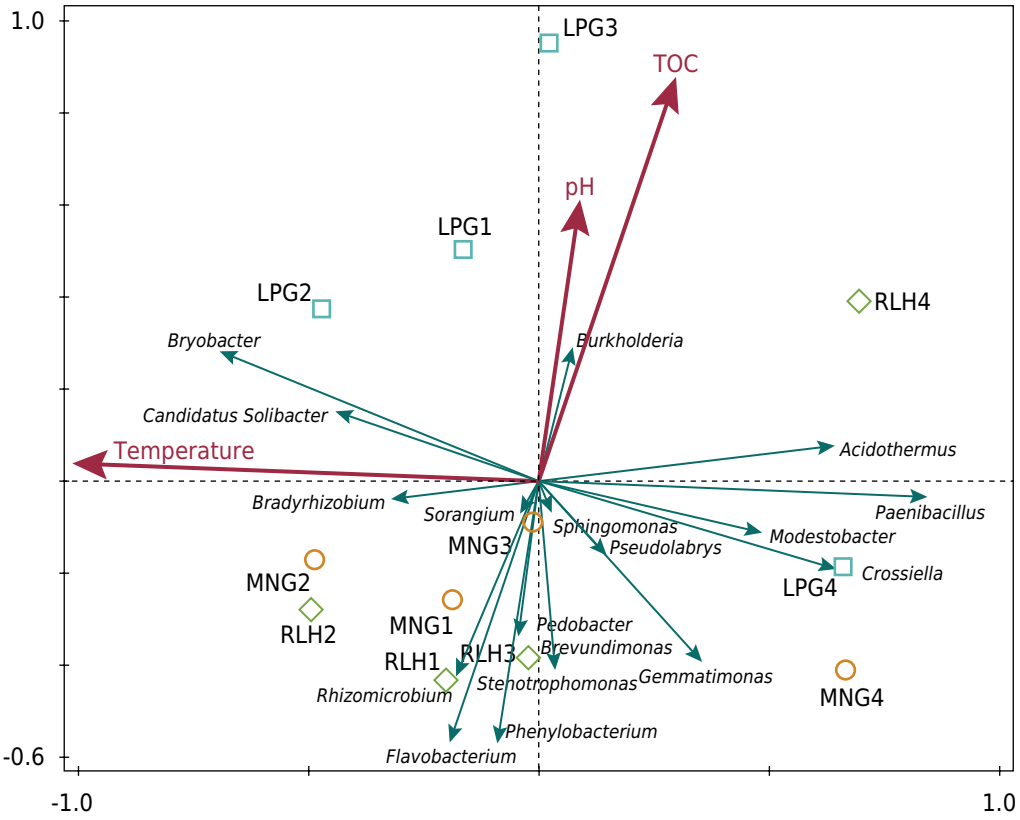

Figure 8. Redundancy analysis (RDA) of the relationship between the soil physicochemical properties and the relative abundance of each bacterial genus of the twelve soil samples ($p<0.05$). Different symbols in the graph represent soil samples from different treatments: Circle: MNG; Square: LPG; and Diamond: RLH. Arrows indicate the direction and magnitude of each variable. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter. Bottle green lines represent bacterial genus; red lines represent environmental factors.

influence *Gymnopus* and *Aspergillus* while negatively influence *Clitopilus*, *Agaricus*, *Scleroconidioma*, and *Conocybe*.

DISCUSSION

The effect of farming patterns on microbial communities

Growing grass is a sustainable management practice that decreases soil bulk densities, increases soil porosity, soil moisture, and nutrient holding capacity (Wang et al., 2013; Jia et al., 2014). This management practice has been adopted in many orchards (Greenham, 1955). These factors can significantly change the microbial communities (Du et al., 2015). There are two major ways to grow grass, including artificially cultivating grass and growing grass without tillage. Both ways have been identified to have beneficial effects on improving soil chemical properties and soil microbial communities (Chalak et al., 2011; Yagioka et al., 2015; Boukhdoud et al., 2016). Several researchers have identified that poultries can contribute to improvement of soil fertility and soil structure, such as increasing soil nitrogen and phosphorus, and improving soil porosities and soil water content (Wilkins, 2008; Lin et al., 2013; Xu et al., 2014; Wang et al., 2016b). These changes are able to provide a more appropriate environment for microorganisms. In this study, the TOC and pH in LPG treatment were significantly higher than in other treatments. Xu et al. (2014) found that there were no remarkable changes in the soil during the first year of raising laying hens but the concentration of nutrients in the soil increased considerably when after 2 to 4 years. Wild grass was found to create more nutrients

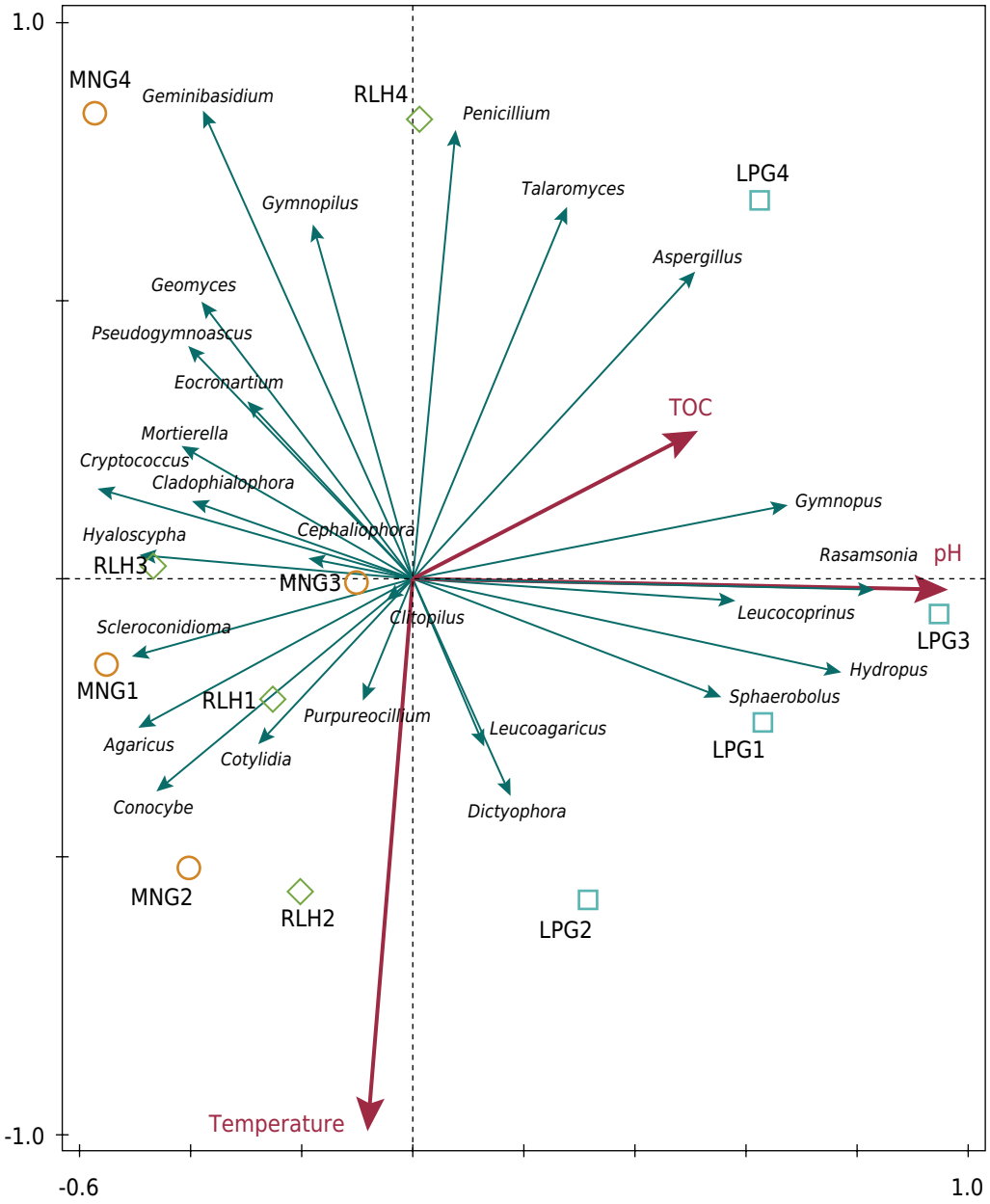

Figure 9. Redundancy analysis (RDA) of the relationship between the soil physicochemical properties and the relative abundance of fungal genus of the twelve soil samples ($p<0.05$). Different symbols in the graph represent soil samples from different treatments: Circle = MNG; Square = LPG; and Diamond = RLH. Arrows indicate the direction and magnitude of variables. Note: RLH: raising laying hens under forest; LPG: cultivating *Lolium perenne* grass under forest; MNG: maintenance of native grass under forest. The number after the treatment symbol means: 1 = spring; 2 = summer; 3 = autumn; 4 = winter. Bottle green lines represent fungal genus; red lines represent environmental factors.

than artificially cultivated grass in an apple orchard (Yan et al., 2014). The differences between Yan's research and ours might be caused by the differences in dominant cultivars, grass species, and soil properties. Although the TOC and pH in LPG were remarkably higher than in other treatments, the bacterial diversity and richness had no significant difference among the three treatments, which is in agreement with Tao et al. (2011). However, the stability of bacterial richness in LPG was the best while that in RLH was the worst during whole year, indicating that the grass cover could provide a relatively stable environment for soil bacterial communities.

Except for the unknown phyla, *Ascomycota* and *Basidiomycota* were the dominant fungal phyla in all treatments, which agreed with most previous studies (Buée et al., 2009;

Weber et al., 2013; Coats et al., 2014). Previous studies have found that most pathogenic microbes were fungi (Agrios, 2005; Raaijmakers et al., 2009; Liu et al., 2016). Many researchers have reported that manure could dramatically increase bacterial activity while decrease fungal activities (Bittman et al., 2005; Liu et al., 2013). Compared to the control, the relative abundance of *Ascomycota*, which was linked to many kinds of pathogenic fungi by previous studies (Toledo et al., 2007; Vujanovic and Labrecque, 2008; Rodrigues et al., 2016), decreased under the laying hens treatment. The relative abundance of *Agaricomycetes* and *Eurotiomycetes*, which was similar to Zhou's research (Zhou et al., 2016), in LPG were significantly higher than in other treatments. *Agaricomycetes*, known as the decomposer of lignin, could increase soil nutrients but could also lead to plant white rot or soft rot (Morgenstern et al., 2008). *Eurotiomycetes* was found in high N content (Zhou, et al., 2016). Therefore, we could speculate that there was more N in LPG than in other treatments, and the fungi in LPG could facilitate the decomposition of those nutrients whereas the saprophytic fungi also increased the risk of plant root disease.

Acidobacteria and *Proteobacteria* were the major phyla identified in soil bacterial communities in this study and as reported by previous studies (Barns et al., 1999; Janssen et al., 2002; Jones et al., 2009). *Acidobacteria* was regarded as oligotrophic bacteria. Smit et al. (2001) found a high ratio of *Acidobacteria* to *Proteobacteria* when the soil nutrients were low. Surprisingly, in the RLH treatment, the relative abundance of *Acidobacteria* in spring and winter were remarkably lower than other treatments, indicating that the activities of laying hens might improve content of some other soil nutrients except TOC.

Seasonal variation of microbial communities

According to the redundancy analysis, temperature was the primary factor influencing microbial communities, which indicated that microbial communities were strongly affected by seasonal dynamics. Additionally, TOC was also influential on bacteria while pH was influential on fungi.

Regardless of treatment, the highest TOC content was found in winter, which was identical to the findings of Aanderud et al. (2010). However, Laudon et al. (2004) found that the highest TOC content was observed during spring. In RLH, activity from the hens, such as scraping soil to find insects and producing manure, enhanced microbial activity but drastic seasonal variations in microbial communities were observed due to the absence of grass covering. In LPG, the fluctuation range of microbial communities was smaller than in other treatments throughout the year. The single-species grass covering provided relatively stable microbial communities. As temperature decreased, many grass residues provided nutrients to bacteria, however, further declining temperatures would inhibit microbial activity. In MNG, the diversity of weed species made the micro-environment unstable but the residues of these weeds lasted longer in comparison to single grass. In winter, cold-hardy grass would tolerate the low temperatures and could create relatively suitable micro-environments for microorganisms.

Proteobacteria and especially *Alphaproteobacteria* remained stable throughout the year while *Acidobacteria* had the lowest relative abundance during winter and *Actinobacteria* had the lowest relative abundance during spring and summer, which was similar to the observations of Lipson and Schmidt (2004). As indicated by Lazzaro et al. (2012), seasonal changes had no effect on *Alphaproteobacteria*. The most dominant bacteria were found in spring and winter. *Xanthomonadales* and *Burkholderiales* were phytopathogens (Campos et al., 2016) predominant in RLH1. This means the activity of the hens during the spring may not improve the soil nutrition but increase the risk of plant disease.

Similar to other studies (Schmidt et al., 2013; Weber, et al., 2013), the relative abundance of *Ascomycota* and *Basidiomycota* in our study were the predominant phyla during all seasons. The relative abundance of *Agaricomycetes* in summer and autumn was higher

than in spring and winter, indicating that the disease rate of infection of plants in summer and autumn was higher than in spring and winter.

CONCLUSION

Raising laying hens may provide manure to increase the soil organic matter; however, a stable micro-environment for microorganisms was not achieved without grass cover. Also, maintaining native grasses resulted in limited quantity of soil TOC. Cultivating grasses under oil tea trees was the best among all options in this study for the improvement of the microenvironment, such as increasing TOC content and pH, stabilizing microbial communities, and reducing pathogenic microorganisms. In addition, cultivating grasses increased the relative abundance of microorganisms. These changes in soil microenvironment may benefit the growth of oil tea trees and further study is needed to quantify the beneficial effect on the performance of oil tree trees.

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Funding Acquisition: Jun Yuan.

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