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Changes in soil bacterial communities in response to the fairy ring fungus *Agaricus gennadii* in the temperate steppes of China

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ABSTRACT

Fairy rings (FRs), mysterious circles of fungal fruiting bodies that occur in open grassy areas, have long been reported, but scientists know little about their effects on the physiochemical properties of soil and the associated bacterial communities. We investigated three concentric zones of *Agaricus gennadii* FRs in June, August and October 2016 in a temperate steppe of China outside of rings (OUT), within rings (IN) and adjacent to fruiting bodies (ON) to determine the effects of FR fungus on soil properties and bacterial community structure. Significantly higher aboveground biomass was only observed in the ON zone because of an increase in available soil P ($P < 0.05$). Soil total N, total P and inorganic N were lower in the ON and IN zones than in the OUT zone. The bacterial Shannon and Chao 1 indices were significantly higher in samples associated with the rings in August and October ($P < 0.05$). At the family level, only the abundance of *Microbacteriaceae* was significantly higher in the ON zone than in the OUT zone during the three-month study period ($P < 0.05$), indicating that species of the *Microbacteriaceae* are associated with *Agaricus gennadii* FRs. Moreover, the changes of soil pH and soil available P caused by FRs were found to be better indicators of changes in soil bacterial communities than total N, total P and inorganic N in soil.

1. Introduction

Basidiomycete fungi frequently form regular rings or arcs of fruiting bodies that, coupled with greener plants are commonly observed in grasslands (Edwards, 1984; Fox, 2006). This pattern, often referred to as “fairy rings” (FRs), has been described for natural plant communities in several habitat types, including sand dunes (Abesha et al., 2003), grasslands (Bonanomi et al., 2012; Caesar-TonThat et al., 2013; Edwards, 1984, 1988; Xu et al., 2011) and the undergrowth of temperate forests (Peter, 2006). These FR fungi often mineralize soil nitrogen (N) and phosphorus (P), and thus stimulate plant growth stimulation in the surrounding soil (Edwards, 1988; Fisher, 1977). Previous studies have shown that concentrations of organic matter, N and P in soil declined from outside to inside a ring (Edwards, 1984), while soil concentrations of NH_4^+-N were lower inside than outside FRs, and the content of organic matter and P did not differ significantly from outside to inside (Caesar-TonThat et al., 2013). Moreover, plant growth enhancement in the stimulation zone on the ring area was recently attributed to changes in microbial community functionality or the promotion of selected species of microorganisms (Bonanomi et al., 2012). Plant growth stimulation may also be attributed to enhancement of aggregation in bulk soil by the fungus as well as its effects on the amount and functionality of specific predominant soil microbial communities (Caesar-TonThat et al., 2013). FR fungus change the physiochemical properties of soil from outside to inside the ring, which will greatly affect the bacterial community in soil. However, the causal relationships among plant growth, soil physiochemical properties and soil bacterial communities caused by FR fungus remain unclear.

Fungi and bacteria play major roles in soil biogeochemical cycles (Alexandre et al., 2017); very diverse fungal–bacterial interactions also contribute to many ecosystem processes (Worrich et al., 2017). Fungi are often thought to dominate soil microbial biomass under drought and nutrient limited conditions (Ritz and Young, 2004) because of their efficient resource translocation between spatially separated sources and sink regions in their mycelia (Allen, 2007; de Vries et al., 2012; Harms et al., 2011; Six, 2012). Fungi and bacteria share the same resources in soil and are therefore almost certain to frequently interact in the soil (de Boer et al., 2005; Lisboa et al., 2014; Warmink et al., 2009). For example, bacteria are known to adhere to the hyphae of arbuscular mycorrhizal fungi (AMF) and feed on hyphal exudates (Bianciotto et al.,

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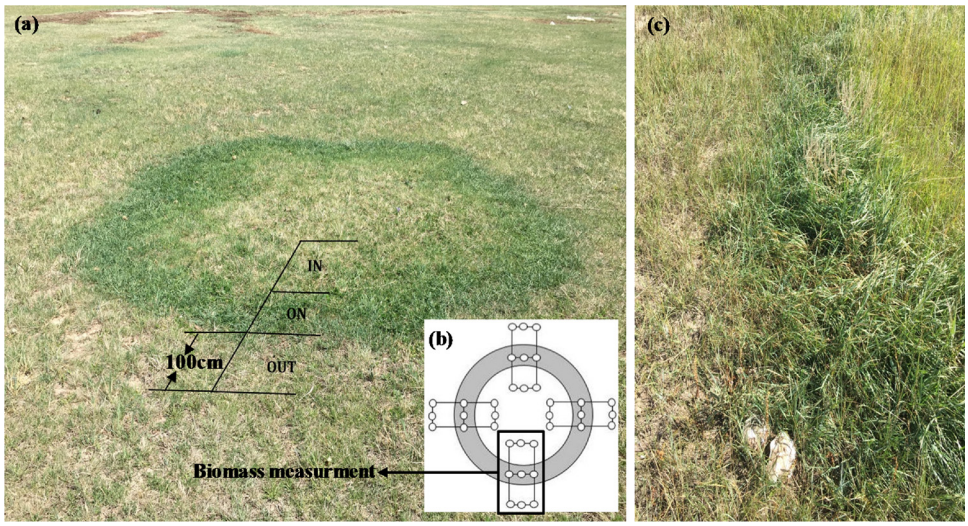

Fig. 1. Three zones of an *Agaricus* fungus fairy ring as evidenced by dark-green vegetation: (a) outside of rings (OUT), adjacent to fruiting bodies(ON), and within rings (IN) where the *Agaricus* has already grown through. (b) Soil samples were collected from topsoil (0–15 cm) in four directions and in the above three zones. (c) *Agaricus* fungus fruiting bodies appeared in the outer edge of the ON zone. Images by Chao Yang on May 26 (a) and August 28 (c), 2016 in Guyuan County, Hebei Province, China (41°44′N, 115°40′E).

1996). Moreover, a previous study reported an increase in the total number of bacteria in both the rhizosphere and hyphosphere because of AMF fungi (Ravnskov and Jakobsen, 1999). In addition to AMF, the fruiting bodies of basidiomycetes can influence soil bacteria. Estimates of the mycelial biomass of basidiomycetes, which represent the bulk of the soil fungal biomass, typically range from 100 to 600 kg ha^{-1} (Cairney, 2012; Hendricks et al., 2016; Wallander et al., 2004). However, considering the quantitative importance of dead fungal mycelia, surprisingly little is known about the plants and microorganisms responsible for mycelial decomposition.

The basidiomycete fungus *Agaricus gennadii*is known to develop FRs in the temperate steppes of China. The vegetation in the fungal growth zone grows vigorously (Fig. 1); therefore, its physiochemical properties were analyzed, and the diversity and composition (order to family levels) of bacterial communities in three zones (outside of rings, OUT; adjacent to fruiting bodies, ON; within rings, IN) over a three-month growing season were compared. Moreover, the relationship between the physiochemical properties of the soil and the bacterial community structure was examined. We hypothesized that (1) the aboveground plant biomass and soil properties would differ significantly in these three FR zones; (2) the effects of the ON zone of FRs would increase the diversity of the soil bacterial community and alter the bacterial composition more than the OUT and IN zones; and (3) associations would exist between soil physiochemical properties and bacterial communities in different FR zones.

2. Materials and methods

2.1. Study sites

This study was conducted at the National Field Station for Grassland Ecosystems in Guyuan County (41°44′N, 115°40′E, elevation 1430 m), Hebei Province, China. The typical temperate climate of this area is characterized by a mean annual precipitation and temperature of 430 and 1.4 °C, respectively. The minimum and maximum monthly mean air temperature in the study area -18.6 °C in January and 21.1 °C in July, respectively. Precipitation mainly occurs during the growing season (June to August), which coincides with the highest temperatures. The plant community is dominated by *Leymus chinensis* (Trin.) Tzvel. The site has a calcic–orthic Aridisol soil with a loamy-sand texture according to the ISSS Working Group RB (1998) (Chen et al., 2015).

2.2. Plant and soil sample collection

Three regular regularly shaped FRs with an internal radius of ~5 m were investigated.. For each of the three replicate rings, three different zones were identified across four transects, proceeding from the outer to the inner areas of rings (OUT, ON and IN zones (Fig. 1a)). The ring-producing fungus (Fig. 1c) was identified by DNA based molecular authentication as *Agaricus gennadii* (see Fig. A1 for details).

Plant and soil samples were collected during three sampling periods (June, August and October 2016). The samples of each subsequent period were collected from areas adjacent to the site of sample collection from the previous period in the same rings, and the distance was 50 cm. During each sampling period, soil samples were collected from the topsoil (0–15 cm soil depth and 5-cm diameter) in four different directions for each ring (Fig. 1b). These four samples collected from each zone and ring were then combined into a single composite sample, on which subsequent analyses were performed for a final total of nine soil samples (three rings and three zones) obtained from each sampling period. All fresh soil samples were divided into two parts with one stored at 4°C for later chemical analysis and another stored at −20°C for DNA extraction. To reduce the damage to the FRs, one quadrat (20 × 20 cm) was established in only one direction to determine the aboveground biomass of the community. Nine vegetation samples (three rings and three zones) were also collected during June, August and October 2016. The aboveground biomass was cut at the soil surface, then oven dried at 65 °C for 72 h before the aboveground biomass was calculated.

2.3. Soil chemical property analysis

Soil moisture content (SMC) was measured by oven-drying soil at 105 °C for 24 h, while soil pH was measured using a glass electrode in a 1:2.5 soil/water suspension. Additionally, the soil organic carbon (SOC) concentration was measured using an auto-analyzer (TOC, Elementar, Germany) while soil total N was determined using a FOSS Kjeltec 2300 analyzer unit (FOSS, Hillerød, Sweden). Soil inorganic N (NH_4^+-N and NO_3^--N) was extracted from 10 g subsamples using 50 ml of 2 mol/L KCl, and then measured using a Flow-Solution analyser (Flowsys, Ecotech, Germany). Total soil P was determined using the sodium hydroxide smelting-molybdenum antimony colorimetric method. Finally, the soil available P (P Olsen) was extracted by shaking 1.5 g of dry soil for 30 min at 20 °C in 100 ml of 42% $NaHCO_3$ at pH 8.5 according to the method of Carter and Gregorich (2008).

2.4. DNA extraction and PCR amplification

Genomic DNA was extracted from soil samples using an E.Z.N.A.® stool DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the

manufacturer's instructions. The quality of extracted DNA was checked by 1% agarose gel electrophoresis and spectrophotometry (optical density at 260 nm/280 nm ratio). All extracted DNA samples were stored at -20 °C for further analysis. The V3-V4 hypervariable regions of the 16S rRNA gene were subjected to high-throughput sequencing by Beijing Allwegene Tech, Ltd. (Beijing, China) using the Illumina Miseq PE300 sequencing platform (Illumina, Inc., San Diego, CA, USA). The V3-V4 region of the bacteria 16S rRNA gene was amplified with the following universal primers: forward 338 F (5′-ACTCCTACGGGAGGC AGCAG-3′); reverse 806R (5′-GACTACHVGGGTWTCTAAT-3′) (Li et al., 2015). These primers contained a set of 8-nucleotide barcode sequences unique to each sample. Samples were subjected to the following PCR program: 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and then a final extension of 72 °C for 10 min. PCR reactions were performed in triplicate 25 ml mixtures containing 2.5 ml of 10× Pyrobest Buffer, 2 ml of 2.5 mM dNTPs, 1 ml of each primer (10 mM), 0.4 U of Pyrobest DNA Polymerase (TaKaRa), and 15 ng of template DNA. The amplicon mixture was applied to a MiSeq Genome Sequencer (Illumina).

2.5. Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions; amplicons were then quantified using QuantiFluor™ -ST (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Allwegene, Beijing) using the standard protocols.

2.6. Processing of sequencing data

The sequencing data were subjected to bioinformatic analysis. Raw FASTQ files were de-multiplexed and quality-filtered using QIIME (ver. 1.8) with the following criteria: (i) the 300-bp reads were truncated at any site that obtained an average quality score of < 20 over a 10-bp sliding window; then truncated reads shorter than 50 bp were discarded; (ii) only those with exact barcode matching were employed, while two nucleotide mismatches in primer matching and reads containing ambiguous characters were removed; (iii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. Reads that could not be assembled were discarded. The unique sequence set was classified into operational taxonomic units (OTUs) under the threshold of 97% identity using UCLUST software. Chimeric sequences were identified and removed using Usearch software (ver. 8.0.1). The taxonomy of each 16S rRNA gene sequence was analyzed by UCLUST against the Silva119 16S rRNA database using a confidence threshold of 90%. The raw readings were deposited into the NCBI Sequence Read Archive (SRA) database under bioproject accession number PRJNA392001.

2.7. Statistical analysis

An evaluation of the normality of data from each zone before analysis using a one-sample Kolmogorov-Smirnov (K–S) test revealed that all variables exhibited a normal distribution. The effects of the two primary factors (months and zones) and their interactions on plant and soil properties were tested using a two-way ANOVA with a $P < 0.05$ taken to indicate significance. All three zones of plant biomass, soil physicochemical properties, bacterial Shannon index, bacterial Chao 1 richness index and the abundance of bacteria at the family level were analyzed using one-way analysis of variance (ANOVA); the level of significance of least-significant difference tests was $P < 0.05$.

The overall structural changes in soil bacterial classes were evaluated using nonmetric multidimensional scaling (NMDS) based on Bray-Curtis similarity matrices, and the permutational multivariate analysis of variance (PERMANOVA) was performed to identify significant differences between the community structures of each zone using the vegan package in R (Oksanen et al., 2016). To examine the relationship between environmental variables and bacterial communities, we used both detrended correspondence analysis (DCA) and redundancy analysis (RDA) techniques. First, we conducted DCA to enable us to choose between linear and unimodal methods (where the length of gradient > 4.0 = CCA and length of gradient < 4.0 = RDA). The gradient lengths of the first two axes were 0.98 and 0.52, 0.90 and 0.46, and 0.78 and 0.50 for June, August and October, respectively; therefore, we used redundancy analysis (RDA) (Dong et al., 2017). The RDA evaluated the significance of the effects of each variable, based on its eigenvalue, using a Monte Carlo Permutation test, and the resulting significance level was determined by the F ratio and P value (Bostrom et al., 2006). Pearson's correlation was also used to determine the correlation between soil physicochemical properties and nine selected families of bacteria.

The rarefaction curve that was based on Mothur 1.21.1 was identified to reveal the sequencing depth. Diversity analysis was conducted to reveal the Shannon, Chao 1 richness (Dong et al., 2017), and Coverage indices using QIIME 1.8. These three were calculated as follows:

$$\text{Shannon} = -\sum\left(\frac{\text{Ni}}{\text{N}}\right)\ln\left(\frac{\text{Ni}}{\text{N}}\right);\ \text{Chao 1} = \text{S}_{\text{obs}} + \frac{\text{F}_1(\text{F}_1-1)}{2(\text{F}_2+1)};\ \text{Coverage} = 1-\text{F}_1/\text{N}$$

where N is the total OTUs of the sample, Ni is the number of individuals in group i, S_{obs} is the observed number of OTUs, F_1 is the number of OTUs with only one sequence, and F_2 is the number of OTUs with only two sequences.

A K–S test, one as well as two-way ANOVA and Pearson's correlation analysis were performed using SPSS 19.0 (IBM, Armonk, New York, USA), while NMDS and RDA analysis were performed using CANOCO 5 (Microcomputer Power, Ithaca, NY, USA). The values presented in the figures were means of three field replicates ± standard errors (mean ± SE) that were determined using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Plant biomass and soil physicochemical properties

A two-way ANOVA showed that sampling months, FR zones, and their interactions had significant effects on plant biomass and all the soil physicochemical properties (Table 1, Fig. 2). Plant aboveground biomass was significantly higher in the ON than in the OUT and IN zones for June ($F_{2,6} = 699$, $P < 0.001$), August ($F_{2,6} = 84$, $P < 0.001$), and October ($F_{2,6} = 23$, $P = 0.002$) (Fig. 2). The physicochemical properties of the soils are summarized in Table 1. The soil pH value was significantly higher in the ON and IN zones than in the OUT zone, except for October ($P < 0.05$). In addition, the soil available P concentration also increased significantly from the OUT to the IN zone for June, August and October ($P < 0.05$). In contrast, the soil concentrations for total N and total P were significantly lower in the ON and IN zones than in the OUT zone, except for total P in October ($P < 0.05$).

3.2. Soil bacterial diversity and community structure

A total of 285,583 quality-filtered reads were obtained from the 27 FRs samples, and the number of OTUs ranged from 1242 to 1398 across all samples (Table A1). In addition, the rarefaction curves and coverage calculations indicated that the sequencing effort per sample was sufficient to detect > 95% of the predicted richness of each sample (Table A1, Fig A2). A two-way ANOVA showed that FR zones, months and zones interactions had significant effects on Shannon and Chao 1 richness index, while no significant difference in sampling months

Table 1
Least-significant difference (LSD) tests of soil physicochemical properties, and the two-way analysis of variance (ANOVA) used to test the effects of months and zones on soil physicochemical properties. Significant relationships at $P < 0.05$ are indicated by *.

Months	Zones	SMC (%)	pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	ION (mg kg^{-1})	AP (mg kg^{-1})
June	OUT	15.20 (0.01) a	9.16 (0.01) c	17.11 (0.37) a	3.06 (0.04) a	0.61 (0.03) a	41.44 (0.33) a	7.68 (0.29) b
	ON	11.83 (0.13) b	9.24 (0.01) b	14.07 (0.27) b	2.73 (0.02) b	0.50 (0.01) b	28.99 (0.53) b	10.94 (0.37) a
	IN	9.60 (0.12) c	9.33 (0.01) a	6.59 (0.06) c	2.63 (0.01) c	0.53 (0.03) b	21.32 (0.21) c	9.60 (0.60) a
August	OUT	12.40 (0.17) b	9.41 (0.02) b	14.72 (0.29) a	2.72 (0.06) a	0.46 (0.01) a	23.68 (2.44) ns	7.98 (0.14) b
	ON	12.83 (0.03) ab	9.48 (0.01) a	15.55 (0.13) a	2.74 (0.04) a	0.48 (0.01) a	18.82 (0.58) ns	9.53 (0.24) a
	IN	13.20 (0.31) a	9.53 (0.02) a	12.78 (0.32) b	2.31 (0.02) b	0.40 (0.01) b	21.27 (0.30) ns	8.54 (0.25) b
October	OUT	10.07 (0.03) a	9.21 (0.01) ns	12.60 (0.39) ns	2.34 (0.01) a	0.36 (0.02) ns	16.54 (0.38) ab	7.34 (0.19) b
	ON	10.40 (0.15) a	9.32 (0.01) ns	13.07 (0.09) ns	2.22 (0.06) a	0.29 (0.02) ns	15.76 (0.15) b	7.84 (0.12) a
	IN	9.52 (0.11) b	9.25 (0.01) ns	12.72 (0.08) ns	2.07 (0.02) b	0.30 (0.03) ns	17.31 (0.12) a	7.49 (0.07) ab
Two-way ANOVA results								
Months		*	*	*	*	*	*	*
Zones		*	*	*	*	*	*	*
Months × Zones		*	*	*	*	*	*	*

Values represent the means (± SE, n = 3). SMC, soil moisture content; pH, soil acidity; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; ION, soil inorganic nitrogen; AP, soil available phosphorus. Different letters in the same column indicate a significant difference at $P < 0.05$ using LSD tests; ns: no significant difference between zones.

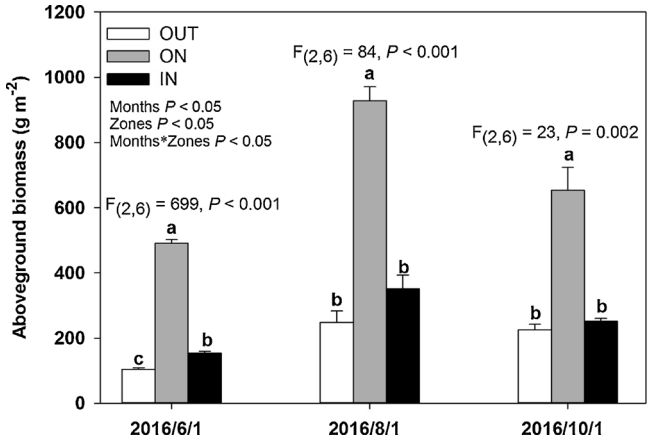

Fig. 2. Aboveground plant biomass differed significantly among the three zones [outside (OUT) and within (IN) the ring, and adjacent to fruiting bodies (ON)] for June, August and October. Means (± SE, n = 3) with different lowercase letters are significantly different based on the LSD test ($P < 0.05$).

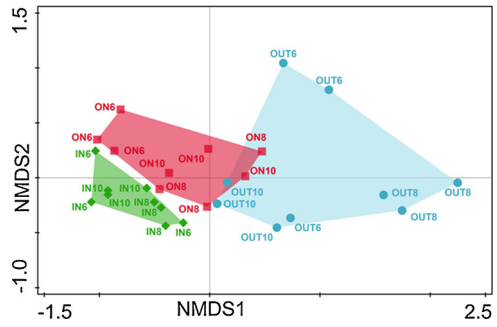

Fig. 4. Two-dimensional nonmetric multidimensional scaling (NMDS1 and NMDS2, stress = 0.11) plots of bacterial community structures based on Bray – Curtis similarity matrices of the square root-transformed relative abundances of bacterial classes. Communities are indicated by colored symbols as follows: blue circles, outside the ring (OUT); red squares, adjacent to the fruiting bodies (ON); green diamonds, within the ring (IN). Each area bounded by samples from the same zone is shaded in the corresponding color to indicate clustering. Numbers indicate the month of sampling (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fig. 3). The Shannon index for the three zones revealed similar trends for August and October, with significantly higher values observed in the ON and IN zones than in the OUT zone for August ($F_{2,6} = 5.4$, $P = 0.047$) and October ($F_{2,6} = 14.5$, $P = 0.005$); however, no significant difference was observed for June (Fig. 3a). In addition, the Chao 1 richness index values were significantly higher in the ON than the IN zones for June ($F_{2,6} = 5.2$, $P = 0.049$), August ($F_{2,6} = 8.1$, $P = 0.02$) and October ($F_{2,6} = 5.1$, $P = 0.05$) (Fig. 3b). Soil bacterial communities were distinct from the three different zone counterparts on NMDS plots (Fig. 4). These results confirmed that the bacterial communities were significantly different in the three zones according to the PERMANOVA ($F = 6.97$ and $P < 0.001$). At the family level, most taxa were not observed to be different, but the abundances of

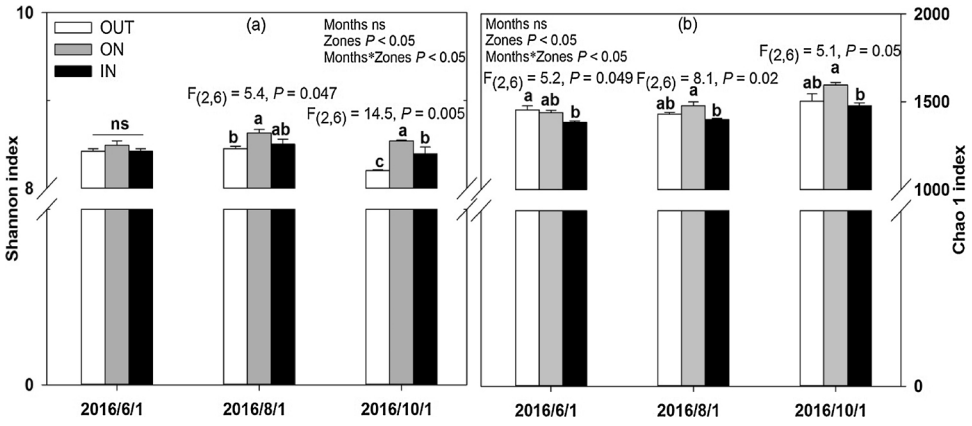

Fig. 3. Shannon diversity (a) and Chao 1 richness (b) indices for soil bacterial among the three zones [outside (OUT) and within (IN) the ring, and adjacent to fruiting bodies (ON)] for June, August, and October 2016. The values were calculated based on the observed number of operational taxonomic units. Means (± SE, n = 3) with different lowercase letters are significantly different based on LSD test ($P < 0.05$); ns: no significant difference between treatments.

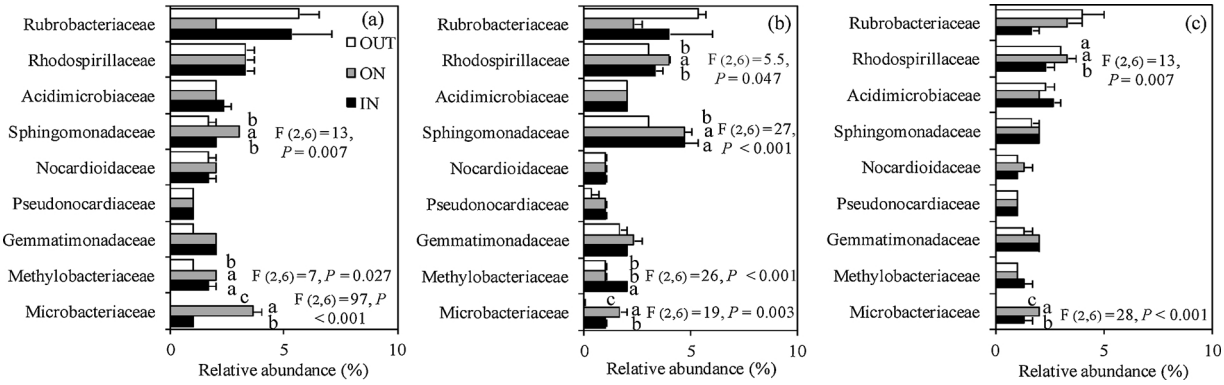

Fig. 5. Soil bacterial community bar plot (means ± SE, n = 3) showing the relative read abundances of different bacterial families within different zones in June (a), August (b), and October (c), 2016. Means (± SE, n = 3) with different lowercase letters are significantly different based on LSD test ($P < 0.05$).

Rhodospirillaceae ($F_{2,6} = 5.5$, $P = 0.047$), *Sphingomonadaceae* ($F_{2,6} = 27$, $P < 0.001$) and *Methylobacteriaceae* ($F_{2,6} = 26$, $P < 0.001$) were significantly higher in the ON zone than in the OUT zone in August. In particular, the abundance of *Microbacteriaceae* was significantly higher in the ON zone than in the OUT zone for June ($F_{2,6} = 97$, $P < 0.001$), August ($F_{2,6} = 19$, $P = 0.003$) and October ($F_{2,6} = 28$, $P < 0.001$; Fig. 5).

3.3. The relationships between soil physiochemical properties and bacterial communities in different FR zones

RDA biplots were used to assess how the physicochemical properties influenced bacterial community structures and seven major environmental factors (including SMC, pH, SOC, TN, TP, ION and AP) (Fig. 6). The first axis (horizontal) of the bacterial communities versus the environmental factors explained 76.3%, 80.6%, and 67.2% of the total variability in June (Fig. 6a, b), August (Fig. 6c, d) and October 2016 (Fig. 6e, f), respectively. According to the Monte Carlo permutation test, soil available P had the strongest correlation with the OTU bacterial composition ($F = 7.20$, $P = 0.002$ for June; $F = 4.71$, $P = 0.002$ for August; $F = 3.32$, $P = 0.002$ for October), followed by soil pH ($F = 2.89$, $P = 0.002$ for June; $F = 7.18$, $P = 0.002$ for August; $F = 2.17$, $P = 0.234$ for October) (Table A2). The results of correlation analysis between the seven soil properties and nine selected family level of bacteria are shown in Table 2. Soil available P was significantly positively correlated with *Microbacteriaceae*, *Nocardioidaceae*, *Methylobacteriaceae*, *Rhodospirillaceae* and *Sphingomonadaceae* ($P < 0.05$), and soil pH was significantly positively correlated with *Sphingomonadaceae* and *Gemmatimonadaceae* ($P < 0.05$).

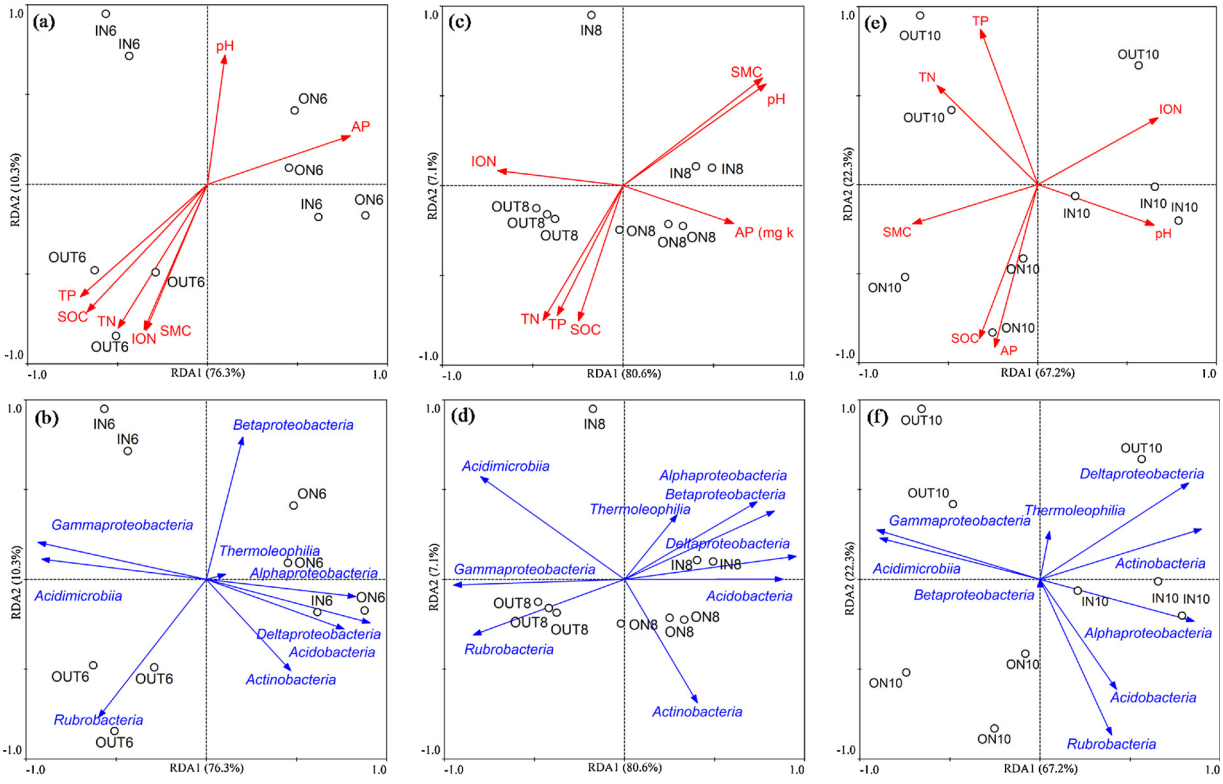

Fig. 6. Ordination plots from the redundancy analysis (RDA) results, which were used to explore the relationships between bacterial abundance and the selected soil properties for the OUT, ON and IN zones in June (a, b), August (c, d), and October (e, f), 2016. SWC, soil moisture content; pH, soil acidity; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; ION, soil inorganic nitrogen and AP, soil available phosphorus. The Monte Carlo permutation test of the RDA is shown in Table A1.

Table 2
Pearson's correlation analysis of relationship between seven soil properties and nine selected families of bacteria. Level of significance: * $P < 0.05$.

	Nocardioidaceae	*Methylobacteriaceae*	*Microbacteriaceae*	*Gemmatimonadaceae*	*Sphingomonadaceae*	*Rhodospirillaceae*	*Pseudonocardiaceae*	*Rubrobacteriaceae*	*Acidimicrobiaceae*
AP (mg kg^{-1})	0.55*	0.49*	0.67*	0.28	0.38*	0.43*	0.16	−0.16	−0.29
pH	−0.37	0.21	−0.08	0.44*	0.84*	0.31	−0.22	0.01	−0.20
ION (mg kg^{-1})	0.49*	0.06	−0.10	−0.49*	−0.14	0.12	−0.03	0.30	−0.26
TN (g kg^{-1})	0.39*	−0.10	−0.12	−0.31	0.08	0.39*	−0.18	0.34	−0.36
TP (g kg^{-1})	0.48*	0.10	−0.10	−0.28	0.06	0.35	−0.07	0.37	−0.31
SOC (g kg^{-1})	0.32	−0.26	−0.18	−0.27	0.01	0.37	−0.12	0.30	−0.28
SMC (%)	0.10	−0.06	−0.19	−0.31	0.37	0.34	−0.11	0.23	−0.45*

SMC, soil moisture content; pH, soil acidity; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; ION, soil inorganic nitrogen; AP, soil available phosphorus.

4. Discussion

4.1. Effects of Agaricus fungi on plant biomass and soil physicochemical properties

The results showed that the passage of *Agaricus gennadii* through soil as a FR fungi in a temperate steppe of China had a clear effect on soil biotic and abiotic properties. Plant aboveground biomass was significantly higher in the ON than the OUT and IN zones, which was consistent with the results of other studies (Bonanomi et al., 2013, 2012; Caesar-TonThat et al., 2013; Edwards, 1984, 1988; Xu et al., 2011). We further observed that this was associated with an increase in available soil P. The contents of SOC, total N and total P decreased after fungal passage. Similar results have been reported for FRs produced by other fungi, such as *Agaricus arvensis* (Edwards, 1984), *Marasmius oreades* (Fisher, 1977) and *Agaricus campestris* (Bonanomi et al., 2012). Many basidiomycetes have high nutrient requirements during the production of their abundant sporophores. Edwards (1984) suggested that soil colonization by fungi enhances organic matter mineralization and the accumulation of N and P in the mycelium, which in turn decreases the soil's total nutrient pool. In addition, when the fungus completes its life cycle, N and P compounds released into the soil ammonium pool may be taken up by plants. A previous study found higher concentrations of soil inorganic N and soil available P in the ON zone (Caesar-TonThat et al., 2013), which are only partly inconsistent with our results. The results of the present study suggest that there are lower concentrations of soil inorganic N and higher concentrations of soil available P in the ON zone. One possible explanation for this soil response is that presence of the may stimulate the growth of plants. In the temperate steppes of China, leaf P was significantly lower than global averages (Han et al., 2005), indicating a lack of P in plants. In the present study, FR fungi released large amounts of available P into the soil, which promoted plant uptake of inorganic N from soil (Gusewell, 2004).

4.2. Relationships between soil physiochemical properties and bacterial communities in different FR zones

Many studies have suggested that the soil physicochemical properties of soil are related to microbial diversity and activity in soils of tropical grasslands (Devi et al., 2014; Lisboa et al., 2014; Waring et al., 2014). However, few studies have investigated the effects of FR fungi on microbial communities in the soil. One study recently showed that *Pseudomonas fluorescens* and *Stenotrophomonas maltophilia* isolates predominated in the ON zone, whereas *Bacillus* isolates predominated in the IN and OUT zones (Caesar-TonThat et al., 2013). In the present study, only the abundance of *Microbacteriaceae* was significantly higher in the ON zone than in the OUT zone, indicating that *Microbacteriaceae* species are associated with FRs of *Agaricus gennadii*. The family *Microbacteriaceae* is a member of the order *Actinomycetales*, and class *Actinobacteria*; this family can be associated with plants and fungi though the mycelium (Evtushenko and Takeuchi, 2006). Such a special vegetation–fungal environment can increase the abundance of *Microbacteriaceae*.

We speculated that FRs of *Agaricus gennadii* change the physicochemical properties of soil, especially soil pH and available P, and then cause changes the structure of the bacterial community in soil. It is well known that bacterial communities can be sensitive to dry–wet gradients (Fierer et al., 2003) and soil moisture content (Ahn and Peralta, 2009). However, in the present study, the soil moisture content was not strongly correlated with bacterial communities under natural conditions, indicating that soil bacteria can adapt to changing soil moisture content under natural conditions. Soil pH has been found to control or influence biogeochemical processes in soil, such as denitrification (Hunter and Faulkner, 2001), and some studies have revealed a significant relationship between bacterial community structure and soil

pH (Fierer and Jackson, 2006; Peralta et al., 2013). Similarly, we found that soil pH was positively correlated with the abundance of *Sphingomonadaceae* and *Gemmatimonadaceae*. Yuan et al. (2013) suggested that bacterial diversity and abundance showed increasing trends coupled with increasing SOC, soil total N, soil total P and soil available N in fertilized soils. In the present study, soil total N, soil total P and soil inorganic N were positively correlated with the abundance of *Nocardioidaceae*. Furthermore, soil available P was positively correlated with the abundance of *Microbacteriaceae*, *Nocardioidaceae*, *Methylobacteriaceae*, *Rhodospirillaceae* and *Sphingomonadaceae*.

5. Conclusions

The present study demonstrates that FRs caused by *Agaricus gennadii* constitute an example of the pronounced influence that a soil fungus can have on the physicochemical properties of soil and the structure of the bacterial community in grasslands. Soil pH and soil available P were higher in the ON and IN zones than in the OUT zone, and soil concentrations of total N, total P and inorganic N were lower in the ON and IN zones than in the OUT zone. This occurred because of changes in soil physicochemical properties that changed the structure of the soil bacterial community. At the family level, only the abundance of *Microbacteriaceae* groups were significantly higher in the ON zone than the OUT zone during each sampling period of the months of the present study. Moreover, our results suggest that soil pH and soil available P could provide better indicators of changes in soil bacteria communities than other properties. However, further studies are needed to fully examine why those two properties are most closely associated with changes in specific bacterial community structures.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.pedobi.2018.05.002.

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