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Shift in the rhizosphere microbial communities between growing- and low-temperature dormant seasons in a northern hardwood forest

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(Acknowledgments)

We thank Dr. Takeshi Taniguchi, Dr. Takahito Yoshioka, Dr. Kazuya Kobayashi, Dr. Shunsuke

Matsuoka, Dr. Yoriko Sugiyama, and the Forest Information Laboratory of Kyoto University members
for their valuable suggestions. We also thank Tomoyuki Nakagawa, Jun Yanagimoto, Ken-ichi Ohta,
Masaru Okuda, Akira Yamanaka, Yuta Miyagi, Syuichi Sato, and Yukie Kawamura as the staff of
Hokkaido Forest Research Station, Field Science Education and Research Center, Kyoto University
for helping the experiments. This study was supported by JSPS-KAKENHI (No. 20J12464, 20H03034,
20KK0134) and the Fund of Joint Research Program of Arid Land Research Center, Tottori University
(No. 02C2002).

Abstract

Background and Aims

Microbial communities within the soil surrounding plant roots (rhizosphere) are distinct from those in the non-rhizosphere soil because of root exudation and symbiosis with plant roots and play important roles in plant growth. However, seasonal variations in these rhizosphere microbial communities are not well known, especially during the plant's dormant season in cool temperate forests.

Methods

We investigated the rhizosphere microbial communities in a cool-temperate deciduous broad-leaved forest at the growing season (mid-summer) and at the beginning and end of the plant's dormant season (early winter and early spring) using metabarcoding of prokaryotic 16S rRNA genes and fungal ITS region, microbial functional prediction, and co-occurrence network analysis.

Results

The rhizosphere dominant prokaryotic subgroup (occupying $19.4 \pm 3.2\%$ of relative read counts) had non-significant seasonal fluctuations, and their relative read counts were correlated with physicochemical properties, including pH and water content. In contrast, the relative read count of ectomycorrhizal fungi was 1.4 times higher in the rhizosphere than that in the bulk soil in mid-summer. However, the relative read counts of ectomycorrhizal fungi within rhizospheres in winter and spring ($28.5 \pm 18.9\%$ and $20.8 \pm 13.8\%$, respectively) were similar to that in bulk soil ($25.8 \pm 18.7\%$ and $22.4 \pm 17.1\%$, respectively).

Conclusion

Ectomycorrhizal fungi reduced their occupancy within the rhizosphere during the plant's dormant seasons, whereas rhizosphere-dominant prokaryotic communities were stable during the seasonal change, implying the importance of prokaryotic rather than ectomycorrhizal fungal communities in the processes occurring within the rhizosphere during dormant seasons.

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58 Keywords

59 Rhizosphere soil, Dormant season, Ectomycorrhizal fungi, Prokaryotic community, Rhizosphere

60 effect

Introduction

Microbial communities, such as fungi and bacteria, within the soil area that are strongly affected by the fine roots of plants (rhizosphere; Kuzyakov and Razavi 2019) are distinct from those in the non-rhizosphere soil. Carbon supply from plants to the rhizosphere microbial communities and symbiotic relationships with fungi, including ectomycorrhizal fungi, partly explain the rhizosphere specific microbial community. In the rhizosphere, nutrient cycling processes, such as decomposition and mineralization, are accelerated by these rhizosphere microbial communities (Rhizosphere effect: Henneron et al. 2020; Phillips and Fahey 2006), which increases nutrient availability to the plants (Dijkstra et al. 2009; Moreau et al. 2019). Furthermore, mycorrhizal fungi, which have symbiotic relationships with more than 90% of the terrestrial plants (Brundrett and Tedersoo 2018) and generally have a higher relative abundance within the rhizosphere (Baldrian 2009; Kuzyakov and Razavi 2019), also play a significant role in nutrient acquisition by host plants (Liese et al. 2018; Paterson et al. 2016; Qin et al. 2020). In addition to providing nutrients, rhizosphere microbes play a significant role in plant stress tolerance and defense against pathogens (Bais et al. 2006; Berendsen et al. 2012; De Zelicourt et al. 2013; Nwokolo et al. 2021). Therefore, rhizosphere microbial communities play pivotal roles in plant growth and health.

The microbial communities in the rhizosphere are strongly affected by carbon supply from plant roots (Philippot et al. 2013; Zhalnina et al. 2018). Furthermore, soil physicochemical properties, such as pH, carbon and nutrient availability, and water conditions, also affect soil microbial communities (Nakayama et al. 2019; Rousk et al. 2009, 2010; Wan et al. 2015). For example, Rousk et al. (2010) demonstrated that the relative abundance of Proteobacteria increased with pH, whereas certain groups of Acidobacteriota decreased their occupancy. Distinct physicochemical properties in the rhizosphere, such as lower pH and higher carbon and nitrogen contents compared to bulk soil

(Nakayama and Tateno 2022), are also key factors shaping rhizosphere microbial communities (Fierer et al. 2007; Philippot et al. 2013).

The ecology in the winter season has been the focus of recent studies due to global warming and climate change (Groffman et al. 2011; Makoto et al. 2014). In contrast to the traditional view that winter is a dormant season, studies have revealed that microbial communities remain active in winter, even at subzero temperatures (Clein and Schimel 1995; Isobe et al. 2018). Furthermore, trees, including deciduous trees, take up nutrients from the soil during the dormant season after leaf abscission (Ueda et al. 2010; Zavišić and Polle 2018), and these nutrients are partly used for spring growth (Ueda et al. 2015). Therefore, nutrient cycling processes and microbial activities, especially within the rhizosphere, during winter are possibly important for plant growth in northern hardwood forest ecosystems. Indeed, acceleration of the nutrient cycle by the rhizosphere effect has been observed even during the plant dormant season (Nakayama and Tateno 2022; Yuan et al. 2020). However, although root exudates, the drivers of the accelerated nutrient cycle in forest ecosystems (Meier et al. 2017; Wang et al. 2021), are secreted throughout the year, including in winter (Nakayama and Tateno 2018; Phillips et al. 2008), the quantities of root exudates from deciduous trees in winter are lower than those in summer because of limited photosynthetic activities (Nakayama and Tateno 2018). Therefore, although similar rhizosphere effects have been observed (Nakayama and Tateno 2022), the microbial mechanisms may be completely different between the growing and dormant seasons. It is also possible that even during the dormant season, rhizosphere microbes and their relationships with rhizosphere effects could differ between the start (i.e., early winter) and end (i.e., early spring) of the dormant season. However, there is a large knowledge gap regarding the rhizosphere microbial communities during the dormant season.

In a previous study, we investigated the seasonal rhizosphere effects of Japanese oak and dwarf bamboo on enzymatic activity and microbial gene abundance, including during the dormant

season. We found that the enzymatic activities of the nitrogen and phosphorus cycles were higher in the rhizosphere than in the bulk soil, even during the dormant season (Nakayama and Tateno 2022). Furthermore, the total fungal gene abundance in the rhizosphere was higher than that in the bulk soil, regardless of the season, whereas the total bacterial and archaeal gene abundances were similar between the rhizosphere and bulk soil (Nakayama and Tateno 2022). However, the differences in microbial communities between the rhizosphere and bulk soil have not been the focus of previous studies. Adding microbial community data will improve our understanding of the roles of microbes in rhizosphere processes and plant nutrient acquisition.

Therefore, this study aimed to reveal the shift in microbial communities between the growing and dormant seasons in a northern hardwood forest in northeast Japan, focusing on the tree rhizosphere. For this purpose, we investigated the microbial communities in the rhizosphere of the Japanese oak, an ectomycorrhizal-symbiotic tree that dominates the natural broad-leaved forest of northeast Japan, and in the bulk soil in early winter (after leaf fall and before snow cover), early spring (after snowmelt and before leaf flush), and mid-summer. We defined early winter, early spring, and mid-summer as the start and end of the dormant season and the middle of the growing season, respectively (Nakayama and Tateno 2022). In addition to microbial community structure and function analyses, we constructed a microbial co-occurrence network to assess the detailed microbial dynamics (Nakayama et al. 2021). We also identified the microbial communities that possibly regulate enzymatic activity based on correlational analysis using modules in the co-occurrence network. In this study, we hypothesized that (1) fungal and prokaryotic communities differ largely between rhizospheres and bulk soils, regardless of season, because positive rhizosphere effects on nutrient cycles have been reported throughout the year (Nakayama and Tateno 2022; Yuan et al. 2020). However, because mycorrhizal fungi and some rhizosphere prokaryotes are affected by the carbon supply from their symbiotic host tree (Kaiser et al. 2010; Zhelnina et al. 2018; Žifčáková et al. 2017), we also

133 hypothesized that (2) the microbial community structures in the rhizosphere vary between growing
134 season and low-temperature dormant season, and even between the start and end of the dormant season.

Materials and Methods

Study site

This study was conducted in a deciduous broadleaved forest in eastern Hokkaido, Japan (43°24.2' N, 144°38.5' E), managed by the Shibecha Branch of the Hokkaido Forest Research Station, Field Science Education and Research Center, Kyoto University. The mean annual air temperature and precipitation at a nearby meteorological station (43°19' N, 144°37' E) were 6.3 °C and 1,176 mm (1991–2020), respectively. At this site, the daily average temperature is below 0 °C from December to February, and leaf abscission in Japanese oak (*Quercus crispula* Blume) occurs from September to November (Nakayama and Tateno 2018, 2021). Snowfall occurs from December to mid-April (Watanabe et al. 2019), and the maximum snow depth is approximately 70 cm (Hosokawa et al. 2017). The soil is usually frozen throughout the winter (from late November to late April; Watanabe et al. 2019), and the soil temperatures at 0 and 5 cm depth were approximately 0 °C under snow cover (Isobe et al. 2018; Watanabe et al. 2019). The soils at this site are classified as Andosols (IUSS Working Group WRB 2015). Detailed information on the study site has been previously described (Christopher et al. 2008; Hosokawa et al. 2017; Nakayama et al. 2019).

Soil sampling and physicochemical characteristics and enzymatic activities of the soil

Soil samples were collected from four plots (15 m × 15 m) established in our previous study (Nakayama and Tateno 2018). The plots consisted of pure and naturally regenerated stands of *Q. crispula*. The forest floor had a dense cover of dwarf bamboo (*Sasa nipponica* (Makino) Makino & Shibata). More detailed information on the study plots are provided in previous reports (Nakayama

and Tateno 2018, 2021, 2022).

Soil sampling was conducted on four days during each sampling season: November 21, 23, 27, and 29, 2019 (early winter); April 29 and May 1, 3, and 5, 2020 (early spring); and August 23, 25, 28, and 30, 2020 (mid-summer). On each sampling date, a soil block was collected from a depth of 0–10 cm (A horizon) at a random point in each plot using a shovel after manual removal of the organic horizon. There were 7–10 individuals of *Q. crispula* in each plot (Nakayama and Tateno 2021), and each sampling point was near (1–2 m apart) the base of *Q. crispula*. A total of 16 soil blocks were collected during each sampling season. In the laboratory, the soil samples were sieved through a 4 mm mesh sieve, and the fine roots on the sieve were carefully picked using forceps and hand (nitril gloves were used). The tools were changed for each sample to avoid cross-contamination. We separated the fine roots of *Q. crispula* and *S. nipponica* based on their morphological traits, such as mycorrhizal type and color. The soil that passed through the sieve was considered as bulk soil, and the soil attached to the fine roots of *Q. crispula* on the sieve was considered as rhizosphere soil. The soil samples for DNA extraction and physicochemical measurement were stored at –20 °C and 4 °C, respectively, until further processing. More details on soil sampling and sample treatment are available in a previous study (Nakayama and Tateno 2022). The physicochemical properties and enzymatic activities of the soil samples were measured and reported previously (Nakayama and Tateno 2022) and are summarized in Tables 1 and 2.

DNA extraction and sequencing

Soil DNA was extracted from 0.25 g of each soil sample using a DNA extraction kit (DNeasy PowerSoil Pro Kits; QIAGEN, Hilden, Germany) following the manufacturer's protocol. The extracted DNA was stored at –20 °C until further analysis. Microbial gene abundances were measured

and reported previously (Nakayama and Tateno 2022) and are summarized in Table 2.

An Ion Personal Genome MachineTM with the Ion 318TM Chip V2 (Thermo Fisher Scientific, Waltham, MA, USA) was used to sequence the extracted DNA. Details of the gene sequencing and data processing procedures are provided in the Supplementary Methods section of Online Resource 1. The raw sequence results were deposited in the DDBJ Sequence Read Archive (DRA) under the accession number DRA016089.

The fungal trophic modes (e.g., saprotrophs and symbiotrophs) and guilds (e.g., ectomycorrhizae) were predicted using FUNGuild (Nguyen et al. 2016) based on the fungal taxonomic data of the ITS sequences. The genus *Sebacina* has been classified as a saprotroph-symbiotroph by FUNGuild. However, we classified the operational taxonomic unit (OTU) of the genus as ectomycorrhizal fungi based on a previous report from the study site (Matsuoka et al. 2020).

Statistical analysis

We used a two-way permutational analysis of variance (PERMANOVA) to test for differences in microbial community structure among soil positions (rhizosphere or bulk soil) and sampling seasons (early winter, early spring, and mid-summer) using adonis function in R package “vegan” (ver. 2.6-4; Oksanen et al. 2022). After categorizing by the sampling season, we conducted a one-way PERMANOVA to detect differences in the microbial community among the soil positions in each season. Permutational analysis of multivariate dispersions (PERMDISP) using betadisper function in R package “vegan” (ver. 2.6-4; Oksanen et al. 2022) was used to test the dispersion of microbial community dissimilarity among the soil positions and sampling seasons. Two-way analysis of variance (ANOVA), followed by multiple comparisons using Tukey’s adjusted *p*-value, was used to test for differences in the relative read counts of microbial phyla and classes, fungal trophic modes, and guilds

among soil positions and sampling seasons using the default R function (ver. 4.1.3; R Core Team 2022) and R package “emmeans” (ver. 1.8.4-1; Lenth 2023).

A microbial co-occurrence network was constructed using rarefied prokaryotic and fungal sequencing data (Online Resource 1) using R package “igraph” (Csárdi and Nepusz 2006). Details of the microbial co-occurrence network construction and module-level correlation analysis have been provided in previous studies (Nakayama et al. 2019, 2021). Briefly, after the reduction of very rare OTUs (observed in less than 1/3 samples), Spearman’s rank correlations were calculated for all pairs of OTUs, and p -values were adjusted based on the false discovery rate (FDR). We identified the pair of OTUs as having a significant positive co-occurrence relationship if the Spearman’s rank correlation coefficient of $\rho > 0.6$ and FDR q -value < 0.001 (Barberán et al. 2012; Chao et al. 2016; Nakayama et al. 2019). OTUs with at least one significant co-occurrence relationship with other OTUs were shown as nodes in the network, and co-occurrence relationships were represented as edges. The modules in the microbial co-occurrence network were detected based on the fast greedy modularity optimization algorithm (Clauset et al. 2004). The network was visualized using Gephi ver. 0.9.5 (Bastian et al. 2009).

The relationships between the modules and factors (chemical properties, enzymatic activities, sampling season, and rhizosphere or bulk soil) were assessed using correlation analysis (Deng et al. 2012; Nakayama et al. 2021). The relative read count matrices of the modules were standardized to a mean of 0 and variance of 1, and the module eigenvectors were calculated using singular value decomposition (Langfelder and Horvath 2007). The signs of the eigenvectors were fixed by assigning a positive correlation to the average relative read count across the OTUs within the modules (Langfelder and Horvath 2007). Pearson’s correlation between the module eigenvalues and factors was calculated as the correlation between the modules and factors. Categorical factors (soil positions and sampling seasons) were transformed into 0 or 1 dummy vectors before calculating correlation coefficients. We also conducted the same correlation analysis between the factors and

modules using the estimated absolute abundance of the modules. The analysis based on relative read counts would help to understand the relative preferences of module members for the environments and their relative importance in enzymatic activities. In contrast, the analysis based on the estimated absolute abundance may improve knowledge about the absolute preferences and the relationships between enzymatic activities and modules. The absolute abundance of each module was calculated by multiplying each relative read count by the total prokaryotic 16S rRNA (bacterial 16S rRNA + archaeal 16S rRNA) and fungal ITS gene abundances reported by Nakayama and Tateno (2022) and summarized in Table 2.

All statistical analyses were performed using the R software (ver. 4.1.3; R Core Team 2022).

Results

Variations in microbial community structures and functions among seasons and soil positions

The total number of prokaryotic and fungal OTUs was 600 (165 ± 11 for each sample) and 2028 (130 ± 28 OTUs for each sample), respectively. The rarefaction curve is shown in Fig. S1 in Online Resource 2.

Prokaryotic and fungal communities varied significantly among the sampling seasons (Table S1 in Online Resource 3). Although the prokaryotic community differed according to soil position (bulk soil vs. rhizosphere; Table S1 in Online Resource 3), the difference in the microbial community was not significant in early winter (Fig. 1). There were no clear differences in the entire fungal community among the soil positions (Fig. 1d, e, f and Table S1 in Online Resource 3). The dispersion of similarity in the prokaryotic and fungal communities did not vary significantly among the soil positions or sampling seasons (Table S1 in Online Resource 3). According to one-way PERMANOVA, no clear seasonal trends were observed in the prokaryotic and fungal communities of the bulk soil and rhizosphere (Fig. S2 in Online Resource 2).

Within the prokaryotic community, Proteobacteria and Acidobacteriota were the dominant phyla occupying $43.5 \pm 3.7\%$ and $25.8 \pm 4.2\%$ (mean $\pm$ standard deviation) of the read counts, respectively, and Alphaproteobacteria and Acidobacteriae were the dominant classes occupying $39.7 \pm 3.7\%$ and $25.7 \pm 4.2\%$ of the read counts, respectively (Fig. 2). However, the relative abundance of these phyla and classes did not vary significantly among soil positions (Fig. 2a, b, and Table S2 in Online Resource 3). On the other hand, the relative read counts of the phyla Actinobacteria and Bacteroidota were significantly higher in the rhizosphere than in the bulk soil (Fig. 2a and Table S2 in Online Resource3), while the trend was the opposite for Firmicutes (Fig. 2a and Table S2 in Online

Resource 3). For the fungal community, Basidiomycota was the dominant phylum occupying $41.1 \pm 16.4\%$ of total fungal reads (Fig. 2c). The relative read count of Basidiomycota was significantly higher in mid-summer than in early winter and spring (Fig. 2c and Table S3 in Online Resource3), particularly in the rhizosphere.

Among the identified fungal trophic modes, the fungal communities were dominated by symbiotrophs, occupying $29.9 \pm 19.0\%$ of the total read counts (Fig. S3 in Online Resource 2). Most symbiotrophic fungi were classified as ectomycorrhizal fungi (64.2–100% of symbiotrophic fungi). Two-way ANOVA showed that the relative read counts of ectomycorrhizal fungi varied significantly among seasons ($F = 8.19$, $p < 0.001$), whereas the effects of soil position and the interaction between the two factors were not significant with respect to the fungal guilds ($F = 1.57$, $p = 0.213$ and $F = 1.34$, $p = 0.267$, respectively). However, multiple comparisons indicated that the relative read count of ectomycorrhizal fungi was significantly higher in the rhizosphere than in bulk soil only in mid-summer ($p = 0.048$; Fig. 3).

Microbial co-occurrence network

The microbial co-occurrence network comprised 107 prokaryotic nodes (OTUs), 42 fungal nodes, and 301 edges (positive co-occurring interactions). Though the network comprised only 17.8% of prokaryotic OTUs and 2.1% of fungal OTUs observed in this study, the total relative read counts of microbes in the network were $74.5 \pm 2.2\%$ and $34.0 \pm 13.0\%$ of prokaryotic and fungal reads, respectively (Fig. S4 in Online Resource 2). Thus, the network represented the core microbial community, at least for prokaryotes. The microbial co-occurrence network was divided into 18 modules (Fig. 4a). Modules 1, 2, 3, 4, 5, 8, 9, 12, and 15 had higher relative read counts (on average, $> 1\%$; Fig. S4), and further analyses focused on the dominant modules. The detailed taxonomic

classifications of the dominant module members are shown in Fig. S5 in Online Resource 2 and Table S4 in Online Resource 3.

Among dominant modules, module 1 had significantly higher relative read counts in the rhizosphere ($19.4 \pm 3.2\%$ for prokaryotes and $2.2 \pm 1.5\%$ for fungi) than in the bulk soil ($17.2 \pm 3.2\%$ for prokaryotes and $1.5 \pm 1.3\%$ for fungi), while module 3 had the opposite trend (Fig. 5a). There were no clear seasonal trends in the relative read counts of modules 1 and 3 (Fig. 5a), and regardless of the season, module 1 had higher relative read counts in the rhizosphere than in the bulk soil (Table S5 in Online Resource 3). The pH, water content, and C:N ratio showed significant positive and negative relationships with the modules (Fig. 5b). In particular, pH, which was significantly lower in the rhizosphere than in the bulk soil regardless of the season (Table 1), was strongly and negatively correlated with module 1 (Fig. 5b; $r = -0.714$, $p < 0.001$).

The estimated absolute abundance of module 1 was higher in the rhizosphere than in the bulk soil, regardless of the season (Fig. 6a), which was similar to the relative read counts of the modules. However, in contrast to the results of the relative read count, module 8, which mainly consisted of fungal OTUs (Table S4 in Online Resource 3), had a higher estimated absolute abundance in the rhizosphere than in the bulk soil, and its abundance shifted seasonally (Fig. 6a) according to the total fungal ITS gene fluctuations (Table 2; Nakayama and Tateno 2022). Similar to the relative read count, the pH and C:N ratio were significantly and strongly correlated with the estimated absolute abundance of each module (Fig. 6b).

Regarding the interactions between microbial subgroups and extracellular enzymatic activities, modules with higher abundances in the rhizosphere than in the bulk soil (modules 1 and 8) were significantly and positively correlated with extracellular enzymatic activities (Fig. 7). Furthermore, modules 8, 9, and 12, which showed seasonal trends in the estimated absolute abundances (Fig. 6b), had significantly positive relationships with enzymatic activities (Fig. 7).

Discussion

Recent studies have highlighted the activities of tree species, microbes, and rhizosphere processes during dormant seasons (Isobe et al. 2018; Nakayama and Tateno 2022; Ueda et al. 2010, 2015; Yuan et al. 2020). However, shifts in rhizosphere microbial communities between the growing and dormant seasons and within the dormant seasons remain unclear. According to previous studies, climate change may alter plant phenology (Linderholm 2006). Such shifts can strongly affect plant growth; for instance, perennial plants utilize nutrients taken up during the dormant season for their spring growth (Ueda et al. 2010). Therefore, revealing the microbial assemblies and nutrient cycling processes within the rhizosphere during the winter season should improve our understanding of the plant-soil interactions and the possible consequences of climatic changes on northern hardwood forests.

Shift in the fungal community in the bulk soil and rhizosphere between growing and dormant seasons

The present study demonstrated that the relative read counts of ectomycorrhizal fungal communities within the rhizosphere of *Q. crispula* vary significantly between seasons (Fig. 3). In contrast to the fungal communities, the rhizospheric prokaryotic communities were relatively stable during these seasons (Figs. 2a, b, and 5). However, the carbon supply from symbiotic host trees to the rhizosphere may change between the dormant and growing seasons because a major fraction of root exudates is produced by photosynthetic activities (Epron et al. 2012; Kuzyakov and Cheng 2001; Nakayama and Tateno 2018) and the rate of photosynthesis decreases during the dormant season (Gamon et al. 2016), especially in deciduous trees, including *Q. crispula*. Thus, our results suggest that climate change, which lengthens the growing season (Linderholm 2006), may have a significant influence on the fungal community, rather than on the prokaryotic community within the rhizosphere.

In a previous study, we reported that fungal abundance was higher in the rhizosphere than in the bulk soil, regardless of the season, possibly because of a higher ectomycorrhizal fungal abundance, irrespective of the season or seasonal shifts in the rhizosphere fungal community (Nakayama and Tateno 2022). Generally, ectomycorrhizal fungi are dominant in the rhizosphere fungal community (Marupakula et al. 2021; Toju and Sato 2018); however, most studies have focused on the fungal community during the plant-growing season. Our results showed that ectomycorrhizal fungi were more abundant in the rhizosphere than in the bulk soil only in summer (Fig. 3), partly confirming hypothesis (2). However, the entire fungal community did not differ significantly between soil positions (Fig. 1), in contrast to the prediction based on hypothesis (1). Other studies revealed similar seasonal patterns in bulk soil samples (Nakayama et al. 2019; Žifčáková et al. 2016, 2017). Because mycorrhizal fungi largely rely on the direct carbon supply from their host tree (Treseder et al. 2006), this seasonal shift could be explained by the decrease in carbon supply from the host tree to mycorrhiza during winter (Birgander and Olsson 2021; Lekberg et al. 2013). Some ectomycorrhizal fungi have saprotrophic potential (Baldrian 2009; Lindahl and Tunlid 2015; Plett and Martin 2011) and can live without a direct carbon supply from their host. However, their ability to produce extracellular enzymes that acquire carbon is much weaker than that of saprotrophic fungi (Baldrian 2009). Therefore, the seasonality of carbon flux from the host tree might strongly regulate seasonal fluctuations in ectomycorrhizal abundance, and the effect would be stronger in the rhizosphere than in bulk soil, as observed in this study. In contrast, the relative read count of module 8, which mainly consisted of fungal OTUs (Table S4 in Online Resource 3), was significantly lower in summer than in winter and spring (Fig. 5), showing opposite trends to those of the ectomycorrhizal fungi. Based on the FUNGuild classification, Fung._OTU_170 and Fung._OTU_33 (families Apiosporaceae and Trichosporonaceae) were classified as saprotrophs and Fung._OTU_12 and Fung._OTU_40 (both from the family Mortierellaceae) were classified as saprotroph-symbiotrophs. Thus, our results suggest

that the saprotrophic fungal subcommunity could be active during the plant's dormant season in the rhizosphere instead of the reduced ectomycorrhizal fungi, similar to the Gadgil effect of litter decomposition (Gadgil and Gadgil 1971).

Shift in the prokaryotic community in the bulk soil and rhizosphere between growing and dormant seasons

In contrast to the fungal community, the prokaryotic community structures within the rhizosphere differed from those within the bulk soil, even during the dormant season (Figs. 1 and 2), supporting hypothesis (1). Furthermore, module 1, which comprised the rhizosphere core bacterial subgroup occupying approximately one-fifth of the rhizosphere prokaryotic community, showed no clear seasonal variations (Fig. 5a and Fig. S4 in Online Resource 2). Unlike the direct carbon supply from host trees to ectomycorrhizal fungi, rhizosphere prokaryotic communities utilize root exudates as plant-derived carbon sources. Although a few studies have reported that trees secrete root exudates even in winter (Nakayama and Tateno 2018; Phillips et al. 2008), the amount could be lower in the dormant season than in the growing season because a major fraction of root exudates are photosynthetically created carbon compounds. However, the core prokaryotic communities (observed as members of module 1) would not be strongly affected by the reduced carbon supply from tree species during the dormant season. On the other hand, the physicochemical properties of soils, especially the pH (Rousk et al. 2009, 2010), can affect the resident bacterial community (Nakayama et al. 2019; Wan et al. 2015). Indeed, the rhizosphere-dominant core microbial subgroup was significantly correlated with pH and soil moisture content in this study (Fig. 5b). In general, the pH value is lower in the rhizosphere than in the bulk soil, partly because of the balance of anion/cation uptake with H^+ and OH^- release by plants (Kuzyakov and Razavi 2019) and the secretion of organic

acids and root exudates from plant roots (Hinsinger et al. 2003). Our previous research at this study site showed that pH trends were maintained regardless of the season (Table 1; Nakayama and Tateno 2022), although the mechanisms of maintaining lower pH in the rhizosphere than in the bulk soil during winter should be addressed in future research. Therefore, the core prokaryotic community in the rhizosphere of *Q. crispula* is mainly regulated by soil properties, which do not significantly change seasonally, but rather by seasonally fluctuating factors, such as temperature and tree phenology.

In this study, the dominant rhizosphere microbial subgroup (module 1) mainly consisted of OTUs belonging to the phyla Proteobacteria and Acidobacteriota, and module 3, with lower abundance in the rhizosphere than in the bulk soil, primarily consisted of OTUs belonging to the phylum Acidobacteriota (Fig. 4). However, the relative read counts of Proteobacteria and Acidobacteriota did not differ significantly between the soil positions (Fig. 2). Therefore, the members of Proteobacteria in module 1 possibly have a copiotrophic ecology and prefer rhizospheric conditions (Fierer et al. 2007, 2012; Peiffer et al. 2013), whereas other Proteobacteria are possibly more abundant in bulk soil. Similarly, although the phylum Acidobacteriota is considered oligotrophic (Fierer et al. 2007), its members have diverse metabolism and ecology. For example, some Acidobacteriota act as plant growth-promoting bacteria (Kielak et al. 2016) and are abundant in the rhizosphere (Da Rocha et al. 2013; Lee et al. 2008). Thus, each microbial OTU has a specific preference for the environments and substrates (Emmett et al. 2020; Zhelnina et al. 2018) and these preferences are important for shaping the rhizosphere-specific microbial community. This was also supported by the phylogenetic diversity of each module, which preferred specific rhizosphere conditions (Fig. 4 and Fig. S5 in Online Resource 2). Although further research targeting the functions of these rhizosphere-specific microbes is needed, analyzing microbial communities regardless of taxonomic classification, as undertaken in this study using modules in co-occurrence networks, will improve our understanding of rhizosphere microbial community assemblies and their functions in the rhizosphere.

Relationships between microbial communities and rhizosphere enzymatic activities

Previous reports have shown that the effect of the rhizosphere on nutrient cycling is positive regardless of the season, even during dormant seasons (Nakayama and Tateno 2022; Yuan et al. 2020). As discussed above, this microbial sub-group could play an important roles in rhizosphere nutrient cycling because the rhizosphere-dominant core prokaryotic community was stable during the shift between the growing and dormant seasons. Our results showed that module 1 had a significant, strong, and positive relationship with most of the observed enzymatic activities (Fig. 6). Similarly, the fungal subcommunity with the highest estimated abundance in the rhizosphere (module 8) was positively correlated with the enzymatic activity. Especially, β -xylosidase activity was significantly and positively correlated only with the relative and estimated absolute abundances of module 8 (Fig. 6), partly because module 8 members were classified as saprotrophs. Although ectomycorrhizal fungi play an important role in plant nutrient acquisition (Talbot and Treseder 2010), our results suggest that both prokaryotic and non-mycorrhizal fungal communities regulate rhizosphere enzymatic activities.

Limitations of this study and future directions

This study revealed a shift in the microbial communities within the rhizosphere of *Q. crispula* between the growing and dormant seasons. However, this study had some limitations. Firstly, the co-occurrence network focused only on core microbial species rather than on rare species (Barberán et al. 2012). Although the co-occurrence network covered $74.5 \pm 2.2\%$ of prokaryotic and $34.0 \pm 13.0\%$ of fungal reads observed in this study (Fig. S4 in Online Resource 2), there is still the possibility that the rare microbial communities play essential roles in the rhizosphere. Detailed research focusing on these rare

species, in addition to the core species, would help understand the rhizosphere microbial community. Secondly, this study investigated only one deciduous tree species, *Q. crispula*. Studies have shown that the carbon supply from trees to the rhizosphere microbial community varies among tree species (Liese et al. 2018; Smith 1976; Sun et al. 2017, 2021). The differences in seasonal changes in root exudates among tree species should be addressed in future research because there are limited studies on winter root exudates from trees. Thirdly, this study was conducted over a period of one year. Therefore, further studies are required to confirm our findings regarding seasonal shifts in the microbial community within the rhizosphere. Furthermore, although root exudate rates could be low during dormant seasons because a major fraction of root exudates is produced by photosynthetic activities (Kuzyakov and Cheng 2001; Epron et al. 2012; Nakayama and Tatenos 2018), there is still a lack of knowledge regarding the seasonal carbon supply from symbiotic host trees to the rhizosphere. Although future studies are needed to improve our understanding of the interactions between microbial species and trees within the rhizosphere, our results highlight the importance of investigating seasonal differences in these relationships.

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Statements and Declarations

Funding

This study was supported by JSPS-KAKENHI (No. 20J12464, 20H03034, 20KK0134) and the Fund of Joint Research Program of Arid Land Research Center, Tottori University (No. 02C2002).

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

All authors contributed to the study's conception and design. Field sampling, analysis, and writing of the first draft of the manuscript were performed by Masataka Nakayama. All authors reviewed and commented on previous versions of the manuscript and approved the final manuscript.

Data Availability

The microbial sequence data are available in the DDBJ DRA (No. DRA016089). Other datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

674 **Tables**

675 Table 1 Basic physicochemical traits of the samples. These data were obtained from Nakayama and Tateno (2022).

	pH	Water content (%)	Carbon content (%)	Nitrogen content (%)	C/N ratio	Extractable nitrogen (mg N kg ⁻¹ dry soil)		
						Organic form	NO ₃ ⁻ -N	NH ₄ ⁺ -N
Winter								
Bulk soil	5.2 ± 0.3 ^a	50.6 ± 5.5 ^b	11.0 ± 2.2 ^b	0.8 ± 0.2 ^b	14.5 ± 0.6	67.8 ± 17.9 ^b	1.0 ± 2.3	10.0 ± 8.7 ^b
Rhizosphere	4.7 ± 0.2 ^b	56.4 ± 3.4 ^a	14.2 ± 1.5 ^a	1.0 ± 0.1 ^a	14.8 ± 0.7	130.4 ± 42.6 ^a	1.6 ± 1.6	18.7 ± 7.7 ^a
Spring								
Bulk soil	5.1 ± 0.2 ^a	54.0 ± 4.2 ^b	11.7 ± 1.4 ^b	0.8 ± 0.1 ^b	14.7 ± 0.9	68.6 ± 20.1 ^b	2.0 ± 2.8	13.8 ± 11.8 ^b
Rhizosphere	4.7 ± 0.2 ^b	57.5 ± 3.7 ^a	14.3 ± 1.2 ^a	1.0 ± 0.1 ^a	14.8 ± 0.9	114.8 ± 19.4 ^a	2.8 ± 3.5	25.7 ± 14.8 ^a
Summer								
Bulk soil	5.0 ± 0.2 ^a	48.2 ± 3.3 ^b	10.2 ± 1.9 ^b	0.7 ± 0.1 ^b	14.6 ± 0.6	63.5 ± 12.8	0.2 ± 0.5	5.8 ± 4.7 ^b
Rhizosphere	4.5 ± 0.2 ^b	54.3 ± 4.0 ^a	14.0 ± 2.0 ^a	1.0 ± 0.2 ^a	14.6 ± 0.7	77.9 ± 30.0	0.4 ± 0.5	17.6 ± 8.9 ^a

676 Note: Values are mean ± standard deviations. Letters (a, b) represent significant differences between bulk and rhizosphere soils within each season.

Table 2 Microbial gene abundances and extracellular enzymatic potential activities of the samples. These data were obtained from Nakayama and Tateno (2022).

	Microbial gene abundances (g ⁻¹ dry soil)			Extracellular enzymatic potential activities (nmol g ⁻¹ dry soil h ⁻¹)					
	Bacterial 16S (×10 ¹⁰ copy)	Archaeal 16S (×10 ⁹ copy)	Fungal ITS (×10 ⁹ copy)	<i>β</i> -xylosidase	<i>β</i> -glucosidase	Acid phosphatase	NAGase	Phenol oxidase	Peroxidase
Winter									
Bulk soil	5.0 ± 1.1	1.9 ± 0.4	0.8 ± 0.2 ^b	42 ± 38	79 ± 39	718 ± 168 ^b	78 ± 45 ^b	121 ± 160	1747 ± 529
Rhizosphere	5.2 ± 1.2	1.8 ± 0.4	1.1 ± 0.6 ^a	50 ± 47	113 ± 86	1217 ± 309 ^a	251 ± 98 ^a	159 ± 151	1677 ± 301
Spring									
Bulk soil	5.1 ± 1.5	2.0 ± 0.4	1.1 ± 0.6 ^b	49 ± 13	115 ± 36 ^b	783 ± 181 ^b	120 ± 89 ^b	164 ± 97 ^b	2064 ± 611
Rhizosphere	5.6 ± 1.5	2.0 ± 0.4	1.5 ± 0.9 ^a	53 ± 16	154 ± 56 ^a	1319 ± 401 ^a	259 ± 159 ^a	291 ± 151 ^a	2147 ± 865
Summer									
Bulk soil	4.5 ± 0.8	2.0 ± 0.3	2.2 ± 0.6 ^b	39 ± 14	107 ± 45	952 ± 185 ^b	184 ± 229 ^b	80 ± 70 ^b	1516 ± 350
Rhizosphere	4.4 ± 0.8	1.8 ± 0.3	2.9 ± 0.9 ^a	41 ± 21	122 ± 89	1721 ± 436 ^a	477 ± 517 ^a	231 ± 177 ^a	1463 ± 365

Note: Values are mean ± standard deviation. Letters (a, b) represent significant differences between bulk and rhizosphere soils in each season. NAGase represents *β*-1,4-N-acetylglucosaminidase.

Figure captions

Fig. 1 (a, b, c) Prokaryote and (d, e, f) fungal community structures between rhizosphere soil and bulk soil in (a, d) early winter, (b, e) early spring, and (c, f) mid-summer. The results of the one-way permutational analysis of variance for the difference between soil positions are provided at the bottom of each figure.

Fig. 2 Relative read counts of (a, b) prokaryote and (c, d) fungi in the rhizosphere and bulk soil at the (a, c) phylum level and (b, d) class level. The values shown are means ($n = 16$). BS and TR represent bulk soil and tree rhizosphere, respectively.

Fig. 3 Relative read counts of ectomycorrhizal fungi. The bars and error bars shown are means ($n = 16$) and standard deviations, respectively. BS and TR represent bulk soil and tree rhizosphere, respectively. Significance analysis was performed between bulk soil and tree rhizosphere by multiple comparison, n.s.: $p \geq 0.05$, *: $p < 0.05$.

Fig. 4 (a) The fungal-prokaryotic positive co-occurrence network and (b) the taxa of the operational taxonomic units (OTUs) belonging to each module. Modules that contain less than 1% of relative read counts on average among samples are classified as other modules.

Fig. 5 Relationships between relative read counts of each module and (a) sampling seasons and soil positions and (b) physicochemical properties. The colors represent Pearson's correlation coefficient. White boxes represent non-significant relationships ($p \geq 0.05$). BS, TR, C:N ratio, and EON represent bulk soil, tree rhizosphere, carbon/nitrogen ratio, and extractable organic nitrogen, respectively. Symbols in each box represent the following: *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Fig. 6 Relationships between estimated absolute abundances of each module and (a) sampling seasons and soil positions and (b) physicochemical properties. The colors represent Pearson's correlation coefficient. White boxes represent non-significant relationships ($p \geq 0.05$). BS, TR, C:N ratio, and EON represent bulk soil, tree rhizosphere, carbon/nitrogen ratio, and extractable organic nitrogen, respectively. Symbols in each box represent the following: *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Fig. 7 Relationships between extracellular enzymatic potential activities and (a) relative read count and (b) estimated absolute abundances of each module. The colors represent Pearson's correlation coefficient. White boxes represent non-significant relationships ($p \geq 0.05$). XY, GL, AP, NAG, PO, and P represent β -xylosidase, β -glucosidase, acid phosphatase, β -1,4-N-acetylglucosaminidase, phenol oxidase, and peroxidase, respectively. Symbols in each box represent the following: *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Fig.1

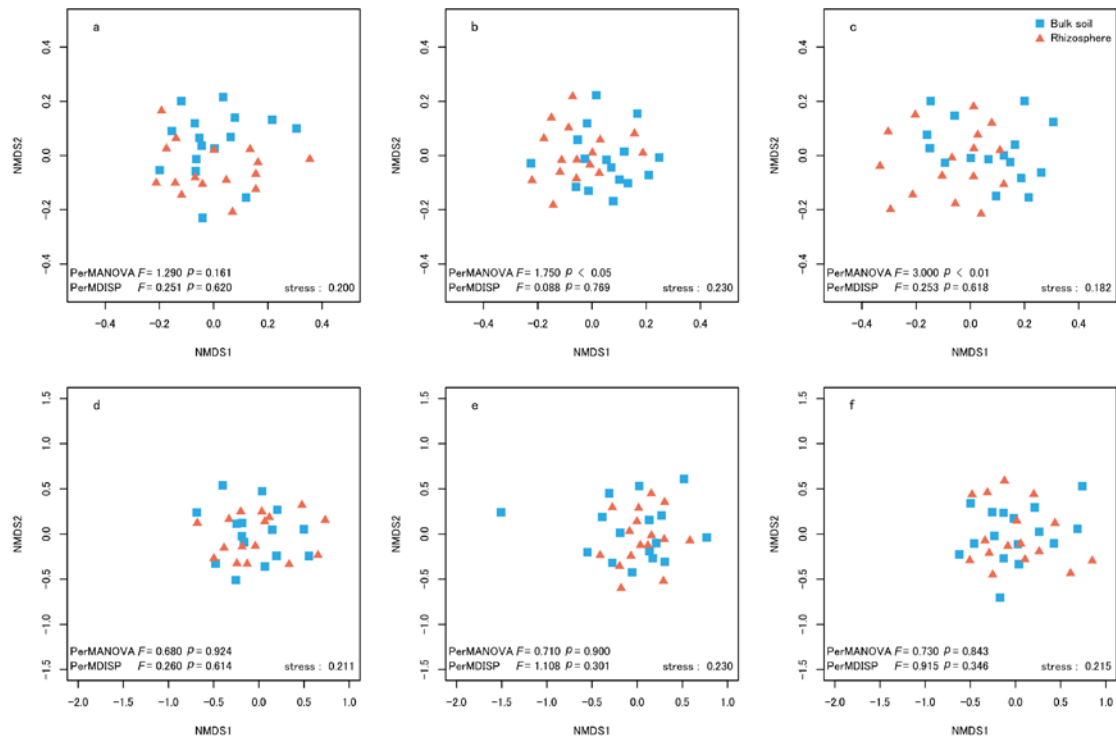


Fig. 2

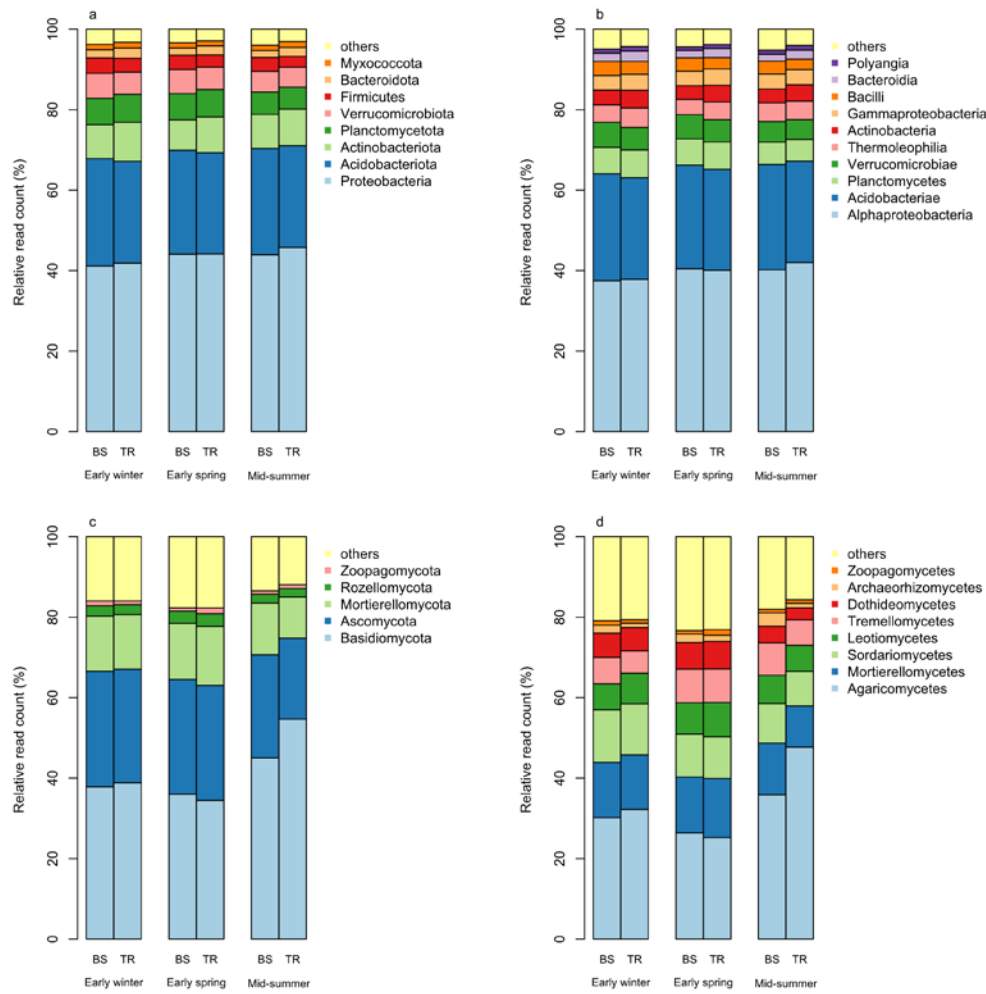


Fig. 3

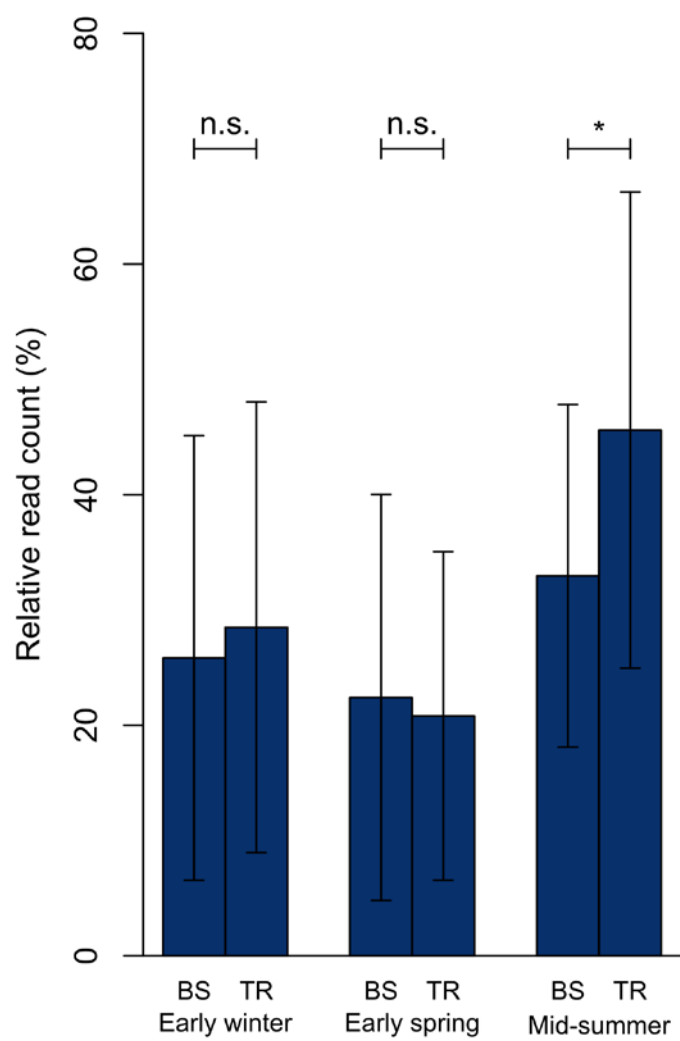


Fig. 4

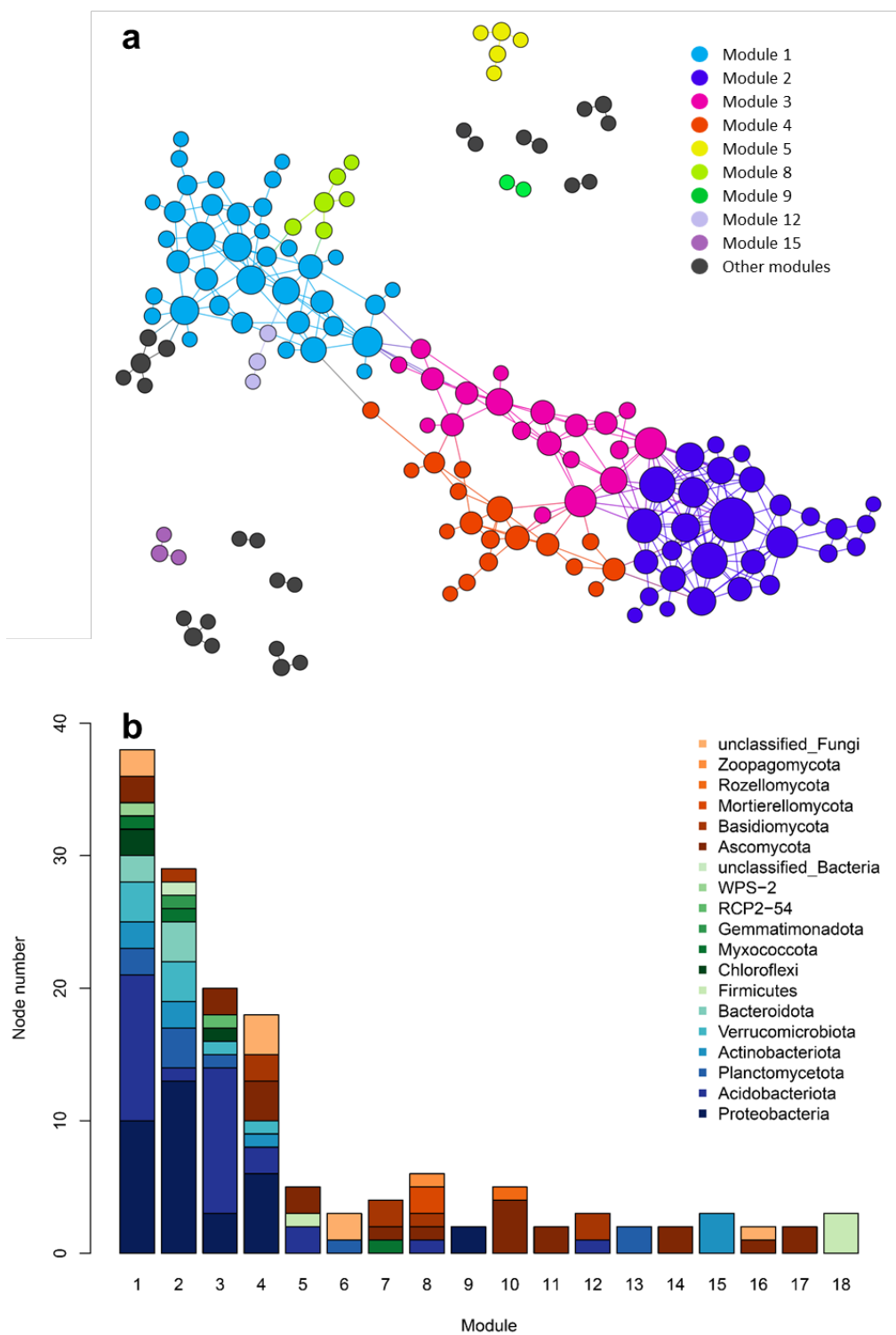


Fig. 5

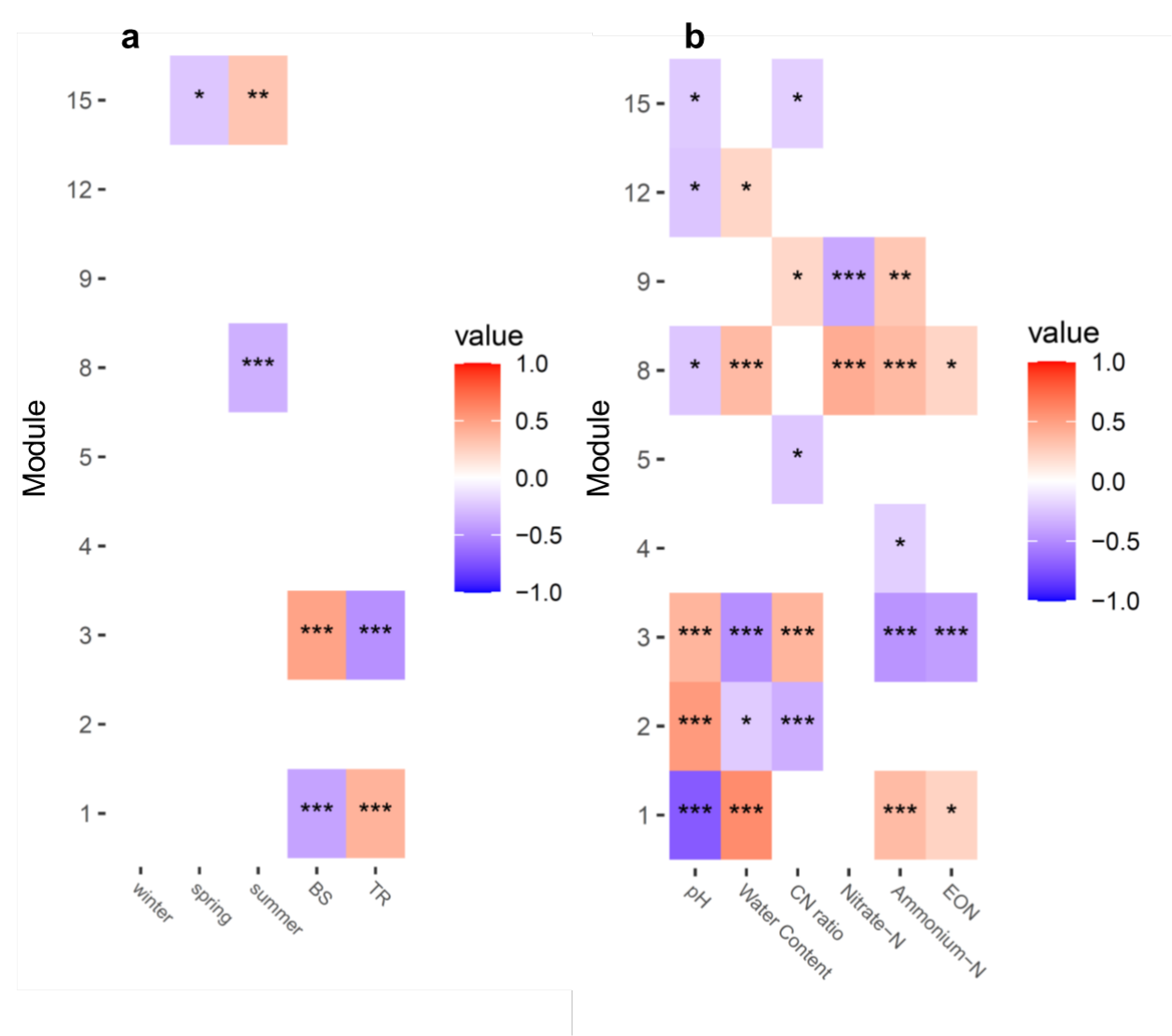


Fig. 6

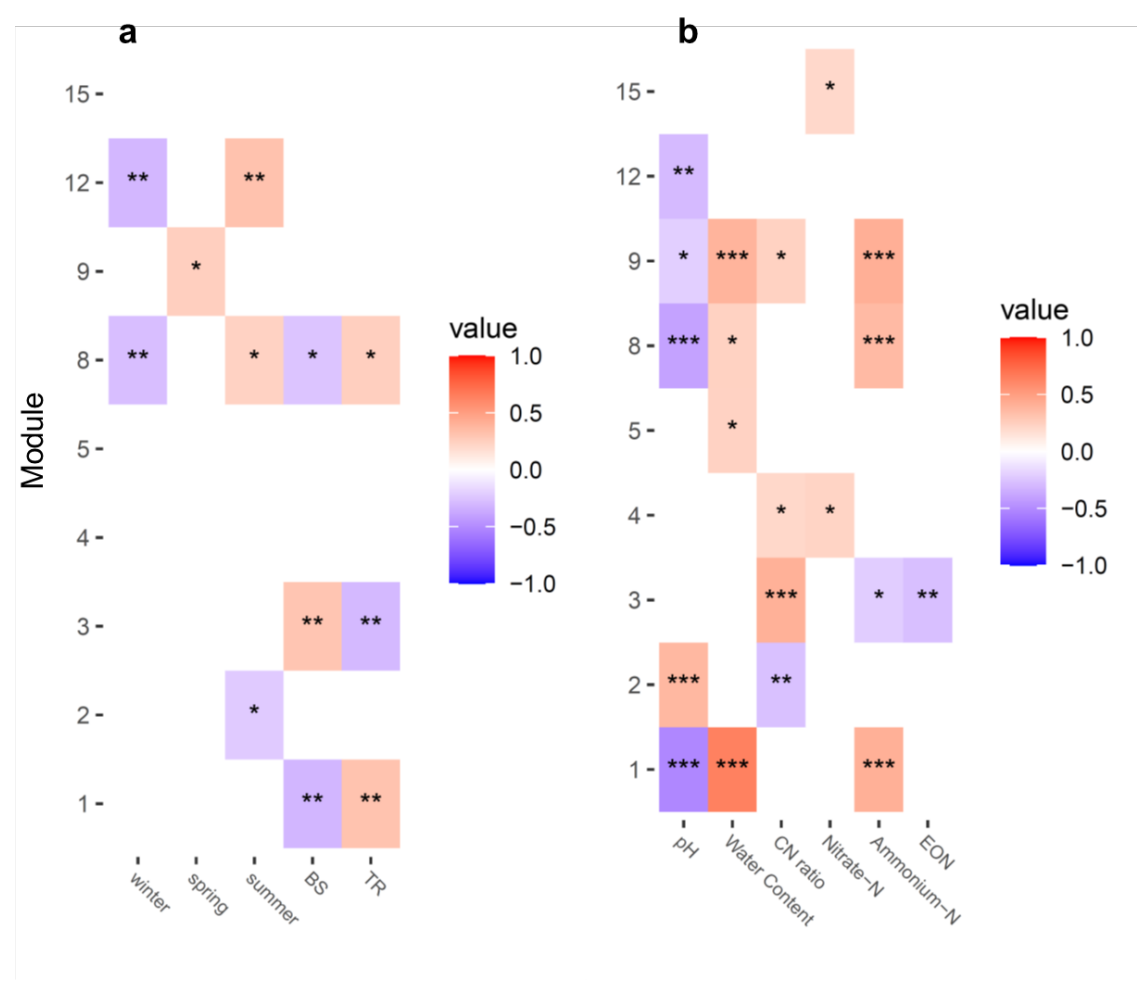
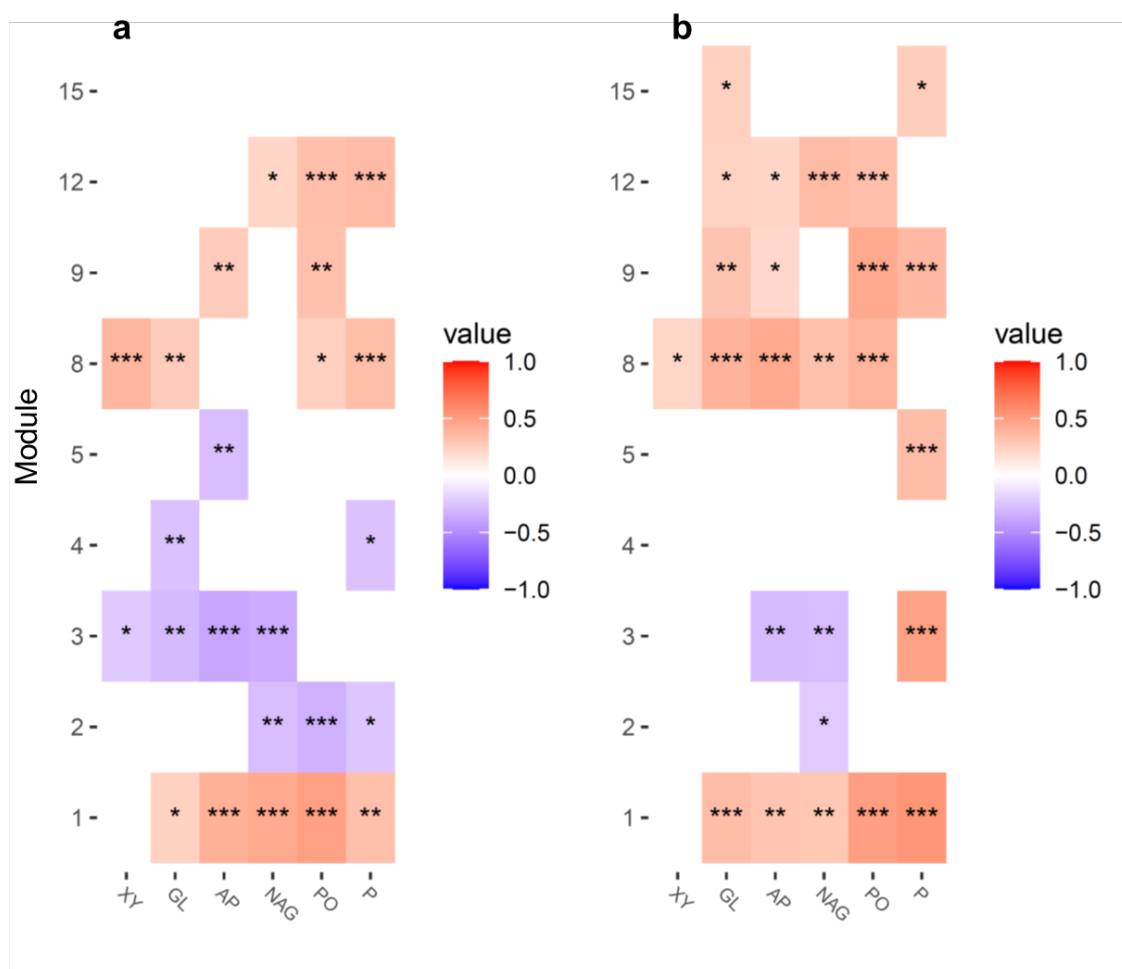


Fig. 7



DNA sequencing

Primer sets U519f (Suzuki and Giovannoni 1996) / U785r (Wang and Qian 2009) and ITS1F_KYO2 / ITS2_KYO2 (Toju et al. 2012) were used to amplify the prokaryotic 16S rRNA genes and fungal internal transcribed spacer (ITS) regions, respectively. We used custom primers with sequence indexes (forward primers only) and sequencing adapter regions. For PCR reaction, 2 µL of 100-fold-diluted samples are mixed with 22 µL of Platinum PCR Super Mix High Fidelity (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL each of 10 mM forward and reverse primers (final volume: 25 µL). The PCR process was performed using T100 Thermal Cycler (BIO-RAD Laboratories, CA, USA) under the following conditions: 95 °C for 3 min of initial denaturation, and 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 68 °C for 1 min. Duplicate PCR was performed for each sample. After confirmation by agarose gel electrophoresis, the duplicate PCR products were combined into a single solution. Following the manufacturer's protocol, the PCR products were purified using AMPure XP (Beckman Coulter Inc., CA, USA). The DNA concentrations of the purified PCR products were determined using the Qubit ds DNA HS Assay Kit (Thermo Fisher Scientific) and mixed into one tube to equalize the DNA quantity in each sample. We checked the size and quality of the DNA in the mixture using an Agilent High Sensitivity DNA Kit and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and diluted the mixed PCR products to approximately 10 pM. Elusion PCR using 25 µL of the diluted library was conducted using Ion PGM OT2 400 Kit (Thermo Fisher Scientific) according to the manufacturer's procedure. The library was enriched and loaded onto an Ion 318™ Chip V2 instrument for the Ion Personal Genome Machine™ (Thermo Fisher Scientific). The machine-accompanied software eliminated low-quality and polyclonal sequences from the original output and exported FASTQ files.

Sequence data processing

Sequences shorter than 200 bp (16S rRNA genes) and 160 bp (fungal ITS region) and/or expected errors greater than 0.5 were discarded. For fungal ITS analysis, ITSx was used to extract fungal ITS sequences (Cole et al. 2005). For the dereplication of sequences, removal of singletons, and clustering of operational taxonomic units (OTUs) at 97% identity, USEARCH (Edgar

2010) was used. UCHIME (Edgar et al. 2011) was used to identify and remove sequencing chimeras. Taxonomic classification was performed using QIIME2 ver. 2021.11 (Bolyen et al. 2019), using SILVA 138 reference sequences (Quast et al. 2012; Robeson et al. 2021; Yilmaz et al. 2014) and UNITE reference sequences (Abarenkov et al. 2021) for the 16S rRNA genes and fungal ITS, respectively.

After quality filtering, 754,269 prokaryotic and 529,287 fungal reads were obtained. The average OTU numbers per sample were 1140.7 for prokaryotes and 446.1 for fungi. Before further processing, OTUs with reads accounting for < 0.1% of the total sample reads were removed because these OTUs could be sequencing/PCR noise (Peay et al. 2015; Toju et al. 2016), or contamination (Toju 2016). A total of 560,345 prokaryotic reads (average OTU number:165.0) and 484,466 fungal reads (average OTU number:131.1) remained after the filtering. Read numbers were equalized to 3767 and 1969 reads for prokaryotes and fungi, respectively, by random pick-up based on the minimum read number using the rarefy function in the vegan package in R (Oksanen et al. 2018).

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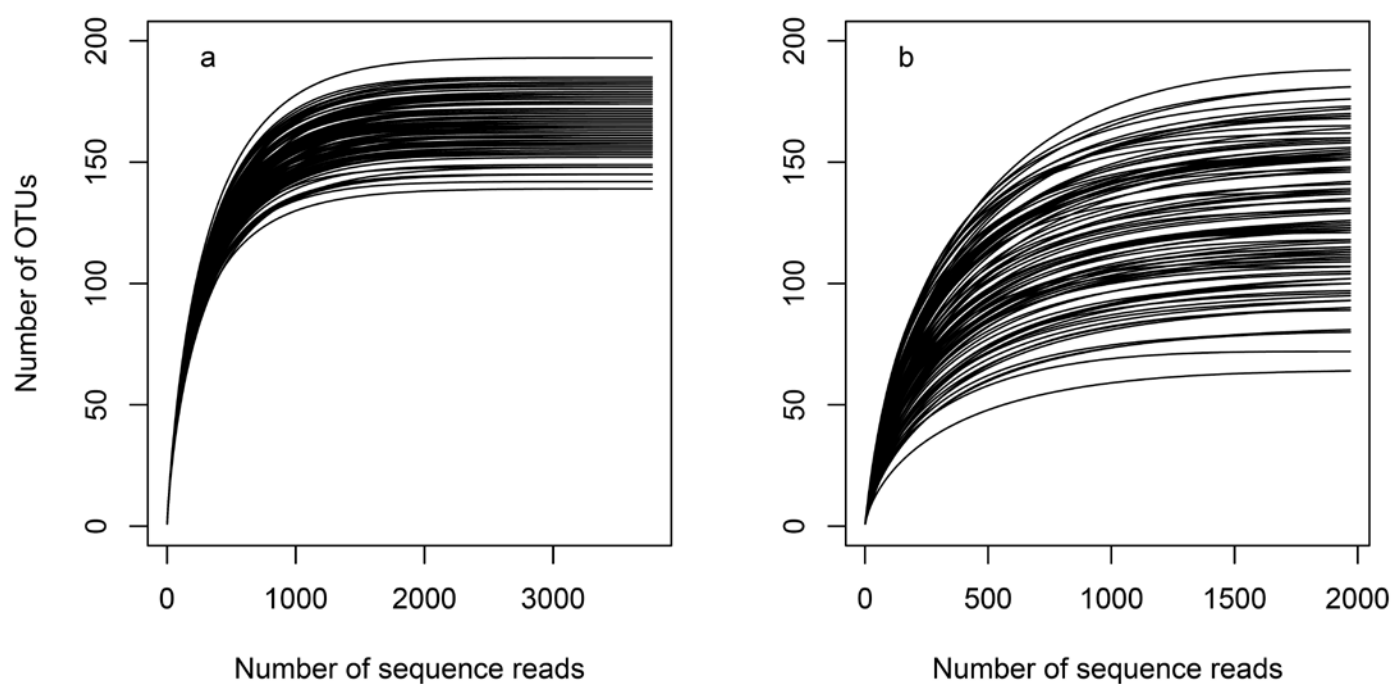


Fig. S1 Rarefaction curves of (a) prokaryotic and (b) fungal OTUs of each sample after rarefied to 3767 and 1969 reads for prokaryotes and fungi, respectively

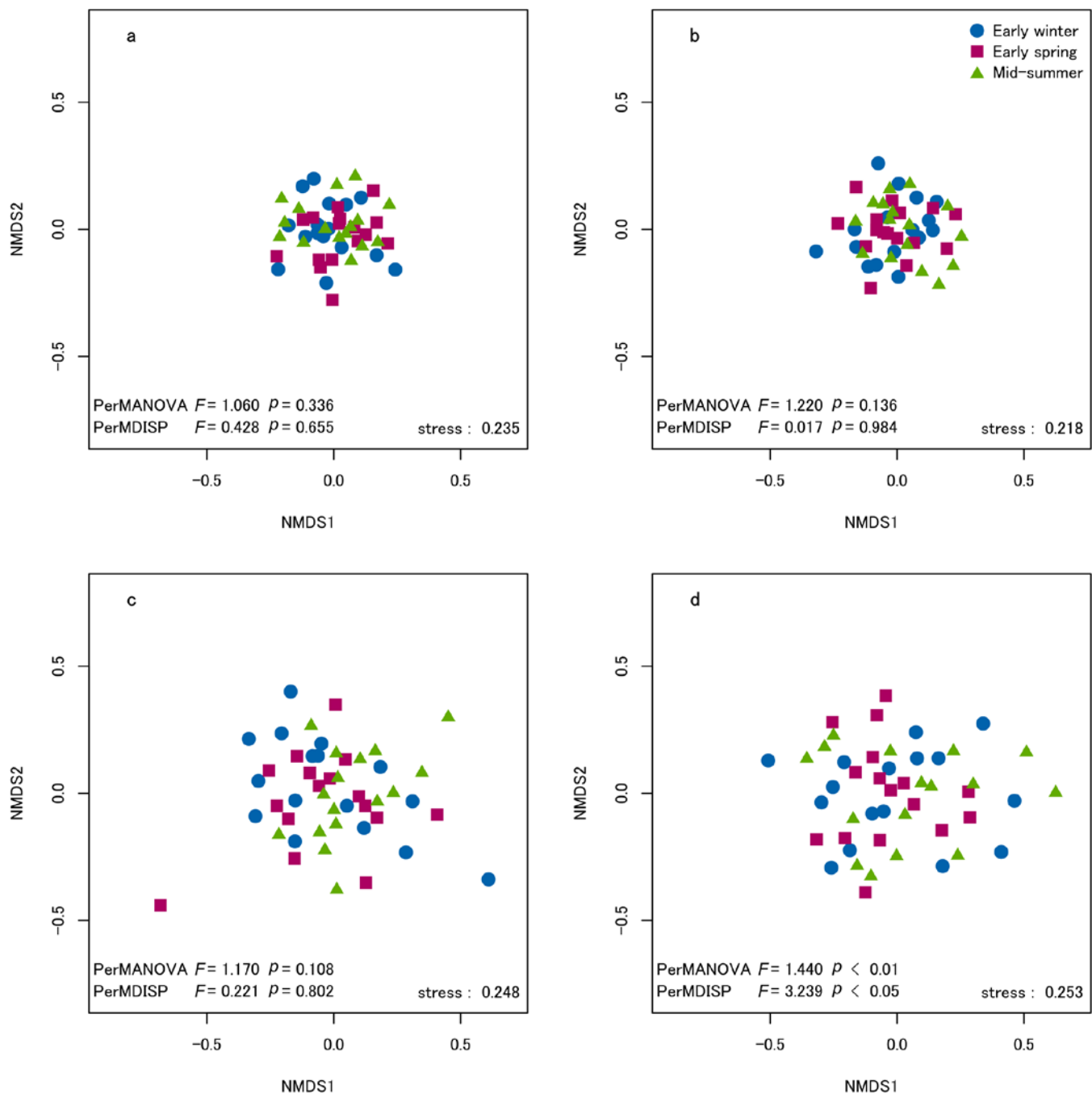


Fig. S2 (a, b) Prokaryote and (c, d) fungal community structures (a, c) in the bulk soil and (b, d) in the rhizosphere. The results of the one-way permutational analysis of variance for the difference among sampling seasons are provided at the bottom of each figure

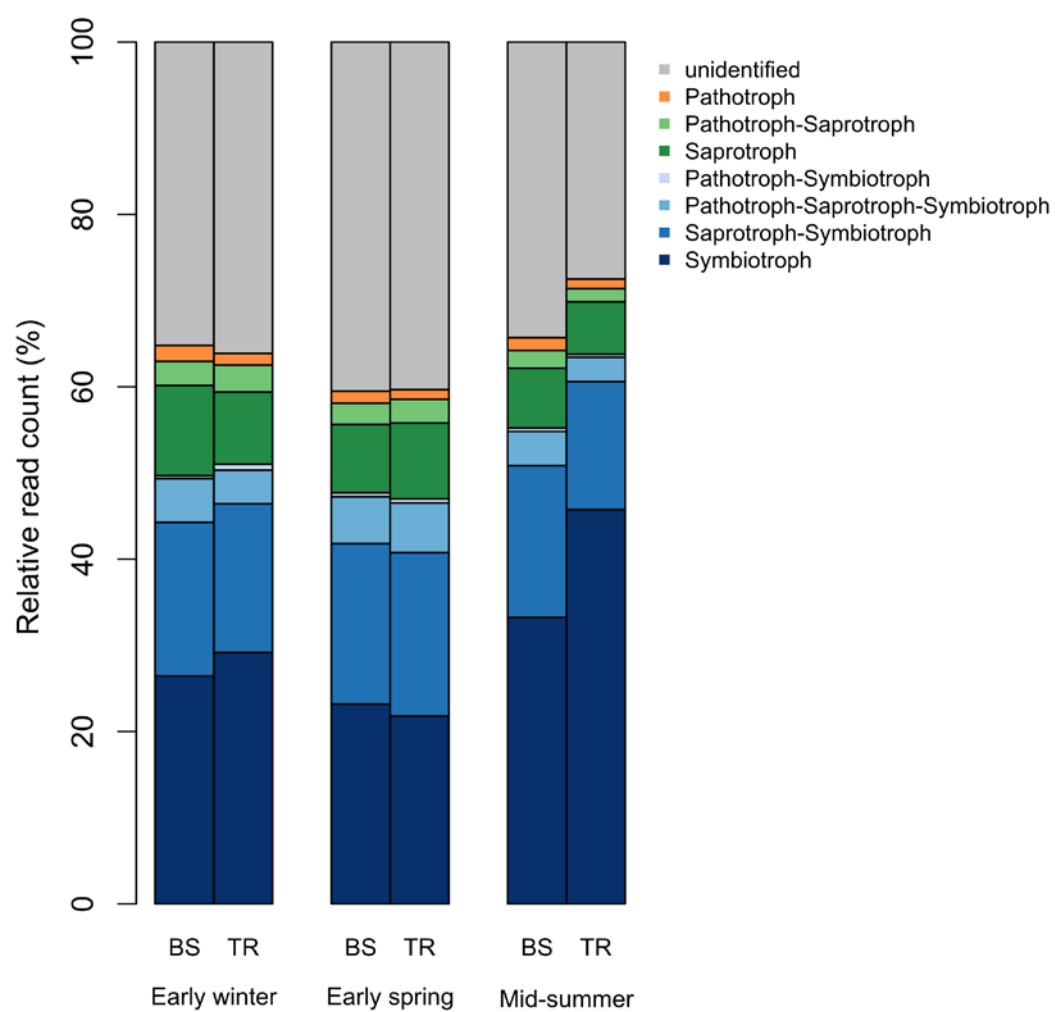


Fig. S3 Relative read counts of fungal trophic mode. The values shown are means ($n = 16$). BS and TR represent bulk soil and tree rhizosphere, respectively

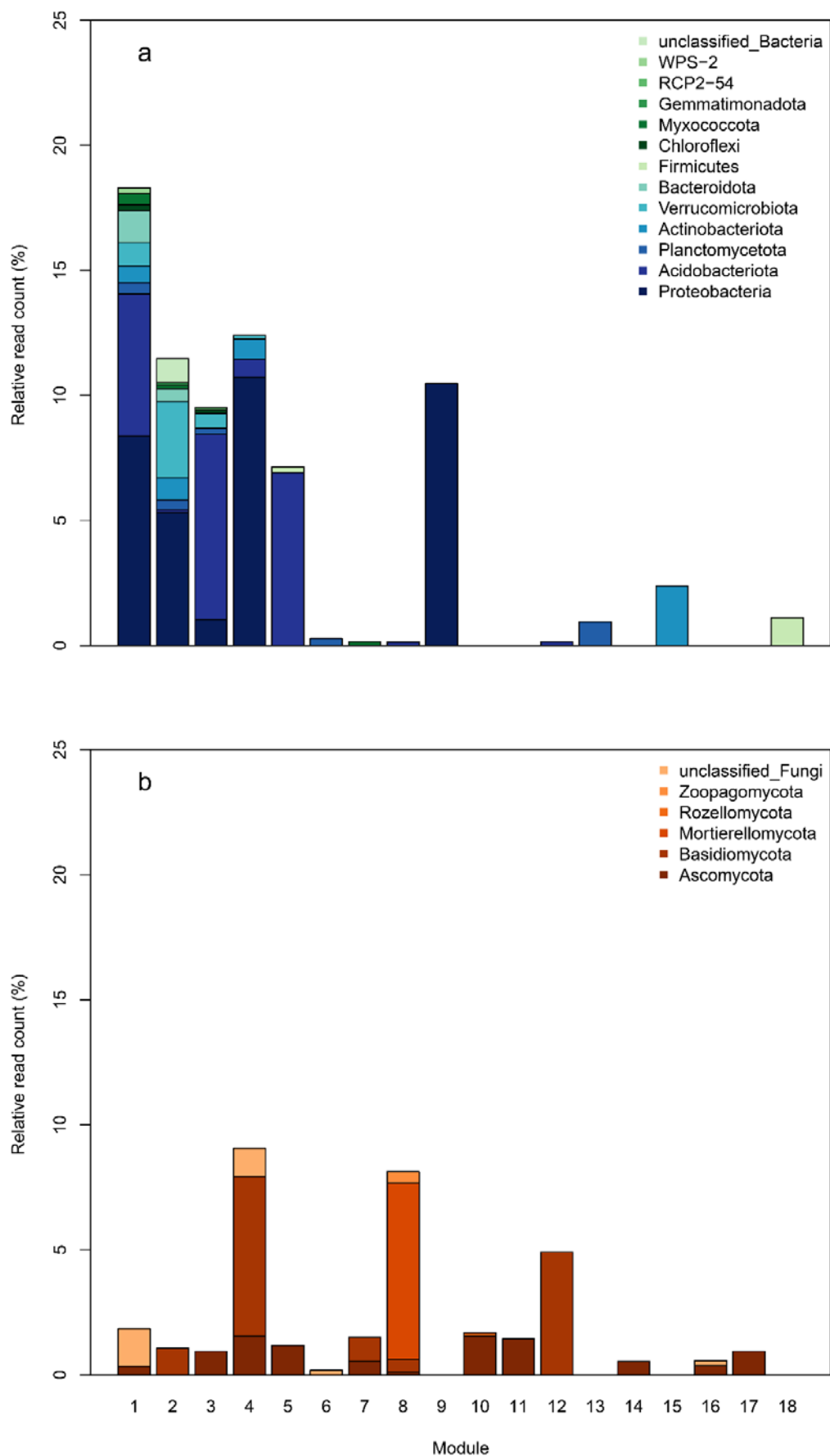


Fig. S4 The average relative read count of each module. The average relative abundances of modules were calculated by summing the average relative abundances of operational taxonomic units (OTUs) belonging to each module

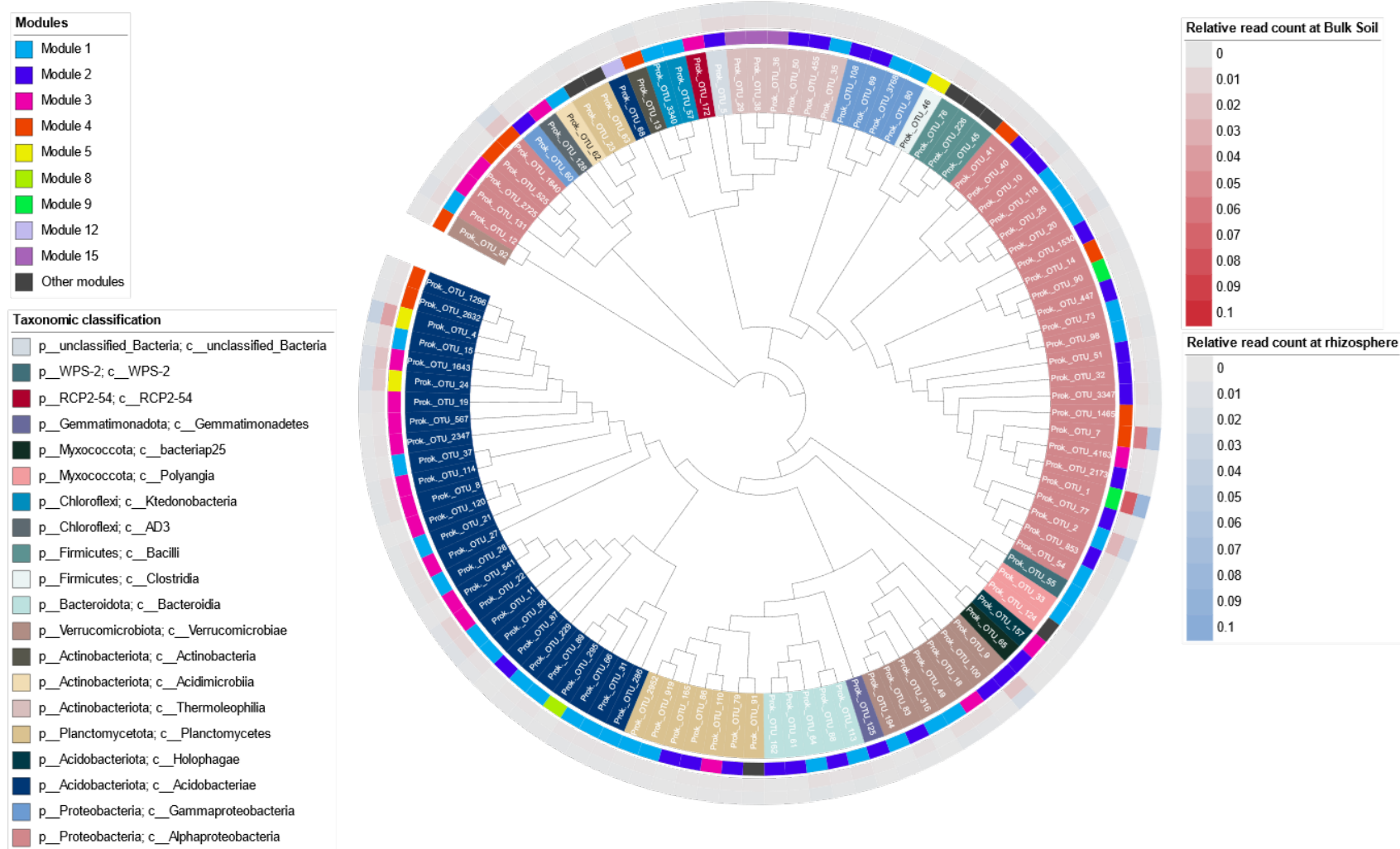


Fig. S5 Phylogenetic tree of prokaryotes appeared in the co-occurrence network in this study. The background color of the labels and the interior ring represent the class and module to which the OTUs belong, respectively. p__ and c__ in the taxonomic classification represent phylum and class, respectively. The middle and outer circles are each OTU's average relative read counts at bulk soil and rhizosphere. iTOL (v5; Letunic and Bork, 2021) was used to visualize the phylogenetic tree

Reference

Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 49, W293–W296. <https://doi.org/10.1093/NAR/GKAB301>

	Soil position		Season		Soil position $\times$ Season		Table
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	
PERMANOVA							S1
Prokaryotes	4.35	< 0.001	1.74	< 0.01	0.53	1.000	Results
Fungi	0.95	0.542	2.22	< 0.001	0.42	1.000	
PERMDISP							of two-way
Prokaryotes	0.001	0.976	0.01	0.994			
Fungi	0.06	0.802	1.86	0.159			

PERMANOVA and PERMDISP for prokaryotic and fungal community

Table S2 Results of two-way ANOVA for dominant prokaryotic phyla and classes

	Soil position		Season		Soil position $\times$ Season	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Phylum						
Proteobacteria	1.54	0.218	8.45	< 0.001	0.54	0.586
Acidobacteriota	1.53	0.219	0.10	0.901	0.06	0.944
Actinobacteriota	9.03	< 0.01	1.91	0.154	0.42	0.661
Planctomycetota	0.44	0.510	9.13	< 0.001	0.36	0.701
Verrucomicrobiota	1.44	0.234	2.37	0.099	0.17	0.846
Firmicutes	5.28	< 0.05	1.35	0.266	0.18	0.839
Bacteroidota	8.93	< 0.01	1.58	0.211	0.05	0.953
Myxococcota	0.16	0.690	0.78	0.463	0.07	0.929
Class						
Alphaproteobacteria	0.71	0.401	8.61	< 0.001	0.85	0.432
Acidobacteriae	1.31	0.255	0.10	0.909	0.05	0.949
Planctomycetes	0.43	0.515	9.17	< 0.001	0.39	0.681
Verrucomicrobiae	1.42	0.237	2.40	0.096	0.17	0.841
Thermoleophilia	1.83	0.18	1.95	0.149	0.83	0.439
Actinobacteria	10.45	< 0.01	0.71	0.497	0.00	0.996
Gammaproteobacteria	1.45	0.232	0.03	0.967	0.35	0.705
Bacilli	3.83	0.053	1.14	0.325	0.17	0.843
Bacteroidia	8.93	< 0.01	1.58	0.211	0.05	0.953
Polyangia	1.32	0.253	0.73	0.487	0.19	0.825

Table S3 Results of two-way ANOVA for dominant fungal phyla and classes

	Soil position		Season		Soil position $\times$ Season	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Phylum						
Basidiomycota	0.92	0.339	7.97	< 0.001	1.16	0.319
Ascomycota	1.15	0.286	4.24	< 0.05	0.95	0.392
Mortierellomycota	0.60	0.439	3.47	< 0.05	1.26	0.289
Rozellomycota	0.00	0.983	1.48	0.234	0.02	0.98
Zoopagomycota	0.55	0.461	0.50	0.611	1.79	0.173
Class						
Agaricomycetes	1.44	0.234	6.94	< 0.01	1.21	0.304
Mortierellomycetes	0.59	0.444	3.47	< 0.05	1.25	0.292
Sordariomycetes	0.30	0.587	3.30	< 0.05	0.08	0.927
Leotiomycetes	0.40	0.527	1.48	0.233	0.50	0.61
Tremellomycetes	0.67	0.417	1.24	0.294	0.17	0.842
Dothideomycetes	0.33	0.566	9.26	< 0.001	0.44	0.645
Archaeorhizomycetes	8.38	< 0.01	1.02	0.364	1.41	0.25
Zoopagomycetes	0.55	0.461	0.50	0.611	1.79	0.173

Table S4 Taxonomic classifications of the members of dominant modules

OTU ID	Module No.	Kingdom	Phylum	Class	Order	Family
Prok._OTU_56	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured
Prok._OTU_28	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured
Prok._OTU_89	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)
Prok._OTU_229	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Koribacteraceae
Prok._OTU_11	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured
Prok._OTU_37	1	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_21	1	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_31	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)
Prok._OTU_66	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)
Prok._OTU_286	1	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)
Prok._OTU_15	1	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_62	1	Bacteria	Actinobacteriota	Acidimicrobiia	IMCC26256	IMCC26256
Prok._OTU_35	1	Bacteria	Actinobacteriota	Thermoleophilia	Gaiellales	unclassified_Prokaryote
Fung._OTU_97	1	Fungi	Ascomycota	Leotiomyces	Helotiales	unclassified_Fungi
Fung._OTU_135	1	Fungi	Ascomycota	Leotiomyces	Helotiales	Hyaloscyphaceae
Prok._OTU_64	1	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae
Prok._OTU_113	1	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae
Prok._OTU_57	1	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	JG30-KF-AS9
Prok._OTU_3340	1	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	JG30-KF-AS9
Prok._OTU_33	1	Bacteria	Myxococcota	Polyangia	Haliangiales	Haliangiaceae
Prok._OTU_2952	1	Bacteria	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae
Prok._OTU_919	1	Bacteria	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae
Prok._OTU_2	1	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae

Prok._OTU_80	1	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae
Prok._OTU_98	1	Bacteria	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae
Prok._OTU_3768	1	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae
Prok._OTU_73	1	Bacteria	Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae
Prok._OTU_54	1	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_20	1	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae
Prok._OTU_12	1	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae
Prok._OTU_118	1	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae
Prok._OTU_25	1	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae
Fung._OTU_104	1	Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi
Fung._OTU_22	1	Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi
Prok._OTU_194	1	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae
Prok._OTU_316	1	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae
Prok._OTU_49	1	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Xiphinematobacteraceae
Prok._OTU_55	1	Bacteria	WPS-2	WPS-2	WPS-2	WPS-2
Prok._OTU_87	2	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured
Prok._OTU_50	2	Bacteria	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured
Prok._OTU_455	2	Bacteria	Actinobacteriota	Thermoleophilia	Gaiellales	unclassified_Prokaryote
Prok._OTU_88	2	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae
Prok._OTU_162	2	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae
Prok._OTU_61	2	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae
Fung._OTU_30	2	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae
Prok._OTU_125	2	Bacteria	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae
Prok._OTU_65	2	Bacteria	Myxococcota	bacteriap25	bacteriap25	bacteriap25
Prok._OTU_79	2	Bacteria	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae
Prok._OTU_86	2	Bacteria	Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae

Prok._OTU_165	2	Bacteria	Planctomycetota	Planctomycetes	Planctomycetales	uncultured
Prok._OTU_447	2	Bacteria	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae
Prok._OTU_77	2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_108	2	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae
Prok._OTU_40	2	Bacteria	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured
Prok._OTU_2173	2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_10	2	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified_Prokaryote	unclassified_Prokaryote
Prok._OTU_51	2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae
Prok._OTU_69	2	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae
Prok._OTU_853	2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_60	2	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae
Prok._OTU_3347	2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_1530	2	Bacteria	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae
Prok._OTU_32	2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylogigellaceae
Prok._OTU_5	2	Bacteria	unclassified_Prokaryote	unclassified_Prokaryote	unclassified_Prokaryote	unclassified_Prokaryote
Prok._OTU_83	2	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae
Prok._OTU_9	2	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae
Prok._OTU_100	2	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae
Prok._OTU_27	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_19	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_8	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_114	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_120	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_2347	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_567	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_1643	3	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2

Prok._OTU_541	3	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured
Prok._OTU_22	3	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	uncultured
Prok._OTU_157	3	Bacteria	Acidobacteriota	Holophagae	Subgroup_7	Subgroup_7
Fung._OTU_29	3	Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae
Fung._OTU_50	3	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_Incertae_sedis
Prok._OTU_128	3	Bacteria	Chloroflexi	AD3	AD3	AD3
Prok._OTU_110	3	Bacteria	Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae
Prok._OTU_4163	3	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_131	3	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_2725	3	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_172	3	Bacteria	RCP2-54	RCP2-54	RCP2-54	RCP2-54
Prok._OTU_18	3	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae
Prok._OTU_1296	4	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_2632	4	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_13	4	Bacteria	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae
Fung._OTU_27	4	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Cucurbitariaceae
Fung._OTU_90	4	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae
Fung._OTU_61	4	Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae
Fung._OTU_2	4	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae
Fung._OTU_9	4	Fungi	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae
Prok._OTU_14	4	Bacteria	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae
Prok._OTU_41	4	Bacteria	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured
Prok._OTU_1465	4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_1640	4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_7	4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_525	4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae

Fung._OTU_79	4	Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi
Fung._OTU_42	4	Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi
Fung._OTU_93	4	Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi	unclassified_Fungi
Prok._OTU_92	4	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Xiphinematobacteraceae
Prok._OTU_4	5	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Prok._OTU_24	5	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup_2	Subgroup_2
Fung._OTU_23	5	Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae
Fung._OTU_134	5	Fungi	Ascomycota	Leotiomycetes	Helotiales	unclassified_Fungi
Prok._OTU_46	5	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae
Prok._OTU_295	8	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)
Fung._OTU_170	8	Fungi	Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae
Fung._OTU_33	8	Fungi	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae
Fung._OTU_12	8	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae
Fung._OTU_40	8	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae
Fung._OTU_68	8	Fungi	Zoopagomycota	Zoopagomycetes	Zoopagales	unidentified
Prok._OTU_90	9	Bacteria	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae
Prok._OTU_1	9	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Prok._OTU_68	12	Bacteria	Acidobacteriota	Acidobacteriae	unclassified_Prokaryote	unclassified_Prokaryote
Fung._OTU_58	12	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae
Fung._OTU_14	12	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae
Prok._OTU_36	15	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae
Prok._OTU_38	15	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae
Prok._OTU_29	15	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae

Table S5 Sum of relative read counts (%) of each module at each season and soil position

	Early winter		Early spring		Mid-summer	
	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere	Bulk soil	Rhizosphere
Prokaryote						
Module 1	17.8 ± 3.2	18.9 ± 3.2	16.6 ± 3.2	19.1 ± 2.8	17.2 ± 3.3	20.2 ± 3.8
Module 2	12.2 ± 4.7	11.4 ± 4.6	12.7 ± 3.6	11.3 ± 3.7	11.2 ± 3.8	10.0 ± 4.4
Module 3	10.1 ± 2.3	7.9 ± 2.1	10.8 ± 2.8	8.5 ± 2.7	11.4 ± 2.4	8.4 ± 2.3
Module 4	11.8 ± 1.7	11.8 ± 1.8	12.9 ± 2.4	12.6 ± 2.3	12.6 ± 2.1	12.6 ± 2.3
Module 5	7.5 ± 2.4	7.2 ± 1.8	7.1 ± 2.0	6.9 ± 1.7	7.0 ± 1.8	7.1 ± 1.5
Module 6	0.3 ± 0.2	0.4 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
Module 7	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
Module 8	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Module 9	10.0 ± 1.4	10.6 ± 1.2	10.3 ± 2.2	10.7 ± 1.6	9.9 ± 2.0	11.4 ± 2.1
Module 12	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
Module 13	0.9 ± 0.3	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
Module 15	2.2 ± 0.6	2.4 ± 0.6	1.9 ± 0.8	2.3 ± 0.8	2.7 ± 0.7	2.7 ± 0.8
Module 18	1.3 ± 1.2	1.0 ± 0.7	1.2 ± 1.1	0.7 ± 0.3	1.4 ± 0.8	1.0 ± 1.1
Fungi						
Module 1	1.7 ± 1.7	2.0 ± 1.6	1.5 ± 1.4	2.8 ± 1.8	1.3 ± 0.9	1.7 ± 1.0
Module 2	1.2 ± 1.7	0.5 ± 0.9	1.3 ± 3.5	0.2 ± 0.5	2.2 ± 5.4	0.9 ± 2.3
Module 3	0.9 ± 0.9	1.0 ± 1.3	1.1 ± 1.8	1.3 ± 1.8	0.7 ± 1.3	0.7 ± 1.2
Module 4	6.5 ± 6.4	9.2 ± 9.6	7.4 ± 9.0	7.3 ± 8.8	12.0 ± 17.1	11.9 ± 20.1
Module 5	0.8 ± 0.8	0.8 ± 0.8	1.1 ± 1.6	1.4 ± 3.1	1.5 ± 1.6	1.4 ± 1.1
Module 6	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.3	0.3 ± 0.3
Module 7	1.7 ± 1.5	1.9 ± 1.7	1.0 ± 1.1	1.4 ± 1.5	1.6 ± 1.6	1.3 ± 1.4
Module 8	8.2 ± 2.4	8.1 ± 3.0	8.3 ± 3.4	10.0 ± 3.1	7.4 ± 3.1	6.7 ± 3.0
Module 10	2.2 ± 2.6	2.4 ± 2.3	1.4 ± 1.2	1.9 ± 1.4	1.2 ± 1.3	1.0 ± 0.8
Module 11	1.5 ± 1.2	1.2 ± 1.0	1.5 ± 1.3	1.7 ± 1.4	1.5 ± 1.7	1.2 ± 0.7
Module 12	3.6 ± 4.5	2.9 ± 3.2	6.6 ± 6.7	6.2 ± 5.4	5.7 ± 6.9	4.5 ± 3.7
Module 14	0.8 ± 0.6	0.7 ± 0.6	0.7 ± 0.7	0.7 ± 0.5	0.2 ± 0.2	0.0 ± 0.1
Module 16	0.5 ± 0.5	0.5 ± 0.5	0.4 ± 0.5	0.3 ± 0.5	0.9 ± 1.3	0.7 ± 1.0
Module 17	1.1 ± 1.0	0.8 ± 0.8	1.4 ± 1.5	1.4 ± 1.1	0.7 ± 0.8	0.3 ± 0.3

Note: The values are mean ± standard deviation.