

# Organic Mulching Alters the Composition, but not the Diversity, of Rhizosphere Bacterial and Fungal communities

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## Research

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# Abstract

**Background:** Organic mulching is an effective forest management technique that provides carbon and nutrient sources to soil ecosystems, thereby improving the soil environment and promoting plant growth. Although the importance of rhizosphere microbiomes in plant and soil ecosystem functions has been widely recognised, the effect of organic mulching on rhizosphere microorganisms and the underlying mechanisms are unclear.

**Methods:** We performed a field experiment in a 15-year-old *Ligustrum lucidum* forest of urban green space. The diversity and composition of the rhizosphere bacterial and fungal communities following organic mulching were assessed by combining 16S ribosomal RNA and internal transcribed spacer amplicon sequencing. The correlations between microbial diversity, composition, and fine-root traits, as well as rhizosphere soil properties, were also analysed.

**Results:** Organic mulching did not significantly affect the diversity of the rhizosphere bacterial or fungal communities. Additionally, organic mulching increased the bacterial diversity after 6 months, with a 20-cm-thick mulch layer showing a greater effect than 5- or 10-cm layers. Organic mulching significantly altered the rhizosphere bacterial and fungal community composition; after 6 months of mulching, the community compositions were significantly associated with fine-root traits (specific root length, nitrogen, and phosphorus concentration) and enzyme (urease and dehydrogenase) activity. Moreover, alterations in the bacterial and fungal communities occurred at the order level within each mulching stage. Bacterial diversity is affected by fungal diversity and rhizosphere soil properties (water content and organic carbon) in time-dependent manners. Hence, organic mulching appears to directly affect the fungal composition while indirectly affecting the bacterial composition via influencing rhizosphere soil properties (dissolved organic carbon and peroxidase activity).

**Conclusions:** Organic mulching affects the rhizosphere bacterial and fungal community composition through different pathways; however, the underlying mechanisms, including the effects of time and soil layers, require further exploration combined with multi-index measurements and long-term dynamic monitoring.

## Background

The understanding of soil microorganisms is limited because of the large number and variety of soil microorganisms. The causes of the vast microbial variation in rhizosphere soil are complex because of the complicated rhizosphere environment, which is directly influenced by living roots. In fact, the nutrient content of rhizosphere soil, as well as its soluble organic matter, enzyme activity, and microbial diversity, are higher than those of bulk soil (Turpault et al. 2007; De Feudis et al. 2017). Thus, the microbial responses in bulk and rhizosphere soils to different soil management practices are distinct (Maarastawi et al. 2018). Although the essential roles of rhizosphere microbiomes (rhizobiomes) have been demonstrated in soil and plants, few studies have considered rhizosphere-related traits, particularly fine-root architecture and characteristics (Kuzyakov and Razavi 2019).

Rhizosphere microorganisms are influenced by fine roots and the rhizosphere soil environment. Plant roots provide effective C and N sources for rhizobiomes by producing exudates and metabolites (Phillips and Fahey 2008). Roots specifically structure their environment to optimise their functions (water and nutrient uptake) while also establishing habitats for microorganisms and their activities (Kuzyakov and Razavi 2019). Thus, root physiological features shape rhizobiomes and exudation (Sasse et al. 2018). For example, root morphology (root surface structure, number, and length) represents a major index for explaining the mechanism by which plants are thought to modulate microbial interactions (Sasse et al. 2018). The C, N, and P contents in roots define the root exudate composition and nutrient availability to some extent (Dotaniya and Meena 2015), as well as dramatically affect the rhizosphere environment and microorganisms. Fine roots (diameter  $\leq 2$  mm) are highly dynamic and vital components of forests that are more sensitive than other roots to environmental change (Yuan and Chen 2010). Further, fine root abrasion serves as one of the main lipids in leachates from the root zone (Jandl et al. 2013). However, little is known regarding fine-root biology and the relationships between fine-root traits and the

rhizosphere environment, including rhizobiomes, limiting the ability to predict the responses of soil microorganisms to environmental changes (Zak et al. 2000).

Although soil physicochemical properties are known to significantly affect soil microorganisms, the predominant factors involved remain controversial. For example, some studies showed that soil pH and permanganate oxidisable C/soil organic C (SOC) represent the major drivers of the microbial community structure (Liu et al. 2017; Ramírez et al. 2020). However, another study indicated that light fractions of organic C and inorganic N are the key factors responsible for regulating the microbial community structure, whereas SOC controls microbial residue accumulation (Jing et al. 2019). External disturbances, such as climate change, precipitation, and fertilisation, can alter the soil environment, including its temperature, moisture, and nutrient elements, while also affecting plant growth and, consequently, soil properties and microorganisms (Hopkins et al. 2014; Wei et al. 2017). This is a reciprocal process in which soil microorganisms also affect plant diversity and productivity while significantly affecting soil fertility (Van Der Heijden et al. 2008; Leff et al. 2015). However, considering the various interactions occurring within this intricate relationship, the changes in soil microorganism communities, particularly within the rhizosphere environment, are complex; therefore, detailed research efforts are needed to resolve these issues.

Organic mulching is an important practice in agricultural soil conservation. Indeed, organic mulching has recently become widely used for urban greening and plantation for soil remediation and plant growth. It not only alters the physical characteristics of soil, including the temperature and bulk density, but also provides C and nutrients to the soil, which in turn influence nutrient uptake by plants (Kader et al. 2017). Changes in the soil environment caused by mulching affect the rhizosphere, as microorganisms are sensitive to various factors such as soil temperature and water content (Dotaniya and Meena 2015). Furthermore, the decomposition of organic mulch and exudate from the roots, occurring after plant growth, provides different nutrients for microorganisms. The thickness of the mulch and decomposed available nutrients decrease over time, thereby weakening the effects on soil physical conditions and nutrient content. Hence, the rhizobiomes and rhizosphere environment are dynamic. The rhizosphere environment also changes according to the natural conditions, including high seasonal variation (Calvaruso et al. 2014). The specific response of rhizobiomes to organic mulching is complicated and not well understood, particularly in the context of forest ecosystems.

Accordingly, the aim of this study was to improve the understanding of microbial diversity and composition after organic mulching of a *Ligustrum lucidum* forest in an urban green space, as well as the relationship between the rhizosphere environment (fine-root traits and rhizosphere soil properties) and rhizobiomes. We aimed to determine how the bacterial and fungal community diversity and composition in rhizosphere soil respond to organic mulching, which biotic and/or abiotic factors in the rhizosphere environment (fine-root traits and rhizosphere soil properties) affect the diversity and composition of rhizobiomes, and how these factors influence the diversity and composition of rhizobiomes.

## Materials And Methods

### Study site

The experimental plot was in Xiaolingwei, Xuanwu District, Nanjing, China (32°02'37"–32°02'39" N, 118°49'41"–118°49'43" E; 37 m a.s.l.). The site was comprised of flat terrain and a northern subtropical monsoon climate with distinct seasons. According to the historical records of Zhongshan Cemetery in Nanjing, the area previously contained buildings that were demolished, after which the region was covered with plantation forestry in 50–60 cm of soil. We analysed 15-year-old pure stands of *Ligustrum lucidum* W.T. Aiton (broad-leaf privet; family: Oleaceae) with tree spacing > 2 m and canopy density approximately 85%. The average tree height was 7.5 m, average crown was 2.5 m, and average diameter at breast height was 10.9 cm. The basic physical and chemical properties of the soil are shown in Table 1.

Table 1  
Physical and chemical properties of the experimental soil used in the study

Soil layer	pH	Total carbon /g·kg <sup>-1</sup>	Total nitrogen /g·kg <sup>-1</sup>	Ammonium /mg·kg <sup>-1</sup>	Nitrate /mg·kg <sup>-1</sup>	Total phosphorus /g·kg <sup>-1</sup>	Total potassium /g·kg <sup>-1</sup>	Available phosphorus /mg·kg <sup>-1</sup>	Available potassium /g·kg <sup>-1</sup>
0–20 cm	7.29	13.12	1.58	2.50	1.71	0.40	1.08	29.02	0.14
20–40 cm	7.31	11.20	1.42	1.43	1.69	0.32	0.95	23.12	0.13

## Experimental design

Four adjacent trees were randomly selected as an experimental plot with 32 experimental plots (128 trees) established. According to the ‘Technical specification for the application of organic mulch on urban and rural greening’ of Shanghai, China and related literature (Dietrich et al. 2019; Zhang et al. 2020), we applied four treatments: 0, 35, 70, and 140 kg of mulch/tree in each plot, which were randomly allocated. The mulch was carefully mounded around each tree as uniformly as possible to a height of 0, 5, 10, or 20 cm above the ground (codes OM0, OM5, OM10, or OM20, respectively). The mulch extended 80 cm away from each trunk, allowing a > 0.5 m buffer between trees. The treatments were applied in November 2017. The organic mulch consisted of composted municipal green waste derived from urban gardens and was produced by Shanghai Moqi Garden Co., Ltd. (Shanghai, China). The basic physical and chemical properties of the mulch are presented in Table 2.

Table 2  
Physical and chemical properties of the organic mulch used in the study

pH	Electrical conductivity /mS·cm <sup>-1</sup>	Organic matter /g·kg <sup>-1</sup>	Dry density /g·cm <sup>-3</sup>	Wet density /g·cm <sup>-3</sup>	Porosity /m <sup>3</sup> ·m <sup>-3</sup>	Total nitrogen /g·kg <sup>-1</sup>	Total phosphorus /g·kg <sup>-1</sup>	Total potassium /g·kg <sup>-1</sup>
6.40	1.35	902.00	0.14	0.79	318.00	23.80	4.30	19.50

## Field sampling

The soil was sampled twice, after 6 and 12 months of organic mulching. During each sampling, soil was recovered from three randomly selected experimental plots (i.e.,  $n = 3$  per treatment, 12 trees) with each experimental plot used only once. The soil profiles were sampled 50 cm away from the tree trunk, and each profile was divided into two layers (0–20 cm and 20–40 cm below the mulch layer). Soil blocks of 20 × 20 × 20 cm<sup>3</sup> were recovered. The fine roots were removed by hand. Rhizosphere soil was collected by gently shaking off the soil adhered to the roots. All fine roots and soil samples were placed in self-sealing bags and immediately transported to the laboratory for analysis. The soil samples were sieved (2 mm) and stored at 4°C until physicochemical analysis. In addition, 5–10 g of rhizosphere soil was collected from each sample, and after removing impurities such as plant roots and animal remains, the soil was placed in sterile centrifuge tubes, which were then placed in an ice box and transported to the laboratory where they were stored at -80°C for subsequent microbial sequencing.

## Laboratory analysis

The physicochemical properties and enzyme activities of the rhizosphere soil were determined as described in our previous study (Sun et al. 2021a, b). These properties included the water content, pH, SOC, dissolved organic C (DOC), total N (TN), dissolved N (DN), ammonium, nitrate, microbial biomass C (MBC), microbial biomass N (MBN), total P (TP), enzyme

(invertase, urease, peroxidase, and peroxidase) activity, and fine-root traits, namely, specific root length (SRL), specific surface area (SSA), root tissue density (RTD), fine root biomass (FRB), and fine root C and N concentrations (FRC and FRN, respectively). Additionally, the total fine root P (FRP) was detected calorimetrically following digestion (Campbell et al. 1991).

Genomic DNA was extracted from rhizosphere samples using the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The DNA purity and concentration were detected and monitored using a Nanodrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Next, 1% agarose gel electrophoresis was performed to assess the DNA quality, and the qualified DNA was stored at  $-80^{\circ}\text{C}$  for subsequent polymerase chain reaction (PCR) analysis. The V4–V5 hypervariable region fragments of the bacterial 16S ribosomal RNA gene were amplified with primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') using a thermocycler PCR system (GeneAmp 9700; Applied Biosystems, Foster City, CA, USA) (Mohd Yusoff et al. 2013); for fungi, the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used for amplification (Adams et al. 2013). PCR was performed in triplicate in 20- $\mu\text{L}$  mixtures containing 4  $\mu\text{L}$  of 5 $\times$  FastPfu Buffer, 2  $\mu\text{L}$  of 2.5 mM dNTPs, 0.8  $\mu\text{L}$  of each primer (5  $\mu\text{M}$ ), 0.4  $\mu\text{L}$  of FastPfu polymerase, and 10 ng of template DNA under the following conditions: 3 min of denaturation at  $95^{\circ}\text{C}$ , followed by 27 cycles (bacteria) or 30 cycles (fungi), for 30 s at  $95^{\circ}\text{C}$ , 30 s of annealing at  $55^{\circ}\text{C}$ , 45 s of elongation at  $72^{\circ}\text{C}$ , and a final extension at  $72^{\circ}\text{C}$  for 10 min. The resulting PCR products were extracted from the 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, Madison, WI, USA) according to the manufacturer's protocol. The purified amplicons were pooled at equimolar levels, and then paired-end sequenced on an Illumina MiSeq platform (San Diego, CA, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

## Statistical analysis

Obtained raw FASTQ files were processed using Trimmomatic software for sequence quality control and filtering. FLASH software was used for stitching according to the overlap relation. After the samples were differentiated, UPARSE software (Edgar 2013) was used for operational taxonomic unit (OTU) clustering according to a 97% similarity level. The species classification annotation determined using the Silva database was compared with the RDP classifier (Pruesse et al. 2007). The diversity index was calculated using Mothur software (Schloss et al. 2009).

All statistical analyses were performed using R v.3.5.3 software (core Team 2018), and the corresponding figures were created using the 'ggplot2' software package in R. Linear mixed effects models were calculated using the R package 'lme4' to evaluate differences in rhizosphere soil bacterial and fungal diversity (Shannon index), rhizosphere soil properties, and fine-root traits among treatments, soil layer, time after organic mulching, and their interactions (Bates et al. 2015). Treatment (four levels), soil layer (two levels), time after organic mulching (two levels), and their interactions (three-way) were treated as fixed factors, whereas the sampling plots were treated as random factors. Bacterial and fungal diversity (Shannon index) between treatments were compared using one-way analyses of variance and Tukey's pair-wise comparison tests.

We used the adonis function in the 'vegan' package (Oksanen et al. 2019) to perform non-parametric multivariate analysis of variance (PerMANOVA) (Anderson 2001) to determine statistical differences in the microbial community membership and abundance among soil properties and fine-root traits. Non-metric multidimensional scaling (NMDS) of the rhizosphere bacterial and fungal community composition and its influencing factors after 6 and 12 months of mulching were analysed relative to the PERMANOVA results. Species showing significant differences in bacterial and fungal community abundance among different treatments in different soil layers were analysed at the order level using a non-parametric factorial Kruskal–Wallis sum-rank test. We also performed principal component analysis based on Bray–Curtis dissimilarities in bacterial and fungal communities using the cmdscale function (Fig S4). Principal component analysis scores were used as proxies for the community composition in subsequent structural equation modelling (SEM) (de Vries et al. 2018).

SEM was used to analyse the relationships by which rhizosphere properties and fine-root traits collectively affect the microbial community diversity and composition. To simplify the modelling, we used composite variables to account for the

collective effects of rhizosphere properties (temperature, water content, pH, SOC, DOC, MBC, TN, ammonium, nitrate, MBN, and TP) and fine-root traits (SRL, SSA, RTD, FRB, FRC, FRN, and FRP) according to previous studies (Grace and Bollen 2008; Xiao et al. 2019). Observed indicators for each composite were selected based on multiple regression analyses for microbial community diversity and composition and Akaike's Information Criterion (AIC) (Grace et al. 2010). To verify the SEM normalisation and reduce normal deviation, all numerical variables in the model were standardised according to the same standard deviation (Grace et al. 2016). All possible interaction paths were pre-validated. When the overall model failed to fit, it was improved by removing meaningless direct or indirect paths. The model was evaluated and reduced based on the goodness of fit, whereas the AIC was applied to ensure optimal selection among different models. That is, the model with the lowest AIC value was selected as the final model. We implemented SEM using the piecewiseSEM package with 'plot' as the random effect to account for autocorrelation among split plots (Lefcheck 2016). All variables were initially tested for a normal distribution.  $P < 0.05$  was considered as statistically significant.

## Results

### Changes in bacterial and fungal diversity after organic mulching

A total of 2,483,649 and 3,197,487 valid 16S/18S ribosomal RNA gene sequences were obtained with an average length of 377 and 248 bp across all samples, respectively. In total, 7332 bacterial OTUs and 4046 fungal OTUs were obtained from the 48 DNA samples.

Time was the only independent variate found to significantly affect the bacterial community Shannon index. The mulching treatment and soil layers, as well as the interactions among treatments, soil layers, and time, had no significant effect on the diversity of the bacterial and fungal communities (Table 3). After 6 months of mulching, the Shannon index for the bacterial community was significantly affected, and bacterial diversity increased with increased mulching amounts (Fig. 1). Moreover, the Shannon index for the bacterial community under OM5 and OM10 after 6 months of mulching, as well as that under OM0 after 12 months of mulching differed significantly between the topsoil and subsoil.

Table 3  
ANOVA of the effects of different treatments, soil layers and time on the diversity of bacterial and fungal communities

	Bacterial diversity	Fungal diversity
Time	24.16***	0.01
Soil layer	2.40	0.09
Treatment	2.82	0.70
Time × Soil layer	2.60	0.20
Time × Treatment	2.04	1.28
Soil layer × Treatment	0.69	2.49
Time × Soil layer × Treatment	2.96	0.31

Note: F values are reported with  $p$  values indicated as follows: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

### Changes in microbial community composition after organic mulching

Organic mulching significantly affected the bacterial and fungal community composition; the interaction of the treatment × soil layer significantly affected the bacterial community composition except for at 6 months (Fig. 2 and Table 4). FRP

significantly affected the bacterial community composition after 6 months of mulching, accounting for 33% of the observed changes, whereas the fungal community composition was significantly influenced by SRL, FRN, and urease and dehydrogenase activity, accounting for 26%, 28%, 33%, and 30% of the changes, respectively (Fig. 2 and Table S1).

Table 4  
PerMANOVA of the effect of treatment and soil layer on bacterial and fungal community composition

		Bacterial composition			Fungal composition	
		Df	PerMANOVA F	R <sup>2</sup>	PerMANOVA F	R <sup>2</sup>
6 months after organic mulching	Treatment	3	1.87***	0.21	1.64**	0.18
	Soil layer	1	1.76*	0.06	1.61*	0.06
	Treatment × Soil layer	3	1.30	0.14	1.84**	0.20
12 months after organic mulching	Treatment	3	1.46*	0.14	2.45***	0.23
	Soil layer	1	1.66	0.05	2.17***	0.07
	Treatment × Soil layer	3	2.75***	0.27	2.32***	0.21

Note: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

*Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi* were the most abundant rhizosphere bacterial phyla (Fig. S2). The orders *Planctomycetales*, *Micrococcales*, *Solibacterales*, and *Rhodocyclales* differed significantly in relative abundance among the treatments after 6 months of mulching, whereas *unclassified\_c\_Acidobacteria*, *norank\_c\_Actinobacteria*, *Sphingobacteriales*, and *Propionibacteriales* differed significantly after 12 months of mulching (Fig. 4). Moreover, *Ascomycota*, *unclassified-k-Fungi*, *Basidiomycota*, and *Zygomycota* were the dominant rhizosphere fungal phyla (Fig. S3). The orders *Sordariales*, *Helotiales*, *Onygenales*, and *Sebacinales* differed significantly in relative abundance among treatments after 6 months of mulching, whereas *Hypocreales*, *Pezizales*, *Agaricales*, and *Onygenales* differed significantly after 12 months (Fig. 3).

## Relationship between microbial community diversity, composition, and rhizosphere fine-root traits

SEM analysis revealed that the water content, SOC, and FRB significantly affected the bacterial and fungal community diversity (Fig. 4a). Bacterial community diversity was significantly affected by fungal community diversity and rhizosphere soil properties (water content and SOC). Rhizosphere soil properties were significantly affected by time. All factors in SEM that affected bacterial community diversity accounted for 44% of the changes. In addition, FRB was significantly affected by time, accounting for 69% of the variation in FRB.

Peroxidase activity, DOC, and FRN were shown by SEM analysis to significantly affect the bacterial and fungal community composition (Fig. 4b). Organic mulching directly affected the fungal community composition, accounting for 9% of its variation, and indirectly affected the bacterial community composition through rhizosphere soil properties (peroxidase activity and DOC). All factors identified by SEM to affect the bacterial community explained 27% of its changes. Soil layers also significantly affected rhizosphere soil properties, accounting for 20% of its changes, together with mulching. FRN was positively affected by time and negatively affected by soil layers.

## Discussion

### Effect of organic mulching on rhizosphere bacterial and fungal community diversity

Although, in general, organic mulching did not significantly affect the diversity of the rhizosphere bacterial and fungal communities, it increased bacterial community diversity after 6 months. Soil environmental and microphysical factors significantly affect the bacterial community, whereas none of these parameters showed a significant correlation with the fungal community (Pudasaini et al. 2017; Tan et al. 2019). Similarly, fungal diversity and rhizosphere soil properties, which include the water content and SOC, were determined to be critical factors affecting bacterial diversity according to the SEM results (Fig. 4a); however, no index significantly affected fungal diversity among our measured variables. Compared to fungi, which control the decomposition of organic matter, rhizosphere bacteria appear to have a greater advantage in terms of organic matter transformation and assimilation because of their interaction with the roots, which leads to greater changes following organic mulching; in contrast, fungi exhibit a positive response to environmental changes (Xiao et al. 2019). Organic mulch provided more available C during the early period of mulching, during which bacteria are predominant because they have the advantage of using labile C, whereas fungi primarily utilise recalcitrant C (Masai et al. 2007; Perotto et al. 2012). Additionally, fungal C utilisation relies primarily on the quantity of recent plant-derived substrates, whereas bacterial access to substrates is additionally controlled by environmental conditions (Preusser et al. 2019).

The water content and SOC were selected as indices affecting the rhizosphere soil properties, indicating that these two factors play important roles in the rhizosphere environment to influence bacterial diversity. The application of organic mulch provides additional nutrients to soil and plants, and the flux and utilisation of nutrients relies on water (Kuzyakov and Razavi 2019), particularly in the rhizosphere, where high element exchange occurs. Addition of organic mulch caused a rhizosphere priming effect, which is a major determinant of SOC turnover in surface soils (Shahzad et al. 2018). Similar to plant litter, the added organic mulch increased C infiltration into the rhizosphere (De Deyn et al. 2008), thereby explaining the increased diversity in rhizosphere bacteria during the early stage of organic mulching. However, these two factors were primarily affected by time, and the increase in the element cycle and energy flow in the rhizosphere after organic mulching may have occurred because of changes in the bacterial community composition and associated function, but not in bacterial community diversity.

Indeed, time (season) is an important factor affecting the soil microbial community (Spedding et al. 2004; Gałazka and Grzadziel 2018). Thus, in our study, although mulching treatment did not significantly affect bacterial or fungal diversity, 6 months of mulching significantly influenced bacterial diversity. Other studies showed similar results, with indirect changes in soil substrate availability due to climate factors exhibiting stronger effects on the microbial community compared to the effects of temperature or moisture (Zhang et al. 2005; Evans et al. 2014). Additionally, the rhizosphere soil properties (water content and SOC), which are related to bacterial diversity, were significantly affected by time. These results are similar to those of other studies indicating that drought can directly affect root bacterial communities by modulating moisture availability (Naylor and Coleman-Derr 2018). Moreover, soil moisture is influenced significantly by season but not by forest management, whereas seasonal dynamics rather than precipitation changes ultimately control the total diversity of the bacterial community (Cregger et al. 2012; Bastida et al. 2017). As observed in a previous study (Ren et al. 2017), FRB is an important factor affecting microbial diversity. Although FRB was selected by SEM analysis in the current study, it did not significantly nor directly affect bacterial or fungal diversity in the final SEM; nevertheless, it may affect fungal diversity through an alternate indirect path.

Because of the complicated rhizosphere environment, the associated microbial diversity is affected by multiple factors. Similar to the results of previous studies, litter species had no or a weak effect on microbial activity or the relative microbial abundance; however, other environmental factors, such as constructed wetlands, may have stronger effects on these factors compared to litter inputs (Ping et al. 2019). Moreover, the indicators included in the current study were not exhaustive. For instance, root exudates also represent an important factor influencing rhizobiomes (Sasse et al. 2018). However, significant indices were selected according to multiple regression analyses for microbial diversity and AIC, with only some of the variables detected by multiple regression analysis included in SEM testing. Furthermore, although microbial biomass is known to rapidly change in response to changes in the external environment (Sinsabaugh et al. 2015), we only collected samples twice over the course of a year; the short time length of analysis is a limitation of this study.

# Effect of organic mulching on rhizosphere bacterial and fungal community composition

The structure of bacterial and fungal communities is influenced by seasonality (Bastida et al. 2017; Hernandez and Menéndez 2019). Here, the microbial community composition exhibited significant changes among the various mulch treatments at different time points; however, only some of the identified variables were related to the microbial community composition after 6 months of organic mulching. Among them, FRP was related to the bacterial community composition, whereas SRL, FRN, as well as urease and peroxidase activities were significantly correlated with the fungal community composition. This observation indicates that fine-root traits and enzyme activity represent essential factors affecting the rhizosphere microbial community composition compared to the rhizosphere soil properties during the early period of organic mulching. Moreover, a potential interaction occurs between the plant roots and rhizobiomes early in the growing season (spring) (Walker et al. 2003).

Addition of organic mulch provides N and P sources for the soil, which are quickly absorbed and utilised by plants through the roots. Hence, variation in the fine-root morphology to adapt to the changing environment as well as the nutrient contents in the fine roots may affect the rhizosphere microbial community structure through root exudates (Sasse et al. 2018). Additionally, urease is associated with the N and P cycles (Xu et al. 2010), whereas peroxidase is secreted by fungi (Hofrichter et al. 2010); hence, changes in the activity of these two enzymes following nutrient addition likely strongly contributed to the microbial community composition during the early period of mulching.

Similar to the results of previous studies (Yu et al. 2013; Garcia-Lemos et al. 2019), *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi* were found to be the main rhizosphere bacterial phyla, whereas *Ascomycota*, *unclassified-k-Fungi*, *Basidiomycota*, and *Zygomycota* were the most abundant and common rhizosphere fungal phyla. Altered fungal community members, including *Sordariales* and *Sebacinales*, are associated with roots and sensitive to variations in root features (Garcia-Lemos et al. 2019), which was supported by the correlations among the fungal community composition, SRL, and FRN (Fig. 3). After organic mulching, the bacterial community, at the order level, exhibited unique differences in the early (after 6 months) and late (after 12 months) stages. Specifically, within the early stage, the bacterial community primarily participated in the decomposition of plant residues, nitrification, and denitrification (Ortiz-Cornejo et al. 2017; Cabello et al. 2019), possibly because of the increase in plant root and soil metabolic activity associated with plant growth and soil recovery at the beginning of the growing season (spring), as well as the decomposition and use of organic mulch. However, according to the altered bacterial communities in the late stage, rhizosphere soil element metabolism and nutrient transformation were much accounted after organic mulching (Araujo et al. 2020; Kalam et al. 2020). Among this community, *unclassified\_c\_Acidobacteria* and *norank\_c\_Actinobacteria* belong to the dominant rhizosphere bacterial community (*Acidobacteria* and *Actinobacteria*), indicating that the composition of the dominant bacterial community contributing to the soil C and nutrient cycles changed after 1 year of mulching.

Mulching significantly and directly influenced the fungal composition, whereas the bacterial composition was affected by rhizosphere soil properties, among which peroxidase activity and DOC were the most important factors. Long-term addition of C substrate triggers the production of enzymes conducive to activating native soil C (Morrissey et al. 2017). Microbes use labile root-derived C more efficiently compared to using recalcitrant SOC (Bicharanloo et al. 2020); thus, labile C fractions, such as DOC, are closely related to the microbial community structure and activities (Cookson et al. 2005; Tang et al. 2021). Peroxidase activity is related to the transformation of organic C (Hofrichter et al. 2010), and addition of organic mulch promotes C conversion between the soil and roots, i.e. more C is cycled in the rhizosphere (De Deyn et al. 2008).

In addition, soil depth significantly affects soil properties. In the current study, soil layers indirectly affected the bacterial composition but not the fungal composition. Hence, to some extent, the composition of the bacterial community had a stronger spatial dependence compared to the fungal community (Yang et al. 2018). As previously mentioned, bacteria may

play important roles in transforming and assimilating organic matter in the rhizosphere, whereas altered C sources at various soil depths affect the microbial community structure (Preusser et al. 2019).

## Conclusions

Our results suggest that the changes in the rhizosphere microbial community composition after organic mulching result from changes in their work or functions rather than in their lack of diversity. In particular, the altered bacterial and fungal communities (at the order level) differed during the various mulching stages. We demonstrated that fine-root traits and enzymatic activity play important roles in the rhizosphere microbial community composition during the early stage of organic mulching. Our study further reveals the regulatory mechanism by which organic mulching affects the rhizosphere bacterial and fungal communities. Multi-index measurements and long-term dynamic monitoring should be performed to continuously explore the mechanism of nutrient exchange and energy flow between soil and plants to provide a foundation for soil improvement and forest productivity.

## Abbreviations

AIC, Akaike's Information Criterion; DN, dissolved nitrogen; DOC, dissolved organic carbon; FRB, fine root biomass; FRC, fine root carbon; FRN, fine root nitrogen; FRP, fine root phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; NMDS, non-metric multidimensional scaling; OTU, operational taxonomic units; PCR, polymerase chain reaction; PERMANOVA, permutational multivariate analysis of variance; RTD, root tissue density; SEM, structural equation modelling; SOC, soil organic carbon; SRL, specific root length; SSA, specific surface area; TN, total nitrogen; TP, total phosphorus

## Declarations

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Availability of data and materials

Not applicable

## Competing interests

The authors declare they have no competing interests.

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# Authors' contributions

Q Guan and X Sun conceived and designed the experiments. X Sun and Y Ye performed the experiments. X Sun and J Liao analyzed the data. X Sun wrote the manuscript; other authors provided editorial advice.

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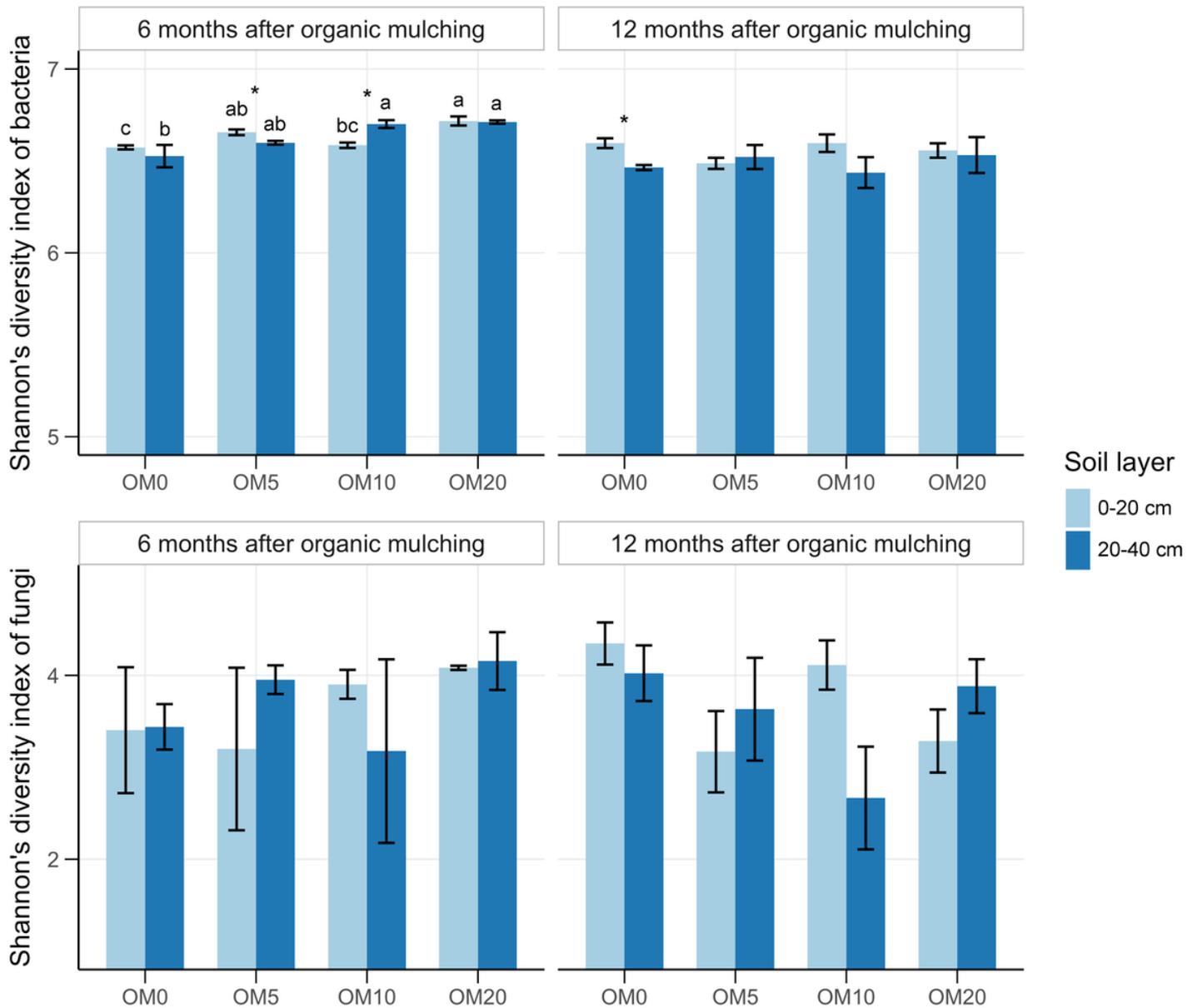
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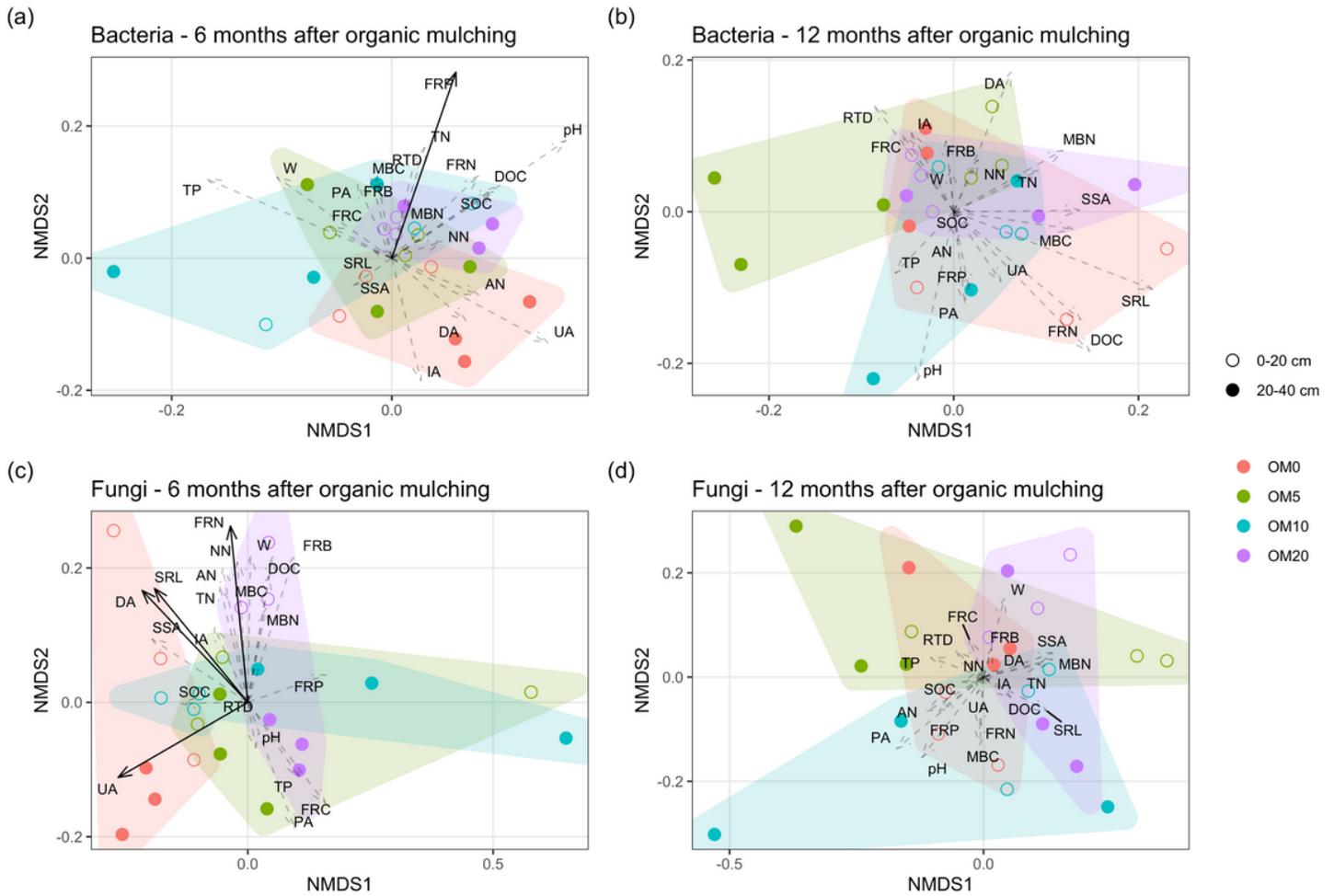
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## Figures



**Figure 1**

Bacterial and fungal community Shannon diversity index and relative abundances (Chao). Values are means  $\pm$  the standard error ( $n = 3$ ). OM0: no mulch; OM5: 5 cm (35 kg) mulch; OM10: 10 cm (70 kg) mulch; OM20: 20 cm (140 kg) mulch. Different lowercase letters represent significant differences among treatments in the same soil layer and time according to Tukey tests ( $P < 0.05$ ), \* represent significant difference between topsoil (0–20 cm) and subsoil (20–40 cm) under the same treatment and time.



**Figure 2**

Non-metric multidimensional scaling (NMDS) graphs of rhizosphere microbial community composition and its influencing factors. Solid arrows represent significant effect, dashed arrows represent non-significant effect. W: water content; SOC: soil organic carbon; DOC: dissolved organic carbon; MBC: microbial biomass carbon; TN: total nitrogen; AN: ammonium; NN: nitrate; MBN: microbial biomass nitrogen; TP: total phosphorus; IA: invertase activity; UA: urease activity; PA: peroxidase activity; DA: dehydrogenase activity; SRL: specific root length; SSA: specific surface area; RTD: root tissue density; FRB: fine root biomass; FRC: fine root carbon concentration; FRN: fine root nitrogen concentration; FRP: fine root phosphorus concentration.

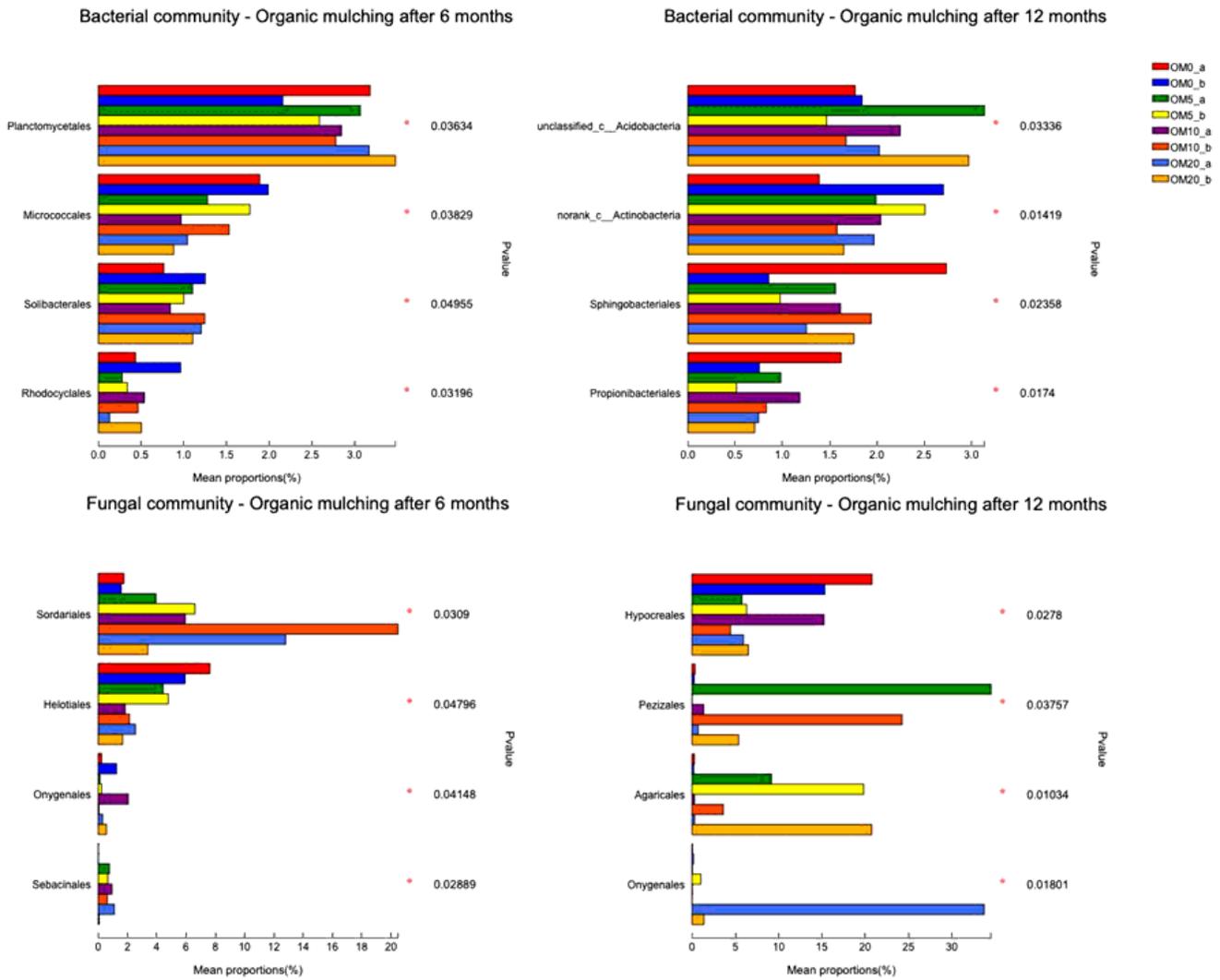


Figure 3

Rhizosphere bacterial and fungal communities with significant differences after organic mulching at the order level. a: Topsoil, b: subsoil. Bacterial and fungal classes containing at least 1% of total OTUs (x axis) for each sampling time are shown in the figure.

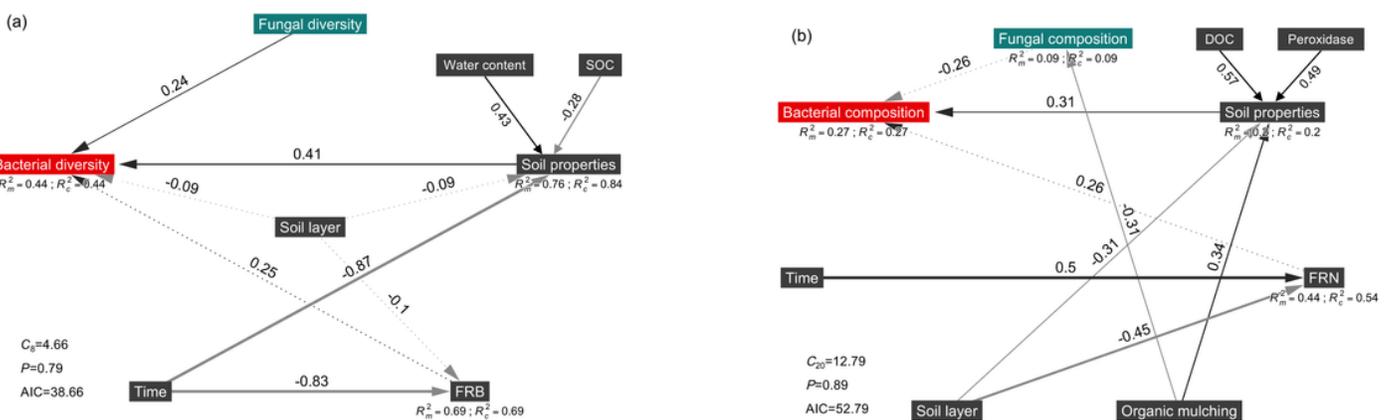


Figure 4

Structural equation model of relationships between microbial community diversity (a) and composition (b), rhizosphere soil properties, and fine root traits. Numbers beside arrows indicate standardized coefficients. Solid arrows represent significant effect, dashed arrows represent non-significant effect. Black and grey lines represent positive and negative relationships, respectively. FRB: fine root biomass; SOC: soil organic carbon; DOC: dissolved organic carbon; FRN: fine root nitrogen concentration; Rm2: Rmarginal2; Rc2: Rconditional2.

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