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Research Article

Amplicon sequencing reveals different microbial communities between growing and non-growing seasons in the soils of *Pinus armandi* forestland in Shennongjia, China

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Abstract

Soil microbial communities are susceptible to climate change due to seasonal alternation. To explore the effects of seasonal variation on soil nutrients and microorganisms, we sequenced the 16S rDNA and 18S rDNA genes of the distinct regions (16S V3-V4, 18S V4) to precisely identify the soil microbial communities in the growing season (Par_S) and non-growing season (Par_W) in *Pinus armandi* forestland, in Shennongjia forest region, China. Eight chemical properties of the soil samples were also determined to elucidate the correlations between the microbial communities and soil characteristics. In Par_S, we identified 36 phyla 348 genera of bacteria, and 58 phyla 197 genera fungi. Par_W's corresponding values were 39 phyla 471 genera and 59 phyla 259 genera, respectively. Par_S owned more abundant bacterial communities than Par_W. The relative abundance of most bacteria and fungi differed significantly between Par_S and Par_W. Most of the top 35 abundant bacterial genera and fungal genera were enriched in Par_S and Par_W, respectively. The soil properties differed significantly between Par_S and Par_W. They were significantly correlated with the variations in the relative abundance of the top 10 bacterial and fungal genera in both Par_S and Par_W. *Rokubacteriales* and *RB41* were dominant among Par_S's top 10 bacterial genera, and were related to the RR of the soil. *Sphingomonas* was dominant among Par_W's top 10 bacterial genera. *Magnoliophyta, Haplotaxida* and *Acari* were dominant among Par_S's top 10 fungal genera, and were related to RR, TK, HN, TP and AP. *Archaeorhizomyces* was dominant among Par_W's top 10 bacterial genera. For the top 10 abundant bacterial genera in Par_S, the relative abundance of *Nitrospira* was negatively correlated with the contents of TN and AK, and *MND1* was negatively correlated with SOM. Regarding the top 10 abundant bacterial genera in Par_W, *SBR1031* was positively correlated with TP and AP, and *MND1* was positively correlated with AP. Regarding the top 10 abundant fungal genera in Par_S, only *Acari* had a positive correlation with TK.

Keywords: 16S rDNA; 18S rDNA; *Pinus armandi* forestland; soil microbial community

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Abbreviation: AK: Available potassium; AP: Available phosphorus; HN: Hydrolyzable nitrogen; Par_S: *Pinus armandi* forestland in growing season; Par_W: *Pinus armandi* forestland in non-growing season; RR: Respiratory rate; SOM: Soil organic matter; TK: Total potassium; TN: Total nitrogen; TP: Total phosphorus

Introduction

Soil is an essential component of terrestrial ecosystems (Ladwig *et al.,* 2016) and a direct source of nutrients for plant growth and development (Etesami and Adl, 2020). Soil microbes maintain soil productivity through biochemical processes such as nutrient cycling (Wu *et al.*, 2020b), metabolic activities, and nutrient transformations of various microorganisms always coincide (Rasmussen *et al.,* 2022). Soil respiration is vital in forest ecosystems (Zhang *et al.,* 2018); like soil microorganisms and nutrients, they are important indicators for assessing soil quality (Xue *et al.,* 2020), which is a long-standing concern.

Shennongjia forest region, as the only administrative region named after a forest area, has a forest coverage of more than 91% and is rich in forest resources. The climate is subtropical, with mild, wet summers and cold, dry winters. Elevation in the study area ranges from 900 to 3105 m. Annual precipitation ranges from 800 to 2,500 mm, most falling between June and August. Snowfall usually lasts five months (from November to March), and annual mean temperature ranges from −19.5 °C in January to 29.4 °C in July, with a temperature difference of nearly 50 °C. Dramatic seasonal shifts in physical and biochemical properties along altitude gradients characterize alpine soil environments. Microbial communities may be shaped seasonally as response to the large and frequent (diurnal) temperature fluctuations, regular soil freeze-thaw and wet-dry events. *Pinus armandi*, a dominant species in the forest area, is a significant timber tree species in China. As a result, the understanding of climatic influences on soil microorganisms can be improved by studies that soil samples from summer and winter are of great importance in understanding the effects of climate conditions on the soil quality.

In this study, we monitored soil respiration rates, briefly determined soil chemical properties, and performed high-throughput sequencing of soil in *P. armandi* forests from the growing season (Par_S) and non-growing season (Par_W), respectively. Our objectives were to (1) compare differences in soil respiration rates between Par_S and Par_W; (2) compare differences in soil chemical properties between Par_S and Par_W; (3) compare differences in soil microbes between Par_S and Par_W. The results will help us understand soil microbial activity, evaluate soil fertility status, and provide a reliable basis for vegetation management.

Materials and Methods

Materials

In August and December 2018, we selected *P. armandi* forestland in the Shennongjia forest region, China. A 30 m × 20 m sample plot was set up, which was divided into six 10 m × 10 m equally spaced grid points, and the intersection of the grids was taken as the sample plots (Figure 1). A total of twelve sampling plots were set up. At each sampling plot, we collected soil samples of 0-10 cm in three replications. These soil samples, the backbone of our research, were carefully handled. After removing visible debris such as stones and plant residues, part of the soil samples was put in sterile self-sealing bags and brought back to the laboratory to be air-dried and sieved for 2 mm to determine soil chemical properties; the other part of the soil samples was sealed and stored at low temperatures for microorganism DNA extraction and analysis.

Overview of the test area

The Shennongjia forest region is located in the western part of Hubei province. Its geographical coordinates are 31°15'-31°75' N, 109°56'-110°58' E, and its area is 3,253 km^2. This study area has a humid subtropical monsoon climate with distinct seasons and abundant precipitation. The forest area has rich biological resources, covering more than 3,700 plant types and 90% forest cover.

Dominant understory species in sampling plots

A 2 m × 2 m quadrat centered over each sampling point was set up to investigate the tree, shrub, and herbaceous. Each plant's species name, height, number, and coverage were recorded. Species names and numbers were counted directly; height was determined using a height gauge (Changchun Huamao Instrument Co., Ltd., Changchun, China); coverage was determined visually (Wheeler *et al.,* 2021). A total of 40 species of woody plants (trees and shrubs) and 53 species of herbaceous plants were counted in the area (Table 1). We investigated the height, number, and coverage of the top 10 dominant species in woody and herbaceous plants, respectively. The dominant understory woody plants were *Fraxinus chinensis* Roxb., with an average height of 118 cm and a coverage of 22%. The dominant herbaceous species was *Oplismenus undulatifoius* (Ard.) Beauv., with approximately 392 plants and a coverage of 91%.

Methods

Soil respiration rates

Soil respiration was measured using LI-8100A soil carbon flux meter (Henan Rongcheng United Technology Co., Ltd., Zhengzhou, China). In August-December 2018, a cylinder (16 cm in diameter and 15 cm in height) was made of polyvinyl chloride (PVC) ring material. In the twelve sampling plots, we inserted it into the soil for 13 cm, leaving 2 cm on the ground. The LI-8100A soil carbon flux observation system was used for the continuous positioning of soil respiration rate, which was measured 1-2 times per month; sunny days were selected for continuous day and night observation, with measurements every two hours from 09:00-17:00 and 17:00 to 09:00. Each PVC ring was measured three times, and took the average of the three times (Wei *et al.*, 2021).

Soil chemical properties

TN was measured using the Kjeldahl method (Chen *et al.*, 2023); TP was measured by the sodium hydroxide fusion method (Bilias *et al.,* 2023); TK was measured using the flame photometric method (Zhang *et al.,* 2021); HN was measured using the Kjeldahl method (Yu *et al.*, 2019); AP was measured using the ammonium molybdate colorimetric method (Li *et al.*, 2020); AK was measured using the flame photometric method (Muchane *et al.*, 2020); SOM was measured using the potassium dichromate titration method (Navarro-Pedreño *et al.,* 2021).

Amplicon generation and sequencing

Microbial diversity sequencing is the detection of 16S and 18S microbial species signature sequences amplified by PCR using high-throughput sequencing technology. Soil genomic DNA was extracted from soil samples (0.5 g) using the Biofast Soil Genomic DNA Extraction Kit (Meiji Biotechnology Corporation, Guangzhou, China); the extracted DNA was diluted in TE buffer (10mM Tris-HCl and 1mM EDTA at pH 8.0) and stored at -20 °C until use.

The V3-V4 hypervariable region of bacterial 16S rDNA was amplified using the primer pair 341F/806R (341F: ACTCCTACGGGAGGCAGCAG, 806R: GGACTACHVGGGTWTCTAAT). The V4 hypervariable region of bacterial 18S rDNA was amplified using the primer pair F/R (F: CCAGCASCYGCGGTAATTCC, R: ACTTTCGTTCTTGATYRA). All PCR products were purified using the TruSeq® DNA PCR-Free Sample Preparation Kit for library construction. After the library was

qualified by qubit and Q-PCR, samples were loaded onto the Illumina NovaSeq6000 platform for HTS. The raw data has been uploaded to NCBI sequence read archive under accession number PRJNA1128415.

The raw HTS data, with reads spliced using FLASH software, was processed using QIIME2. Finally, valid data was obtained through tag interception and length filtering, then clustered into operational taxonomic units (OTUs) with 97% sequence identity using VSEARCH software. The OTUs sequences were annotated via database comparisons using Mothur, and the relative content of OTUs in each sample was calculated. Shannon diversity and Chao1 richness were calculated based on the OTUs. A map of bacterial community composition was drawn using R software (v3.6.3, R Foundation for Statistical Computing, Vienna, Austria).

OTU clustering and species annotation

Firstly, the forward and reverse reads from sequencing were merged and filtered out of the low-quality chimeric sequences to obtain valid sequences (Chen *et al.,* 2018). After that, the clustering program QIIME2 (Bolyen *et al.,* 2019) was used to denoise the amplicon data using the DADA2 algorithm (Callahan *et al.,* 2016) and then de-redundancy, which means obtaining the feature data. Feature representative sequences were compared with the corresponding databases to obtain species annotations for all features for each sample. In QIIME2, diversity was calculated using the Shannon and Simpson indices; richness was calculated using the Chao1 and ACE indices, and the Alpha diversity indices from rare samples. Beta diversity was calculated using weighted and unweighted UniFrac. Redundancy analysis (dbRDA) was calculated to show the relationship between microbial communities and environmental factors.

Statistical analysis

SAS9.3 (North Carolina, USA) was used to analyze variance (ANOVA) and Duncan's multiple comparisons on the experimental data. Pearson analyzed the correlation between the soil's physicochemical properties, enzyme activities, and different treatments. Origin 2023 (OriginLab, Massachusetts, USA) was used for drawing; Spearson analysis was performed on the correlation between soil physicochemical properties and soil bacterial genera using SAS9.3.

Alpha diversity was analyzed using five metrics, Shannon, Simpson, Chao1, ACE, and Goods_coverage, to analyze the sample's complexity of species diversity. All these metrics in our sample were calculated using QIIME2 and displayed using R software (V3.6.3). Chao1 and ACE were chosen to identify community richness. Shannon and Simpson were used to identify community diversity. Goods_coverage was used to determine sequencing depth. The closer to 1, the more reasonable the sequencing depth.

Beta diversity refers to the variability of species among different environmental communities. Principal coordinate analysis (PCoA) was performed to get principal coordinates and visualize them from complex, multidimensional data. PCoA on weighted Unifrac was displayed by WGCNA package, stat packages and ggplot2 package in R software (V3.6.3). Distance-based redundancy analysis (dbRDA) was performed using CANOCO4.5 to examine the relationship between the frequency of enriched genera and samples and the chemical properties of selected soil samples.

Results

Respiration rates

The data showed that soil respiration rates differed significantly ($P<0.05$; Table 2) between the Par_S and Par_W. The former possessed a higher soil respiration rate than the latter. Soil respiration rate varied from 1.85 to 4.17 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the Par_S, with a more considerable variation of 2.32 $\mu mol \cdot m^{-2} \cdot s^{-1}$ between the highest and the lowest values. In comparison, soil respiration rate varied from 0.75 to 2.23 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the Par_W, with a much smaller variation of 1.48 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Table 1. Survey on the top 10 understory vegetation species of the *Pinus armandi* forest

	Species name	Height/cm	Number	Coverage/%
Tree/Shrub layer	*Fraxinus chinensis* Roxb.	118	16	22
	Litsea ichangensis Gamble	140	11	12
	Lonicera stanishii Carr	110	5	10
	Rubus innominatus S.Moore	120	7	18
	Helwingia japonica (Thunb.) F. Dietr.	68	8	24
	Viburnum betulifolium Batalin	137	7	24.5
	Toxicodendron vernicifluum (Stokes) F. A. Barkley	85	7	18.9
	Prunus obtusata Koehne	187	4	14
	Castanea henryi (Skan) Rehder & E. H. Wilson	165	4	31.2
	Rubus lambertianus Ser.	70	6	33
Herb layer	*Astilbe chinensis* (Maxim.) Franch. & Sav.	51	26	19
	Parathelypteris nipponica (Franch. & Sav.) Ching	56	138	38
	Aster ageratoides Turcz.	49	34	18
	Oxalis acetosella L.	6	59	21
	Impatiens blepharosepala Pritz.	32	22	17
	Oplismenus undulatifolius (Ard.) Roemer & Schuit.	12	392	91
	Glechoma biondiana (Diels) C. Y. Wu & C. Chen	28	21	75
	Hedera nepalensis var. *sinensis* (Tobler) Rehder	13	22	22
	Duchesnea indica (Andrews) Focke	6.3	72	24
	Sedum amplibracteatum K. T. Fu	9	48	43

Chemical properties

The soil chemical properties were not significantly different (P<0.05) between Par_S and Par_W samples, except of AP (Table 3). TN, HN, TK, and AK contents were a little bit higher in the Par_S than Par_W. It means that seasons slightly affected the most chemical properties of the soil, but dramatically affected the content of AP.

Table 2. Soil respiration rates in Par_S and Par_W

Sample plot	Season	
	Par_S (growing season)	Par_W (non-growing season)
1	2.27±0.10a	0.75±0.02b
2	2.82±0.04a	1.30±0.07b
3	2.90±0.00a	0.95±0.00b
4	1.85±0.03a	2.23±0.06a
5	2.15±0.03a	1.46±0.09a
6	3.14±0.05a	0.97±0.06b
7	3.43±0.06a	0.99±0.02b
8	2.96±0.00a	1.03±0.06b
9	2.00±0.00a	1.18±0.03b
10	1.94±0.01a	1.67±0.20a
11	2.90±0.04a	1.54±0.05b
12	4.17±0.05a	1.27±0.01b

Note: Different letters in the same row indicate significant differences (t-test, $p<0.05$, mean ± S.E., n= 3).

Bacterial and fungal sequencing statistics

Illumina-based analysis of the V3-V4 hypervariable region of bacterial 16S rDNA gene,1402419 total reads were generated, and 475844 valid reads were obtained. Total reads and valid reads in Par_S were 738371 and 211401, respectively; In Par_W, the corresponding reads were 664048 and 264443, respectively. For the V4 hypervariable region of fungal 18S rDNA gene, 2152962 total reads were generated, and 1437104 valid reads were obtained. Total reads and valid reads in Par_S were 1131045 and 715621, respectively. In Par_W, the corresponding reads were 1021917 and 721483, respectively. Based on a 97% similarity, 6974 bacterial and 6468 fungal diversity OTUs were generated from the two samples, respectively. Par_S possessed 4088 bacterial OTUs and 3785 fungal OTUs. Par_W obtained 4607 bacterial OTUs and 4526 fungal OTUs (Figure 2). For bacteria, 1721 OTUs were found in both Par_S and Par_W, while 2367 and 2886 OTUs were only found in Par_S and Par_W, respectively. Regarding Fungi, 1843 OTUs were identified in both Par_S and Par_W, while 1942 and 2683 OTUs were only identified in Par_S and Par_W, respectively.

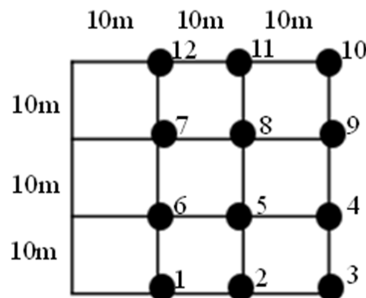

Figure 1. Distribution of the sample plots

Microorganism community

According to the results of species annotation, the biological classification was carried out according to Domain, Phylum, Class, Order, Family, Genus, and Species. The bacteria identified in all the samples were classified into 43 phyla and 683 genera, and the fungi were 65 phyla and 532 genera. In Par_S, the bacteria included 36 phyla 348 genera, and the fungi were 58 phyla 197 genera. In Par_W, the bacteria were classified into 39 phyla 471 genera, and the fungi were 59 phyla 259 genera.

At the phylum level, the top 20 dominant bacterial phyla were Proteobacteria, Acidobacteriota, Chloroflexi, Methylomirabilota, Bacteroidota, Actinobacteriota, Gemmatimonadota, Myxococcota, Firmicutes, Nitrospirota, Planctomycetota, Patescibacteria, Latescibacterota, RCP2-54, Fusobacteriota, NB1-j, Desulfobacterota, Verrucomicrobiota, MBNT15, Elusimicrobiota and others. At the genus level, the top 20 dominant bacterial genera were *Sphingomonas, Rokubacteriales, Subgroup_2, SBR1031, MND1, RB41, mle1-7, Nitrospira, KD4-96, Vicinamibacteraceae, Bryobacter, A4b, Candidatus_Solibacter, Prevotella, KF-JG30-B3, Streptococcus, Bradyrhizobium, Latescibacterota, IMCC26256, Haliangium* and others (Figure 3).

In terms of fungi, the top 20 dominant phyla were Ascomycota, Phragmoplastophyta, Cercozoa, Nematozoa, Mucoromycota, Annelida, Arthropoda, Basidiomycota, Apicomplexa, Amoebozoa, Ciliophora, Cryptomycota, Ochrophyta, Schizoplasmodiida, Incertae_Sedis, Platyhelminthes, Peronosporomycetes, Gracilipodida, Vertebrata, Rotifera and others. The top 20 dominant fungal genera were *Archaeorhizomyces, Magnoliophyta, Haplotaxida, Triplonchida, Mortierella, Acari, Euamoebida, Tylenchida, Gregarinasina, Cercomonas, Dorylaimida, LKM11, Thecofilosea, Collembola, Heteromita, Sebacinaceae, Schizoplasmodiida, Wilcoxina, Vampyrellidae, Arboramoeba,* and others (Figure 4).

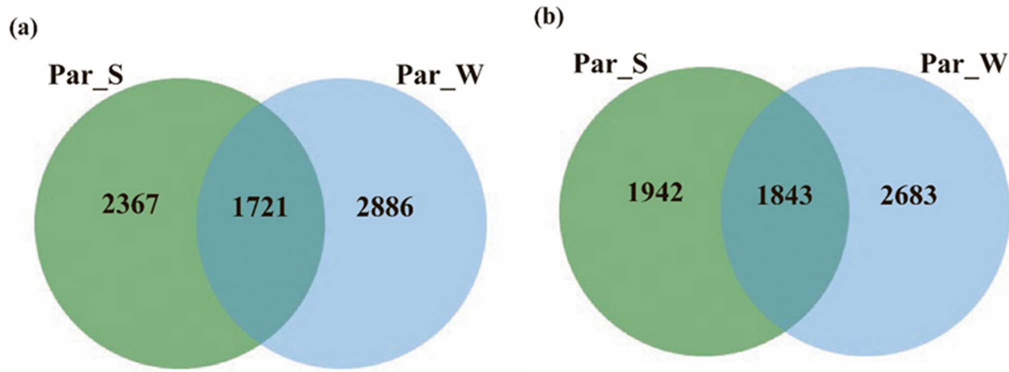

Figure 2. Numbers of operational taxonomic units (OTUs) in Par_S and Par_W samples for 16S rDNA (a) and 18S rDNA (b) sequencing

Note: Par_S: growing season; Par_W: non-growing season; the same below.

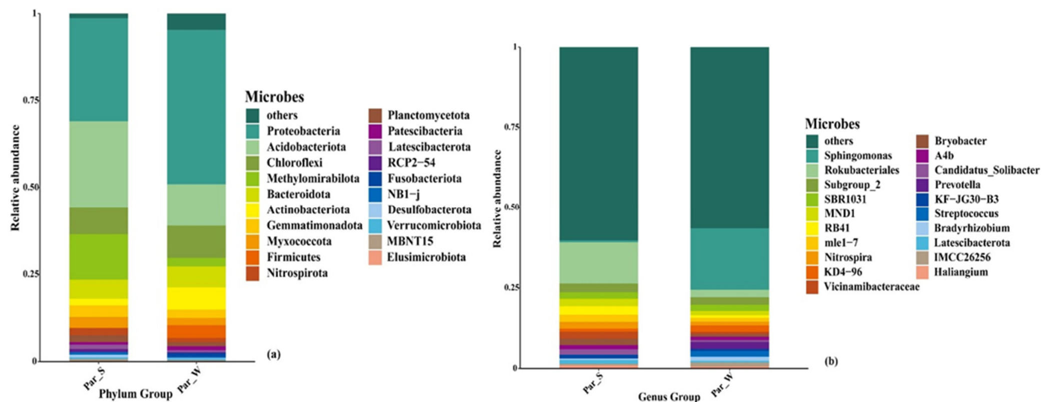

Figure 3. Relative abundance of the top 20 abundant bacterial phyla (a) and genera (b) in Par_S and Par_W samples based on 16S rDNA sequencing (sequences that could not be classified to any known group were labeled "others")

The relative abundance of most bacteria and fungi differed significantly between Par_S and Par_W. Most of the top 35 abundant bacterial genera were enriched in Par_S. Among them, *Subgroup_17, Nitrospira, Puia*, and *Latescibacterota* were the most abundant genera in Par_S, and *Sphingomonas, Alloprevotella, Prevotella, Streptococcus*, and *AD3* were the most abundant genera in Par_W. On the contrary, most of the top 35 abundant fungal genera were distributed in Par_W. Among them, *Harpacticoida, Dorylaimida, Euamoebida, Acari, Elev-18S-1089, Haplotaxida,* and *Collembola* were the most abundant genera in Par_S, and *Rhabditida, Thecofilosea, Mortierella, Sebacinaceae, Mammalia, LKM11, Endogone,* and *Limnomedusae* were the most abundant in Par_W (Figure 5).

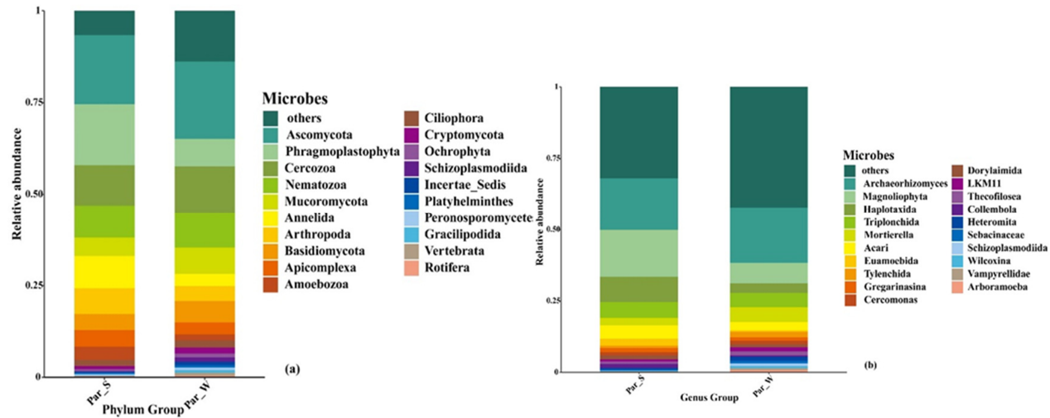

Figure 4. Relative abundance of the top 20 abundant fungal phyla (a) and genera (b) in Par_S and Par_W samples based on 18S rDNA sequencing (sequences that could not be classified to any known group were labeled "others")

Alpha diversity indices

The Goods_coverage of bacteria and fungi in both Par_S and Par_W was around one, indicating a reasonable sequencing depth. The richness indices (Chao1 and ACE) and diversity indices (Shannon and Simpson) showed that the bacterial communities of Par_S were significantly greater ($P<0.05$) than Par_W in Chao1, ACE, and Shannon indices. The Simpson index was also greater in Par_S than Par_W, but the difference was insignificant ($P>0.05$). These results indicated that Par_S owned more abundant bacterial communities than Par_W. Regarding fungal communities, Par_S was significantly greater ($P<0.05$) than Par_W in Chao1 and ACE indices, but Shannon and Simpson indices of Par_S were significantly lower than Par_W ($P<0.05$; Table 4).

Table 3. Chemical properties in Par_S and Par_W

Chemical property	Season	
	Par_S (growing season)	Par_W (non-growing season)
Total nitrogen (TN)	2.33±0.07a	2.28±0.18a
Hydrolyzable nitrogen (HN)	215.42±5.35b	210.58±17.35a
Total phosphorus (TP)	1.43±0.04a	1.43±0.09b
Available phosphorus (AP)	37.54±5.42a	48.56±17.28b
Total potassium (TK)	8.35±0.15a	8.13±0.33b
Available potassium (AK)	131.69±7.87a	131.13±22.20a
Soil organic matter (SOM)	46.80±1.49a	48.44±4.69a

Beta diversity analysis

To further compare the microbiota among different samples, we performed PCoA on the relative abundance of bacterial and fungal genera (Figure 6). The results revealed that the microbial communities varied among Par_S and Par_W. For bacteria, Par_S had a significantly higher value of PC1 (the first component), and both Par_S and Par_W had similar values of PC2 (the second component). Par_S was separated distinctly from Par_W along PC1. As for fungi, Par_S had a slightly higher value of PC1 and PC2, but Par_S could not be separated distinctly from Par_W along both PC1 and PC2.

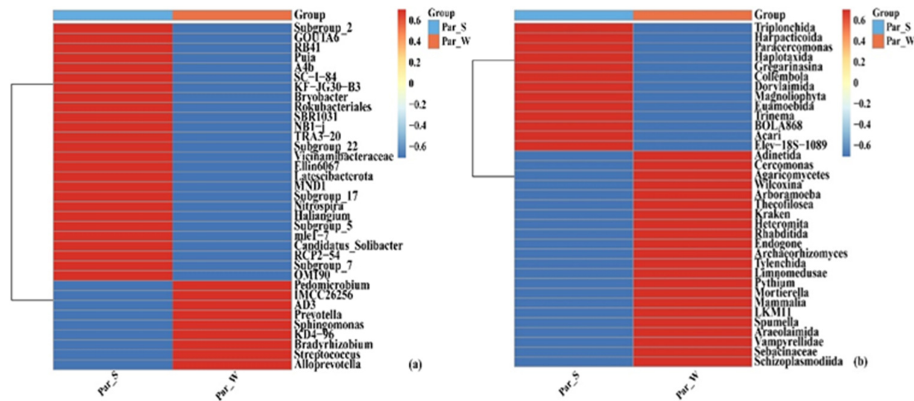

Figure 5. Heatmap analysis of the top 35 abundant genera distribution of bacteria (a) and fungi (b) from Par_S and Par_W samples based on relative abundances

Note: The figures were constructed on the basis of normalized *Z* values. The relative values for the microbial genera are depicted by the color intensity.

Effects of soil chemical properties on abundant genera

Eight soil properties were significantly correlated with the variations in the relative abundance of the top 10 bacterial and fungal genera (P<0.05) in Par_S and Par_W according to distance-based redundancy analysis (dbRDA). As shown by their close grouping and the vectors, *Rokubacteriales* and *RB41* were dominant among the top 10 bacterial genera in Par_S, and were related to the respiratory rate (RR) of the soil (Figure 7). While, *Sphingomonas* was dominant among the top 10 bacterial genera in Par_W, and no soil properties were significantly related to it. With regard to fungi, *Magnoliophyta, Haplotaxida* and *Acari* were dominant among the top 10 fungal genera in Par_S, and were related to the soil respiratory rate (RR), and the contents of total potassium (TK), hydrolysable nitrogen (HN), total phosphorus (TP) and available phosphorus (AP). While, *Archaeorhizomyces* was dominant among the top 10 bacterial genera in Par_W, and no soil properties were significantly related to it.

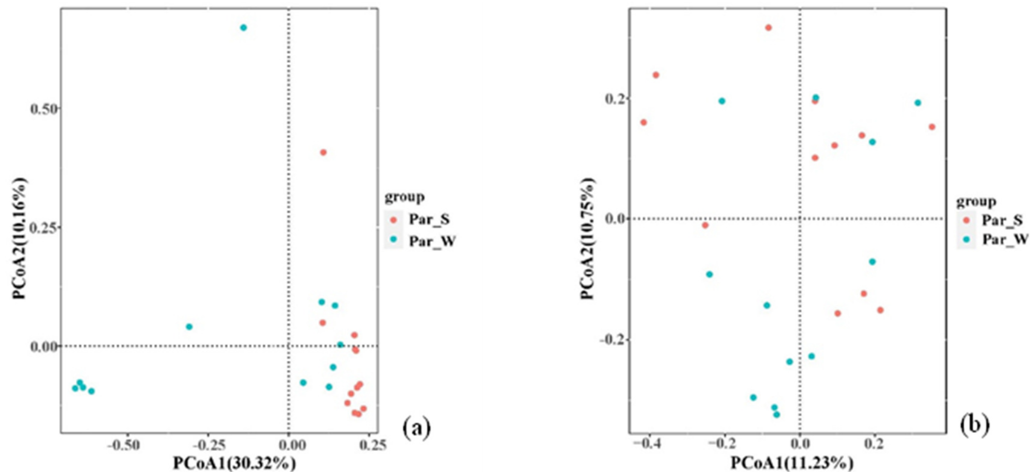

Figure 6. Principal coordinate analysis (PCoA) plots on the basis of 16S rDNA (a) and 18S rDNA (b) sequencing from Par_S and Par_W samples based the distance matrix calculated using the weighted UniFrac algorithm for samples

Note: The scatter plot is of principal coordinate1 (PC1) vs principal coordinate 2 (PC2). The percentage is the percentage of variation explained by the components.

Correlation analysis can reflect the relationship between microbiota community and environmental factors. For the top 10 abundant bacterial genera in Par_S, the relative abundance of *Nitrospira* was negatively correlated with the contents of TN and AK (P<0.05), and *MND1* was negatively correlated with SOM (P<0.05; Table 5). Regarding the top 10 abundant bacterial genera in Par_W, *SBR1031* was positively correlated with TP and AP (P<0.05), and *MND1* was positively correlated with AP (P<0.05). In terms of the top 10 abundant fungal genera in Par_S, only *Acari* had a positive correlation with TK, and no fungi showed significant correlations with the soil properties (Table 6).

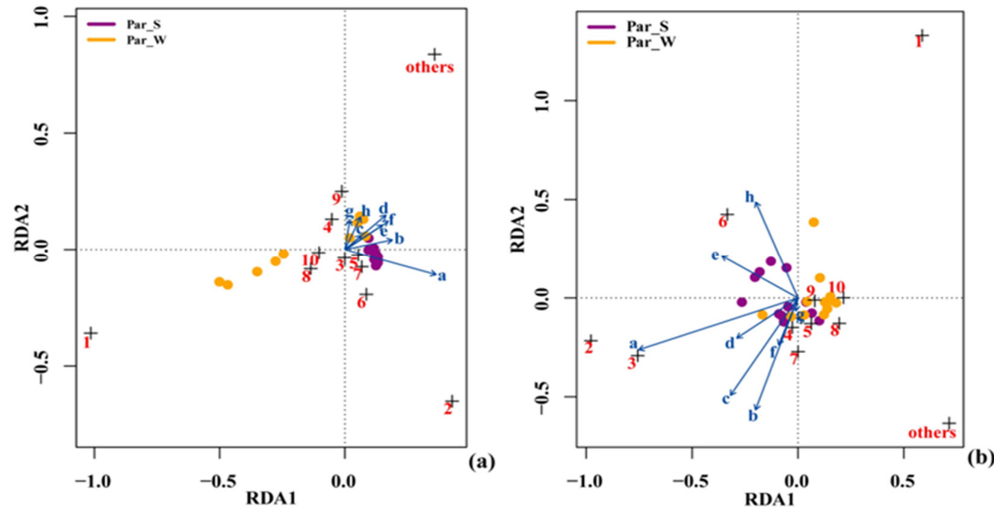

Figure 7. Distance-based redundancy analysis (dbRDA) based on the relative abundance of bacterial (a) fungal (b) genera and selected soil physicochemical properties in Par_S and Par_W samples.
Note: The selected soil physicochemical properties of bacterial and fungal genera include (a) RR, (b) TK, (c) HN, (d) AK, (e) TP, (f) TN, (g) SOM and (h) AP. The top 10 bacterial genera were: (1) *Sphingomonas*, (2) *Rokubacteriales*, (3) *Subgroup_2*, (4) *SBR1031*, (5) *MND1*, (6) *RB41*, (7) *mle1-7*, (8) *Nitrospira*, (9) *KD4-96* and (10) *Vicinamibacteraceae*. The top 10 fungal genera were: (1) *Archaeorhizomyces*, (2) *Magnoliophyta*, (3) *Haplotaxida*, (4) *Triplonchida*, (5) *Mortierella*, (6) *Acari*, (7) *Euamoebida*, (8) *Tylenchida*, (9) *Gregarinasina* and (10) *Cercomonas*.
RR: respiratory rate; TN: total nitrogen; HN: hydrolysable nitrogen; TP: total phosphorus; AP: available phosphorus; TK: total potassium; AK: available potassium; SOM: soil organic matter.

Table 4. Alpha diversity indices of soil bacteria and fungi in Par_S and Par_W

		Par_S	**Par_W**
ACE index	Bacteria	748.78±38.44a	579.46±93.87b
	Fungi	670.67±68.57a	654.59±127.25b
Chao1 index	Bacteria	748.70±38.44a	579.33±93.86b
	Fungi	670.67±68.57a	654.63±127.24b
Shannon index	Bacteria	8.93±0.07a	7.18±0.55b
	Fungi	5.77±0.29b	6.69±0.30a
Simpson index	Bacteria	1.00±0.00a	0.91±0.03a
	Fungi	0.92±0.01b	0.97±0.01a
Goods_coverage	Bacteria	1	1
	Fungi	1	1

Note: Different letters in the same column indicate significant differences (*P*<0.05, Duncan's, mean ± S.E., n= 12).

Table 5. Pearson's correlations (r) between the top 10 abundant bacterial genera and soil properties

Season	Genus	TN	TP	TK	HN	AP	AK	SOM	RR
Par_S	*RB41*	-0.467	-0.298	0.022	-0.293	-0.392	-0.498	-0.533	-0.094
	Subgroup_2	-0.226	-0.383	0.001	-0.320	-0.037	0.115	-0.311	0.124
	KD4_96	-0.510	-0.472	-0.040	-0.469	-0.156	-0.561	-0.171	0.315
	SBR1031	-0.284	-0.390	0.141	-0.365	-0.189	-0.026	-0.386	0.071
	Rokubacteriales	-0.109	-0.386	-0.307	-0.101	-0.324	-0.333	-0.256	0.143
	Nitrospira	-0.706*	-0.546	-0.184	-0.569	-0.290	-0.604*	-0.348	-0.090
	MND1	-0.436	-0.351	-0.332	-0.428	-0.433	-0.361	-0.614*	0.112
	Vicinamibacteraceae	0.035	-0.008	-0.374	-0.004	-0.172	-0.289	-0.042	0.201
	Sphingomonas	-0.139	-0.183	0.046	-0.219	-0.043	0.077	-0.248	0.045
	mle1_7	-0.021	-0.417	0.113	0.053	-0.472	0.072	-0.309	-0.285
	Mean value	-0.286	-0.343	-0.091	-0.272	-0.251	-0.241	-0.322	0.054
Par_W	*RB41*	0.364	0.548	-0.041	0.194	0.469	0.413	0.523	-0.060
	Subgroup_2	-0.390	0.273	0.269	-0.431	0.310	-0.288	-0.272	-0.010
	KD4_96	-0.181	0.338	-0.365	-0.371	0.201	-0.227	-0.162	-0.315
	SBR1031	0.024	0.665*	0.309	-0.089	0.642*	0.052	0.141	0.097
	Rokubacteriales	-0.057	0.347	0.095	-0.219	0.409	0.079	0.070	-0.152
	Nitrospira	-0.024	0.271	0.113	-0.136	0.332	0.173	0.038	-0.238
	MND1	0.222	0.576	0.295	0.008	0.667*	0.274	0.318	0.133
	Vicinamibacteraceae	0.174	0.472	-0.108	-0.031	0.442	0.237	0.303	-0.245
	Sphingomonas	-0.260	-0.060	-0.222	-0.093	-0.159	-0.373	-0.182	0.236
	mle1_7	-0.125	0.231	0.124	-0.292	0.318	-0.004	0.003	-0.093
	Mean value	-0.025	0.366	0.047	-0.146	0.363	0.034	0.078	-0.065

Note: *Correlation is significant at the $P<0.05$. **Correlation is significant at the $P<0.01$. TN: total nitrogen; HN: Hydrolysable nitrogen; TP: total phosphorus; AP: available phosphorus; TK: total potassium; AK: available potassium; SOM: soil organic matter; RR: respiratory rate. The same below.

Table 6. Pearson's correlations (r) between the top 10 abundant fungal genera and soil properties

Season	Genus	TN	TP	TK	HN	AP	AK	SOM	RR
Par_S	*Euamoebida*	-0.336	-0.496	0.149	-0.269	-0.342	-0.202	-0.469	0.262
	Magnoliophyta	0.151	0.441	-0.178	0.188	-0.055	-0.092	0.148	-0.039
	Tylenchida	0.199	0.176	0.058	0.022	0.222	0.080	0.310	-0.442
	Haplotaxida	-0.460	-0.487	0.135	-0.140	-0.394	-0.180	-0.496	0.364
	Triplonchida	0.180	0.054	0.242	-0.010	0.384	0.500	0.043	-0.229
	Gregarinasina	0.238	-0.238	0.305	0.231	-0.163	0.148	0.028	-0.074
	Acari	0.119	0.057	0.611*	0.008	-0.177	-0.068	0.215	-0.336
	Mortierella	0.233	0.289	0.275	0.117	0.467	0.496	0.109	-0.564
	Archaeorhizomyces	0.121	-0.087	-0.332	0.105	-0.123	-0.135	0.014	0.458
	Cercomonas	-0.291	-0.415	-0.050	-0.226	-0.298	-0.179	-0.412	-0.158
	Mean value	0.015	-0.071	0.122	0.003	-0.048	0.037	-0.051	-0.076
Par_W	*Euamoebida*	0.202	-0.036	0.197	0.002	0.019	0.267	0.013	-0.323
	Magnoliophyta	0.023	-0.476	-0.408	-0.109	-0.464	-0.241	0.033	-0.290
	Tylenchida	-0.511	-0.301	-0.171	-0.367	-0.354	-0.435	-0.515	-0.449
	Haplotaxida	-0.065	-0.415	-0.598	-0.266	-0.430	-0.271	0.037	-0.243
	Triplonchida	-0.122	0.075	0.249	-0.376	0.362	-0.030	-0.211	-0.122
	Gregarinasina	-0.135	-0.364	-0.011	-0.103	-0.329	-0.322	-0.189	-0.303
	Acari	0.258	-0.275	-0.403	0.248	-0.290	0.068	0.125	-0.415
	Mortierella	-0.035	0.267	0.417	-0.218	0.454	0.094	-0.140	-0.069
	Archaeorhizomyces	-0.348	0.083	0.247	-0.258	0.040	-0.313	-0.300	-0.098
	Cercomonas	0.286	-0.139	-0.122	-0.014	-0.040	0.248	0.135	-0.355
	Mean value	-0.025	0.366	0.047	-0.146	0.363	0.034	0.078	-0.065

Discussion

Soil microbial communities are susceptible to climatic changes due to seasonal alternations (Fu and Shen, 2022). Previous studies have shown that seasonal variation pronouncedly affects soil microbial community structure in *P. massoniana* forests (Luo *et al.*, 2020). Microbial biomass and respiration were higher in warm-season turf systems than in cool-season ones (Chen *et al.,* 2021). Different vegetation types combined with seasonal effects affected microorganisms' carbon metabolism and enzyme activities in wetland ecosystems (Wu *et al.,* 2021). However, it has also been shown that the impact of seasonally induced climate change on plant-microbe-nutrient interactions still needs to be clarified and requires more detailed studies (Meena *et al.,* 2023). Plant-climate relationships vary by location, especially in mountainous areas (Bi *et al.,* 2020). The response of plants to seasonal variation can only be correctly assessed if the characteristics of the environment in which the species live are well understood (Cao *et al.*, 2019). In the past, however, the effects of human activities on soil microorganisms, such as the effects of agrochemicals on soil microorganisms, have been studied more frequently (Mandal *et al.*, 2020). The impact of vegetation degradation on soil microorganisms has been studied a lot as well (Wu *et al.,* 2020a). However, few studies have examined the effects of seasonal differences on forest soil properties, such as microorganism communities.

In this study, we identified microbiotic communities in soil from different seasons (Par_S and Par_W) by high-throughput amplicon sequencing. 2367 and 2886 bacterial OTUs were only found specifically in Par_S and Par_W, respectively. The corresponding values in fungi were 1942 and 2683 OTUs, respectively. That means bacterial communities were more abundant than fungi, consistent with the previous literature (Anthony *et al.,* 2023). Meantime, Alpha diversity indices (ACE, Chao1, Shannon, Simpson) showed that Par_S had higher bacterial diversity and lower fungal diversity, while Par_W showed lower bacterial diversity and higher fungal diversity. It is consistent with earlier observations that a metabolic division of labor leads to complementarities among microorganisms (Deveau *et al.*, 2018; Wagg *et al.*, 2019).

Previous studies found that soil bacterial abundance increased significantly with increasing temperature (Wang *et al.,* 2015). Moreover, soil fungal communities vary with altitude and soil cover (Ren *et al.*, 2018; Větrovský *et al.*, 2019). Between Par_S and Par_W, not only the microorganism communities were remarkably different, but their relative abundance significantly differed as well (Figure 3 and Figure 5). For bacteria, Proteobacteria and Acidobacteria were the most abundant phylum in both Par_S and Par_W, identical to previous studies (Li *et al.*, 2014; Zhang and Wang, 2017). For instance, Miyashita (2015) found that Proteobacteria and Acidobacteria dominated bacterial communities in different geographic regions and soil types. The relative abundance of Methylomirabilota in Par_S was obviously higher than in Par_W. At the genus level, the dominant genus was *Rokubacteriales* in Par_S, but in Par_W was *Sphingomonas*. Regarding fungi in our results, Ascomycota was the most abundant phylum in both Par_S and Par_W, consistent with previous studies (Yang *et al*., 2020). The relative abundance of Phragmoplastophyta, Annelida and Arthropoda in Par_S was obviously higher than Par_W. Although the abundance of Basidiomycota in this ecosystem is lower than that of Ascomycota, they are two common fungal phyla found in abundance in soil (Johnston *et al*., 2019; Nakayama *et al*., 2019). At the genus level, the dominant genus was *Archaeorhizomyces* and *Magnoliophyta* in both Par_S and Par_W. The relative abundance of *Haplotaxida* in Par_S was remarkably higher than Par_W.

The results of PCoA analysis showed that Par_S and Par_W could be better separated in bacteria but not in fungi, indicating a different bacterial community structure between them. The dbRDA analysis found that the top 10 bacterial genera (*Rokubacteriales* and *RB41*) in Par_S were related to RR of the soil, and the top 10 fungal genera (*Magnoliophyta*, *Haplotaxida* and *Acari*) in Par_W were related to RR, TK, HN, TP and AP. Soil respiration correlates well with soil temperature, increasing with temperature (Yan *et al.*, 2019). While, the correlation analysis did not find significant correlationship between RR, TK, HN and the top 10 abundant

bacterial/fungal genera (P>0.05). Meantime, the eight soil properties were only significantly correlated with few of the top 10 abundant bacterial/fungal genera (P<0.05). For instance, significant correlationship in bacteria was found between TN, AK and *Nitrospira*, between SOM, AP and *MND1*, between TP, AP and *SBR1031*. In fungi, significant correlationship was only found between TK and *Acari* in Par_S. Previous reported that N-induced soil factors, such as nitrogen and carbon contents, negatively correlated with fungal diversity (Bayranvand *et al.*, 2021). While, nitrogen and carbon contents seems were not significantly correlated with the ralative abundance of fungi indicated by the correlation indice between TN, HN and SOM and the top 10 abundant fungal genera in our study. Compared to Par_W, Par_S exhibited higher correlationship with most of the eight soil properties in bacteria (Table 5), but lower correlationship with most of the eight soil properties in fungi (Table 6).

Conclusions

In Par_S, we identified 36 phyla 348 genera of bacteria, and 58 phyla 197 genera fungi. Par_W's corresponding values were 39 phyla 471 genera and 59 phyla 259 genera, respectively. Par_S owned more abundant bacterial communities than Par_W. The relative abundance of most bacteria and fungi differed significantly between Par_S and Par_W. Most of the top 35 abundant bacterial genera and fungal genera were enriched in Par_S and Par_W, respectively. The soil properties differed significantly between Par_S and Par_W. They were significantly correlated with the variations in the relative abundance of the top 10 bacterial and fungal genera in both Par_S and Par_W. *Rokubacteriales* and *RB41* were dominant among Par_S's top 10 bacterial genera, and were related to the RR of the soil. *Sphingomonas* was dominant among Par_W's top 10 bacterial genera. *Magnoliophyta*, *Haplotaxida* and *Acari* were dominant among Par_S's top 10 fungal genera, and were related to RR, TK, HN, TP and AP. *Archaeorhizomyces* was dominant among Par_W's top 10 bacterial genera. For the top 10 abundant bacterial genera in Par_S, the relative abundance of *Nitrospira* was negatively correlated with the contents of TN and AK, and *MND1* was negatively correlated with SOM. Regarding the top 10 abundant bacterial genera in Par_W, *SBR1031* was positively correlated with TP and AP, and *MND1* was positively correlated with AP. Regarding the top 10 abundant fungal genera in Par_S, only *Acari* had a positive correlation with TK.

Authors' Contributions

HC and MZ performed the study, analyzed the data, and edited the manuscript. WH, WT, DF and HL collected and analyzed the data. HC and KD designed the experiments and revised the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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