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Seasonal variations of soil fungal diversity and communities in subalpine coniferous and broadleaved forests

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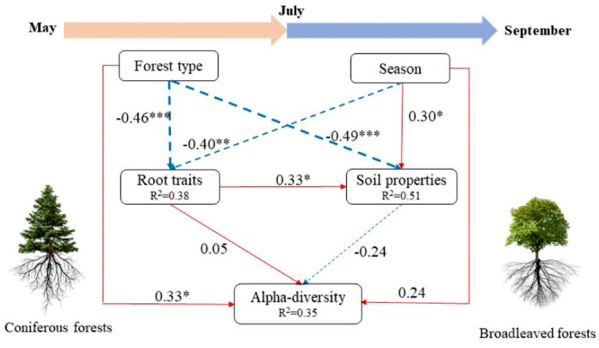
HIGHLIGHTS

- Soil fungal diversity and richness in broadleaved forests were higher than in conifer forests.
- Distinct differences in fungal community composition were observed across forests and seasons.
- Forest type and season affected soil fungal communities by altering soil properties and root variables.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil fungi have essential roles in ecosystems, but the seasonal dynamics of soil fungal communities in forests remain unclear. To explore the pattern and variation of soil fungal community diversity and structural composition across forest types and seasons, and identify the main contributors to soil fungal communities, we collected soil samples from subalpine coniferous (*Picea asperata* and *Larix gmelinii*) and broadleaved plantations (*Betula albosinensis* and *Quercus aquifolioides*) in southwest China in different seasons. Soil fungal community structural composition was determined using the Illumina MiSeq sequencing platform. The results showed that soil fungal diversity and richness in broadleaved forests were higher than in conifer forests. From heatmap cluster analysis, distinct differences in fungal community composition among forest types (coniferous and broadleaved forests) and seasons (May and July, September) were observed. Fungal communities were dominated by Basidiomycota and Ascomycota regardless of forest type and season. Helotiales and Atheliales were abundant in coniferous forests, while Agaricales, Russulales and Thelephorales predominated in broadleaved forests. Fungal community diversity and composition were significantly driven by soil pH, moisture, organic carbon, ammonium (NH_4^+-N), fine root biomass and root tissue density, when controlling for the effects of forest type and season. Thus, forest type and season significantly affected soil fungal community diversity and composition by altering soil properties and root variables.

1. Introduction

As major taxa of soil biome, soil fungi are highly abundant and diverse in terrestrial ecosystems and comprise many eukaryotic microbes which form the important component of soil microbial communities (Tedersoo

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et al., 2014). They play critical roles in ecosystem functions, including regulate soil nutrient cycling (Leff et al., 2018), improve nutrient acquisition for plants (Selosse and Rousset, 2011) and affect plant biomass and productivity (Tedersoo et al., 2014; Matsuoka et al., 2018). Studies have shown that soil fungi also regulate the composition and biodiversity of plant communities (Bittebiere et al., 2020; Sweeney et al., 2021), whereas soil fungal diversity and structure depend on host plant performance (Öpik et al., 2013). As such, exploring fungal community variations across plant species and identifying variation drivers are critical for understanding plant-soil-microbial interactions.

In forest systems, forest type is a fundamental factor affecting soil fungal community and controlling related ecosystem functions (Leff et al., 2018; Porazinska et al., 2018; Sweeney et al., 2021). Forest type can alter soil physicochemical properties (nutrient availability and pH) and affect the composition and assembly of soil fungal community as a result of different growth rates, litters, and root exudates (Geml and Wagner, 2018; Ji et al., 2021). The influence of forest type on soil fungal community highly depends on the quality and quantity of forest litters (root or leaves) (Yang et al., 2020; Gong et al., 2021). For instance, litters of broadleaved forests are generally labile and easy to decompose with low carbon/nitrogen ratios, while conifer forests have recalcitrant litters (Augusto et al., 2015; Dawud et al., 2017). Thus, compared with conifer forests, broadleaved forests with higher nutrient availability and improved soil microenvironments can consequently provide more favourable environments for fungi and increase fungal community diversity (Deng et al., 2019; Ji et al., 2021; Sweeney et al., 2021). Additionally, symbiotic relationships between plant roots and soil fungi (e.g. mycorrhiza) (Smith and Read, 2010) also determine soil fungal community by altering root variables (biomass, length and diameter) and forming soil fungi-dominated networks, which are strongly related to nutrient cycling and litter decomposition (Fierer, 2017; Jiang et al., 2017; Sweeney et al., 2021). Although many studies have compared broadleaved and conifer forests in subalpine areas, including ecological functions, soil properties and physiological traits (Augusto et al., 2015; Dawud et al., 2017; Luo et al., 2017), the effects of forest type on soil fungal community diversity and composition, and main determining factors in subalpine systems are less understood.

Seasonality also influences soil fungal community in forest ecosystems, mainly due to phenological forest alterations (Lugo and Cabello, 2002; Unuk et al., 2019). Seasons usually change climatic conditions and affect nutrient cycling by modifying plant physiology, e.g. autumn and winter are associated with a high input of plant residues (i.e. dead roots and litter) to soil (Rasche et al., 2011; Siles and Margesin, 2017). Tree growth rates and soil fungi metabolic activities tend to be higher in vigorous growing seasons than cold and dry seasons due to adequate water and favourable temperatures in July (Montagnoli et al., 2014; Ji et al., 2021), which can cause more complex and sensitive variations of soil characteristics and fungal community (Ji et al., 2021; Han et al., 2021). Furthermore, connections between plants and soil fungi significantly decline in non-growing seasons (Smith and Read, 2010; Bennett et al., 2013). Taken together, season-related environmental variables and seasonal variations in host plant performance can affect fungal composition by directly controlling fungal activities and indirectly affecting soil properties (Koranda et al., 2013; Yao et al., 2017a; Reyes et al., 2019). Bainard et al. (2014) reported that forest type, soil available phosphate and moisture were key factors driving soil fungal community variation among seasons.

Subalpine forest ecosystem in western Sichuan is located on the eastern Tibetan Plateau, which covers an extensive area with high biodiversity in the transition zone from the Qinghai-Tibet plateau to the Sichuan basin. Since the 1950's, due to serious deforestation and disturbance, most primary forests were clear-felled and degraded to secondary forests and shrubs (Pang et al., 2011). These areas were then replaced by pure restoration plantations, which provided a full range of ecological services (Pang et al., 2004; Wang, 2004; Wang and Wang, 2007). In these plantations, *Betula albosinensis* Burk., *Quercus aquifolioides* Rehd. et Wils. and *Betula platyphylla* Suk. are the main broadleaved species, while *Picea asperata* Mast., *Larix gmelinii* (Rupr.) Kuzen. and *Abies fabri* Mast. Craib. are mid-to-late successional coniferous species. While few studies have focused on soil fungal community of conifer forests in this region (Guo et al., 2021; Xie et al., 2021), it remains unknown whether soil fungal community are associated with forest type (i.e. broadleaved and coniferous forests).

To better understand how forest type and season affect the diversity and composition of soil fungal community, soil samples were collected from four forest types over three seasons (May, July and September) in subalpine forest in southwest China. For soil fungi are host specific (Öpik et al., 2013; Tedersoo et al., 2014), their community structures and functions would be different across forest type and season. Therefore, we hypothesized that: (1) broadleaved forests would promote soil fungal diversity for improved soil microenvironments (nutrient availability and pH) due to easy decomposing litters compared with conifer forests. (2) Variations in soil and root characteristics and fungal community diversity would be more complex in July than in May and September because of favourable temperature and humidity conditions and (3) both forest type and season would alter soil fungal composition.

2. Materials and methods

2.1. Study area

The experiment was surveyed near Maoxian Mountain Ecosystem Research Station (31°42′ N, 103°54′ E, 1820 m a.s.l.), Chengdu Institute of Biology, Chinese Academy of Sciences in Sichuan province, China. This area has a montane temperate climate and the growing season is from late April to late October. Mean annual temperature, precipitation and pan evaporation are 9.3 °C, 825 mm and 969 mm, respectively. More than 70 % of rainfall occurs between May and September. We selected four typical plantation forest types in this region: 30–40 years old evergreen conifers (*P. asperata*), deciduous conifers (*L. gmelinii*), evergreen broadleaved forest (*Q. aquifolioides*) and a deciduous broadleaved forest (*B. platyphylla*) (Fig. 1). In these forests, trees were planted after clear-cutting in the 1980's. Soils in all forests was classified as Calcic Luvisol according to IUSS Working Group WRB classification (WRB, 2014). The understory in forests was dominated by herbs and grasses, e.g. *Heracleum hemsleyanum*, *Impatiens potaninii*, *Impatiens textorii*, *Lamium barbatum*, *Morus australis*, *Polygonum sieboldii*, *Thalictrum aquilegiifolium* and *Rubus setchuenensis*.

2.2. Sampling

In April 2021, about 1 ha plantation area was selected for each forest type, dominated by *P. asperata*, *L. gmelinii*, *Q. aquifolioides* and *B. platyphylla*, respectively. The distance between forests was >1 km. In each forest type, four plots (10 m × 10 m) were established, with intervals of 10–20 m (Han et al., 2021). To avoid edge effects, plots were at least 5 m away from boundaries. For each forest type, ten trees in each plot were randomly selected to measure the diameter at breast height and height (Table 1). Soil samples from plots were collected on May 10th, July 20th and September 20th 2021 and represented early, middle and late growing seasons. Five soil cores, from four corners and the central point in each plot, were collected using a steel drill (5 cm in diameter and 15 cm long) after removing the litter layer, and mixed as one sample. Therefore, 48 samples (4 plots × 4 forest types × 3 seasons) were obtained. Soil samples were sieved through a 2-mm mesh and divided into two subsamples. One subset was transported back to the laboratory at 4 °C to analyse soil properties, while the other was stored immediately at −80 °C for DNA analysis. Root samples in the steel drill were collected, mixed for each plot and taken back to the laboratory at 4 °C to analyse root variables.

2.3. Soil properties and root variables determination

Soil pH was measured in a 1:2.5 (m/v) soil-to-water extract by a digital pH metre (pH 700, Eutech, Singapore). Soil moisture was determined by

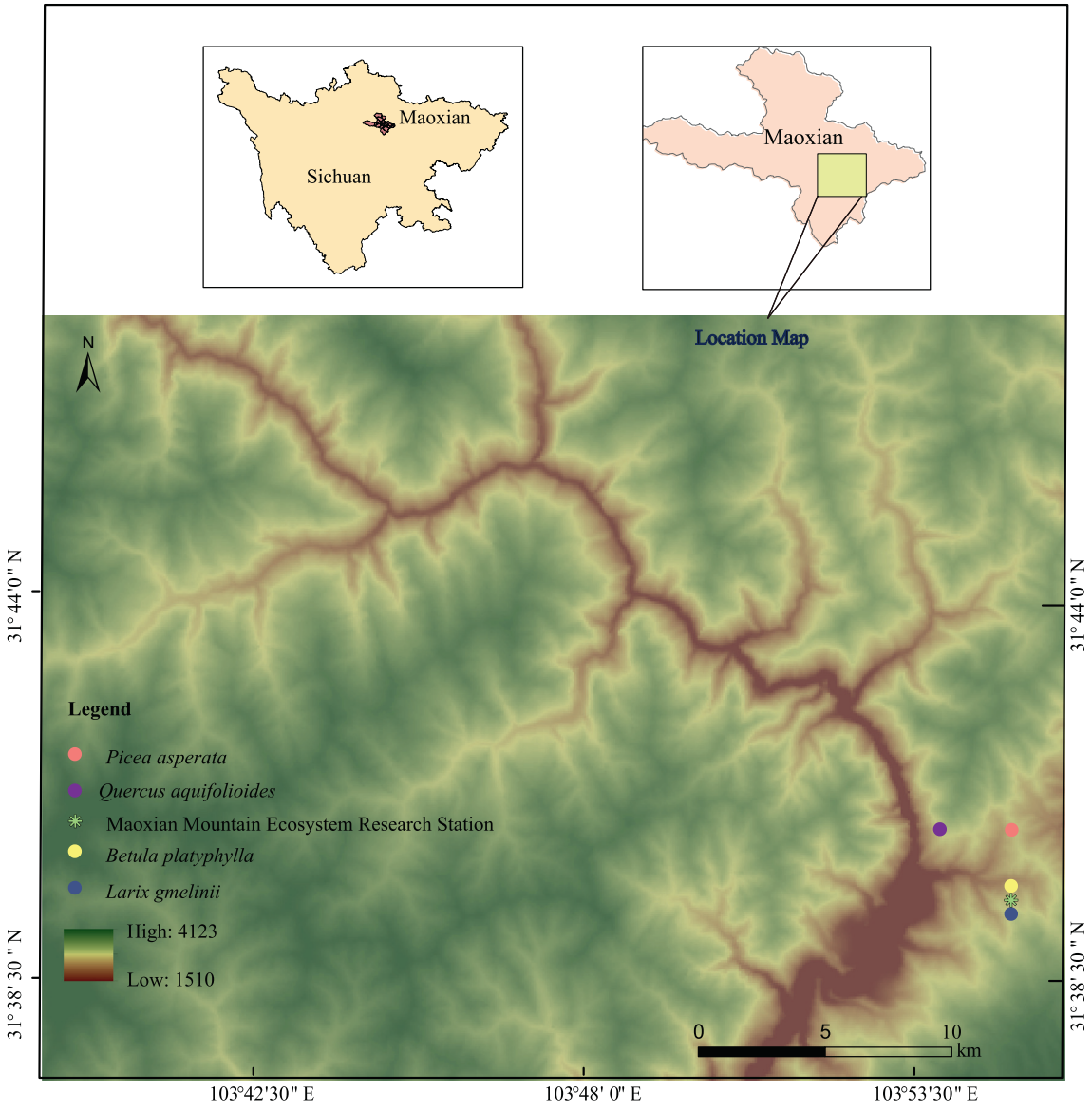

Fig. 1. Location of sampling forest types.

measuring moisture loss after drying samples at 105 °C for 24 h. Soil nitrate (NO_3^--N) and ammonium (NH_4^+-N) were measured using an AutoAnalyser III (SEAL Analytical, Germany). Soil organic carbon (SOC) and total nitrogen (TN) were determined using a Vario MACRO cube Elementary Analyser (Elementar Analysensysteme Vario MACRO Cube, Germany) at 850 °C (Wang et al., 2020).

Roots samples were cleaned by soaking overnight, sieved through a 0.1-mm mesh and roots <2 mm in diameter were carefully dissected and distinguished (Lwila et al., 2021). Living roots were separated from dead roots based on turgescence and colour (Schmid, 2002; Hertel et al., 2013), and then were identified and scanned at 600 dpi using a scanner (Epson EU-88; Seiko Epson Corp., Japan) according to a previous method (Spitzer et al., 2020). The obtained images were processed with WinRHIZO Pro 2007 software (Régent Instruments, Quebec, Canada) to get root length, surface area and volume. After scanning, root samples were oven dried at 60 °C to constant weight to calculate fine root biomass (FB, g m^{-2}). Root tissue density (RTD, g cm^{-3}) was calculated as the root dry biomass divided by the root total volume.

Table 1
Description of the sampling forest types (mean ± SE, n = 4).

Forest type	Longitude	Latitude	Altitude (m. a.s.l)	Density (stems ha^{-1})	Height (m)	DBH (cm)
Picea asperata	103°53′ E	31°42′ N	2140	800–1000	17.5 ± 1.50	27.1 ± 1.75
Larix gmelinii	103°53′ E	31°41′ N	2120	1000–1200	16.1 ± 2.10	22.9 ± 2.08
Betula albosinensis	103°53′ E	31°41′ N	2010	800–1000	14.2 ± 2.24	24.2 ± 2.15
Quercus aquifolioides	103°51′ E	31°42′ N	2420	1000–1200	13.7 ± 2.18	23.1 ± 1.16

DBH: diameter at breast height.

Specific root length (SRL) and area (SRA) were calculated as the ratio of root length and surface area to root biomass, respectively (Pérez-Jaramillo et al., 2017).

2.4. Illumina MiSeq sequencing and bioinformatics processing

Fungal DNA was extracted from soil using the E.Z.N.A® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following manufacturer's instructions. DNA was sequenced using an Illumina MiSeq sequencing system (Thermo Scientific, Wilmington, USA, 2 × 300 base pairs (bp)). Soil fungal internal transcribed spacer (*ITS1*) region was amplified using ITS1F (5′-CTTGGT CATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCATCGATGC-3′) primers (Adams et al., 2013). PCR was performed as follows: initial heating to 95 °C for 3 min, followed by 32 thermal cycles (30 s at 95 °C, 30 s at 55 °C and 45 s extension at 72 °C) and concluded by a 10 min final auto-extension at 72 °C. PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and amplicons were pooled in equal amounts. Paired-end sequencing (2 × 300 bp) was performed using standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) on the Illumina MiSeq sequencing platform (Illumina, San Diego, USA).

The Illumina sequencing data were analysed using FastQC to evaluate original paired-end sequence quality and Quantitative Insights Into Microbial Ecology (QIIME) software was used to filter original data to remove sequences with uncertain bases and an average quality less than Q20 (Caporaso et al., 2010). Sequences were then assigned by barcode (mismatch = 0) using the FASTX-toolkit and quality controlled in QIIME (parameters: max ambigs = 6; max homop = 6; min length = 150; max length = 400), to exclude short and low-quality sequences. Chimeric sequences were removed using Usearch (version 7.0 http://drive5.com/uparse/) and paired ends were merged using FLASH (Magoč and Salzberg, 2011). Operational taxonomic units (OTUs) with 97 % similarity cut-offs were performed using UPARSE (version 7.1 http://drive5.com/uparse/), assigned to taxa using the classification (UNITE) database and subsequently normalised by the minimum number of reads (Abarenkov et al., 2010; Kõljalg et al., 2013). Alpha-diversity of fungal communities, including OTU richness (Sobs), ACE, Shannon's diversity and Simpson's diversity indices, and also phylogenetic diversity were calculated using the Mothur program (version 1.30.2) (Schloss et al., 2009).

2.5. Data analysis

All statistical analyses were performed in R version 3.6.2 (R Development Core Team., 2019). Variance homogeneity and data normality were tested before statistical analyses and log-transformation was used if necessary. Two-way repeated measures ANOVA was used to evaluate the effects of forest type and season on alpha-diversity index, root variables and soil properties. Mean multiple comparisons were then conducted using *post-hoc* tests. Pearson's correlation analyses were used to assess the relationship between fungal alpha-diversity and soil properties and root variables. A collinearity analysis was conducted for all soil and root variables to avoid inter-correlations among variables prior to further data analysis (variance inflation factor <4) (Table S1) (Han et al., 2021).

Venn diagram was constructed to show the distributions of shared and unique OTUs across forest types and seasons. Ordination by nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity ('vegan' package) was conducted to analyse fungal community composition dissimilarity across forest types and seasons. Kruskal–Wallis H tests (fdr adjustments) were used to compare the relative abundance of fungal taxa across forest types and seasons. Redundancy analysis (RDA) was performed to analyse fungal community distribution patterns and test the main factors

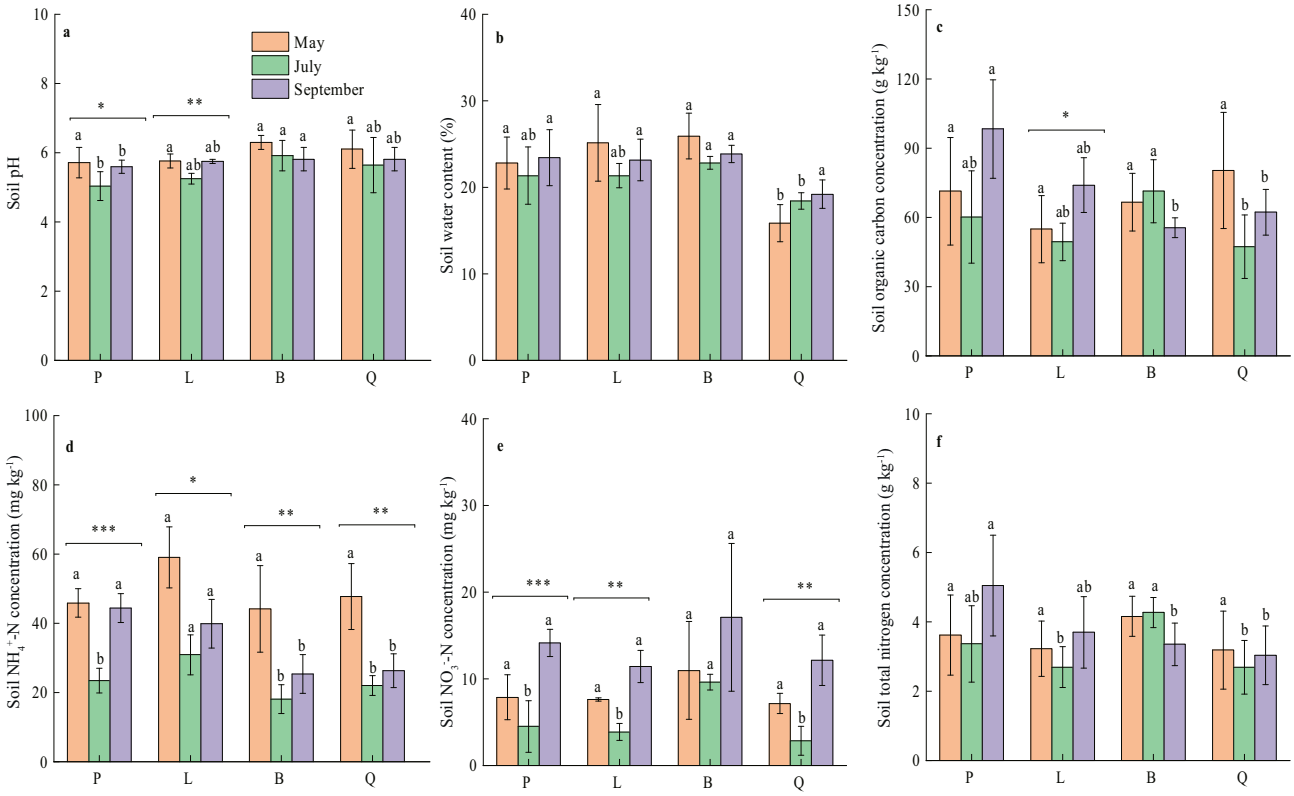

Fig. 2. Variations in soil pH (a), soil water content (b), soil organic carbon (c), and concentrations of ammonium-nitrogen (NH_4^+-N) (d), nitrate-nitrogen (NO_3^--N) (e), and total nitrogen (f) across seasons and forest types (*Picea asperata* (P), *Larix gmelina* (L), *Betula platyphylla* (B) and *Quercus aquifolioides* (Q)). Mean ± SE (n = 4). Asterisks indicate significant differences among three seasons for the same forest type (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$). Different lowercase letters indicate significant differences among forest types in the same season ($P < 0.05$).

determining fungal community diversity and composition. Structural equation modelling (SEM) was conducted to identify the direct and indirect effects of forest type and season on soil fungal diversity, with 999 bootstraps of path coefficients in AMOS (version 20.0). Akaike information criterion (AIC) was used to select specific variables and evaluate the fit of goodness using chi-squared tests.

3. Results

3.1. Soil properties and root variables across forest types and seasons

Forest type and season exerted significant effects on soil properties (Table S2, Fig. 2). In July and September, soil pH of broadleaved forest (*B. platyphylla*) was significantly higher than in conifer forests (*P. asperata*), and soil pH of conifers in July were significantly lower than May and September (Fig. 2a). Soil water content (SWC) was significantly affected by forest type; *Q. aquifolioides* forest had the lowest SWC (Table S2, Fig. 2b). Significant interactive effects between forest type and season were found with respect to SOC (Table S2, Fig. 2c). *L. gmelina* forest had significantly higher SOC in September than in July, while *P. asperata* forest in September had higher SOC than broadleaved forests (Fig. 2c). Forest type and season exerted significant varied effects on soil available nitrogen (Table S2, Fig. 2d–e). Except *P. asperata* forest, soil NH_4^+-N concentration in July and September was significantly lower than in May, while broadleaved forests had significantly lower NH_4^+-N than conifer forests in September and July (Fig. 2d). Likewise, soil NO_3^--N concentration in July was lower than in September and in *B. platyphylla* was higher than other forests in July (Fig. 2e). Soil TN was affected by forest type (Table S2), TN in *P. asperata* forest was significantly higher than broadleaved forests in September (Fig. 2f).

Fine root biomass and RTD were highly affected by forest type, season and their interactions (Table S2). *Q. aquifolioides* forest had significantly higher fine root biomass than other forests across seasons and fine root biomass in May was lower than in July and September for *Q. aquifolioides* and *P. asperata* forests (Fig. 3a). The RTD of all forests in July was lower than in May and September, while *Q. aquifolioides* forest had significantly higher RTD than coniferous forests in May and September (Fig. 3b). *B. platyphylla* forest had significantly higher SRL than *Q. aquifolioides* and *P. asperata* forests in July and September (Fig. 3c). Furthermore, SRA in broadleaved forests in July were significantly higher than in September and no significant changes in May and September (Fig. 3d).

3.2. Alpha-diversity of soil fungal community

Rarefaction analysis showed that sequence data were sufficient for soil fungal community analysis (Fig. S1). Alpha-diversity of soil fungal community was significantly affected by forest type (Table 2). Both Sobs and ACE indices for *Q. aquifolioides* forest were significantly higher than conifer forests in July. However, Shannon's and Simpson's indices displayed no significant changes across seasons and forest types. Also, phylogenetic diversity in broadleaved forests was significantly higher than in conifer forests in July (Table 2).

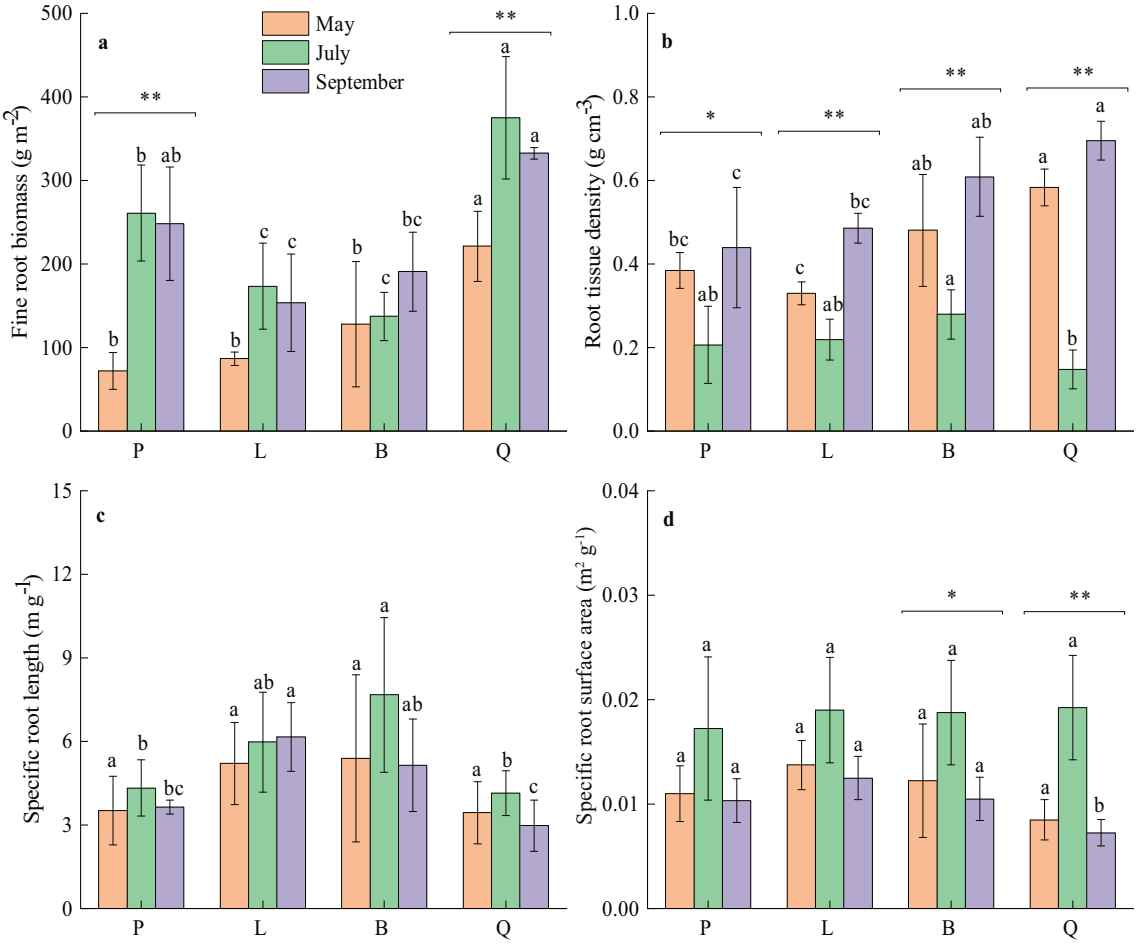

Fig. 3. Variations in root biomass (a), root tissue density (b), specific root length (c), and specific root area (d) across seasons and forest types (*Picea asperata* (P), *Larix gmelina* (L), *Betula platyphylla* (B) and *Quercus aquifolioides* (Q)). Mean ± SE (n = 4). Asterisks indicate significant differences among three seasons for the same forest type (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$). Different lowercase letters indicate significant differences among forest types in the same season ($P < 0.05$).

Table 2
Variations in alpha-diversity of soil fungal community obtained for clustering at 97 % identity (mean ± SE, n = 4) across seasons and forest types. P, L, B, Q stand for *P. asperata*, *L. gmelina*, *B. platyphylla*, *Q. aquifolioides* forest, respectively. Bold values indicate significant effects at $P < 0.05$. Different capital letters indicate significant differences among forest types at the same season ($P < 0.05$). T, forest type effect; S, season effect; T × S, the interaction between forest type and season.

Season	Forest type	Sobs index	ACE index	Shannon's index	Simpson's index	Phylogenetic diversity
May	P	129.25 ± 35.51A	186.123 ± 57.07A	1.88 ± 0.57	0.29 ± 0.17	33.34 ± 5.78A
	L	128.50 ± 29.22A	206.88 ± 36.04A	1.57 ± 0.35	0.36 ± 0.13	33.43 ± 7.19A
	B	191.00 ± 87.30A	252.54 ± 82.78A	2.12 ± 0.68	0.24 ± 0.12	44.87 ± 13.52A
	Q	183.25 ± 70.48A	211.35 ± 69.67A	1.75 ± 0.89	0.39 ± 0.28	42.38 ± 13.22A
July	P	136.25 ± 45.86B	214.79 ± 48.53BC	1.81 ± 0.99	0.38 ± 0.32	36.44 ± 11.79B
	L	138.25 ± 32.91B	179.68 ± 24.35C	1.39 ± 0.52	0.43 ± 0.21	38.31 ± 9.41B
	B	257.33 ± 63.29AB	321.68 ± 67.54AB	2.55 ± 0.72	0.16 ± 0.10	59.28 ± 10.20A
	Q	281.25 ± 141.48A	353.36 ± 123.53A	1.77 ± 1.47	0.21 ± 0.13	61.13 ± 13.78A
September	P	146.75 ± 45.98B	191.31 ± 58.10A	2.16 ± 0.59	0.35 ± 0.15	39.24 ± 11.22A
	L	197.50 ± 44.74AB	273.10 ± 44.62A	1.69 ± 0.38	0.32 ± 0.15	48.59 ± 10.47A
	B	166.00 ± 17.30AB	278.56 ± 57.90A	1.81 ± 0.45	0.47 ± 0.37	38.17 ± 2.51A
	Q	227.00 ± 50.97A	272.56 ± 68.85A	2.14 ± 0.44	0.29 ± 0.12	51.11 ± 9.12A
	T	$P = 0.046$	$P = 0.044$	$P = 0.407$	$P = 0.445$	$P = 0.022$
		F = 3.700	F = 3.759	F = 1.054	F = 0.963	F = 4.870
	S	$P = 0.145$	$P = 0.050$	$P = 0.878$	$P = 0.557$	$P = 0.068$
		F = 2.112	F = 3.436	F = 0.130	F = 0.601	F = 3.040
	T × S	$P = 0.313$	$P = 0.102$	$P = 0.877$	$P = 0.836$	$P = 0.233$
		F = 1.265	F = 2.048	F = 0.391	F = 0.452	F = 1.473

3.3. Soil fungal community composition across seasons and forest types

Across all samples, a total of 2,465,662 (from 30,327 to 128,285) fungal sequences were obtained and identified into 1785 fungal OTUs that changed considerably with season and forest type (Fig. S2a–c). Soil fungal OTUs in May, July and September varied from 1004, 1264 and 1160 across all forest types, respectively. Differences were observed between conifer forests (582 in *P. asperata* and 633 in *L. gmelina* forests) and broadleaved forests (846 in *B. platyphylla* and 1003 in *Q. aquifolioides* forests). Also, soil fungal OTUs in all forest types increased from May to July and September. Soil fungal OTUs were primarily composed of Ascomycota, Basidiomycota and Mortierellomycota

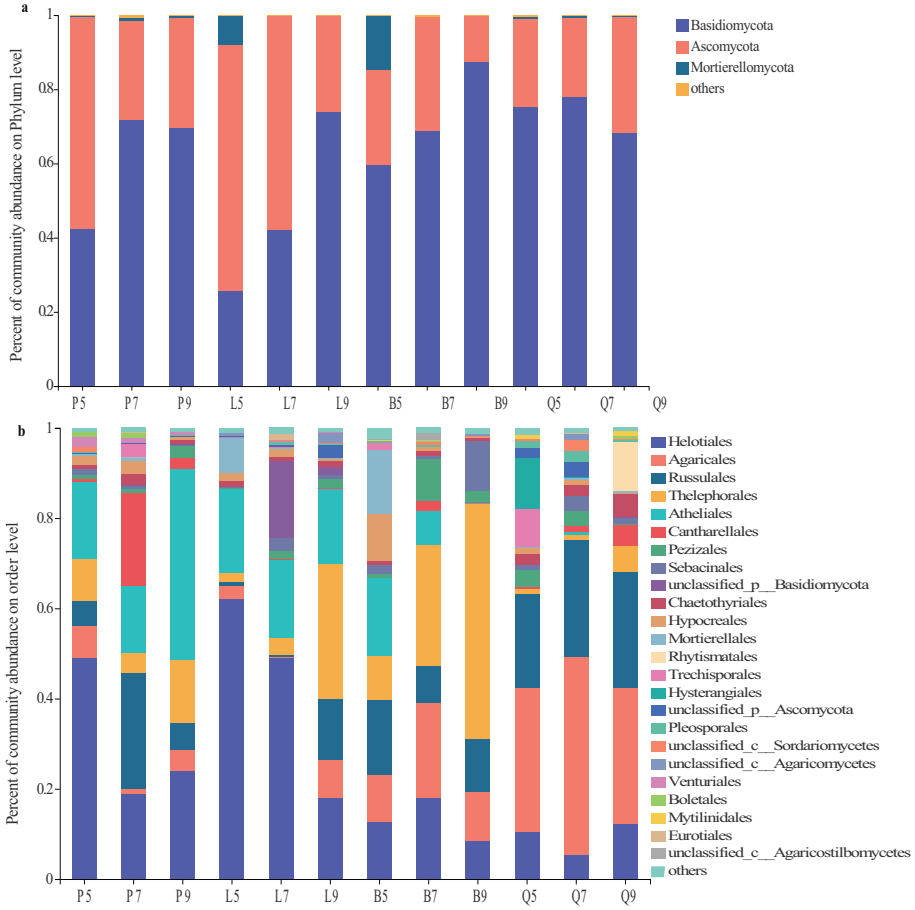

Fig. 4. Relative abundances of soil fungal community composition at the phyla (a) and order (b) levels across forest types and seasons. P, L, B, Q indicate *Picea asperata*, *Larix gmelina*, *Betula platyphylla* and *Quercus aquifolioides*, respectively. 5, 7, 9 stand for sampling in May, July and September, respectively.

at 97 % similarity level and accounted for >80 % of all sequences, belonging to 11 phyla, 43 classes, 106 orders, 244 families and 474 genera. Across all samples, the dominant fungal phyla were Ascomycota and Basidiomycota, with an average relative abundance of 63.7 % and 34.6 %, respectively (Fig. 4a). Furthermore, the dominant fungal orders were: Helotiales, Agaricales, Russulales, Thelephorales, Atheliales, Cantharellales, Sebacinales, Pezizales, Mortierellales and Hypocreales, with average relative abundances of: 24.3 %, 15.7 %, 13.9 %, 11.9 %, 11.8 %, 2.91 %, 2.12 %, 2.39 %, 1.42 %, 1.59 %, respectively, across all samples (Fig. 4b).

NMDS analysis showed a clear separation of soil fungal community between coniferous and broadleaved forests (Fig. 5a). Furthermore, heatmap cluster analysis on OTU level showed distinct differences in fungal community composition across coniferous and broadleaved forests and seasons (May and July were significantly different to September) (Fig. 5b, c). In terms of soil fungal community composition across seasons and forest types, significant differences in abundance proportions of dominant phyla and orders were identified (Fig. 6). The relative abundance of Basidiomycota increased from May to July and September and was higher in broadleaved forests. In contrast, Ascomycota had the opposite trend across seasons and dominated in conifer forests (Fig. 6a, b, c). Also, the relative abundance of Thelephorales in September was significantly higher than in May and July and the relative abundance of five dominant orders varied with forest type (Fig. 6d–f). Specifically, Helotiales and Atheliales were abundant in conifer forests, while Agaricales, Russulales and Thelephorales were abundant in broadleaved forests (Fig. 6e, f).

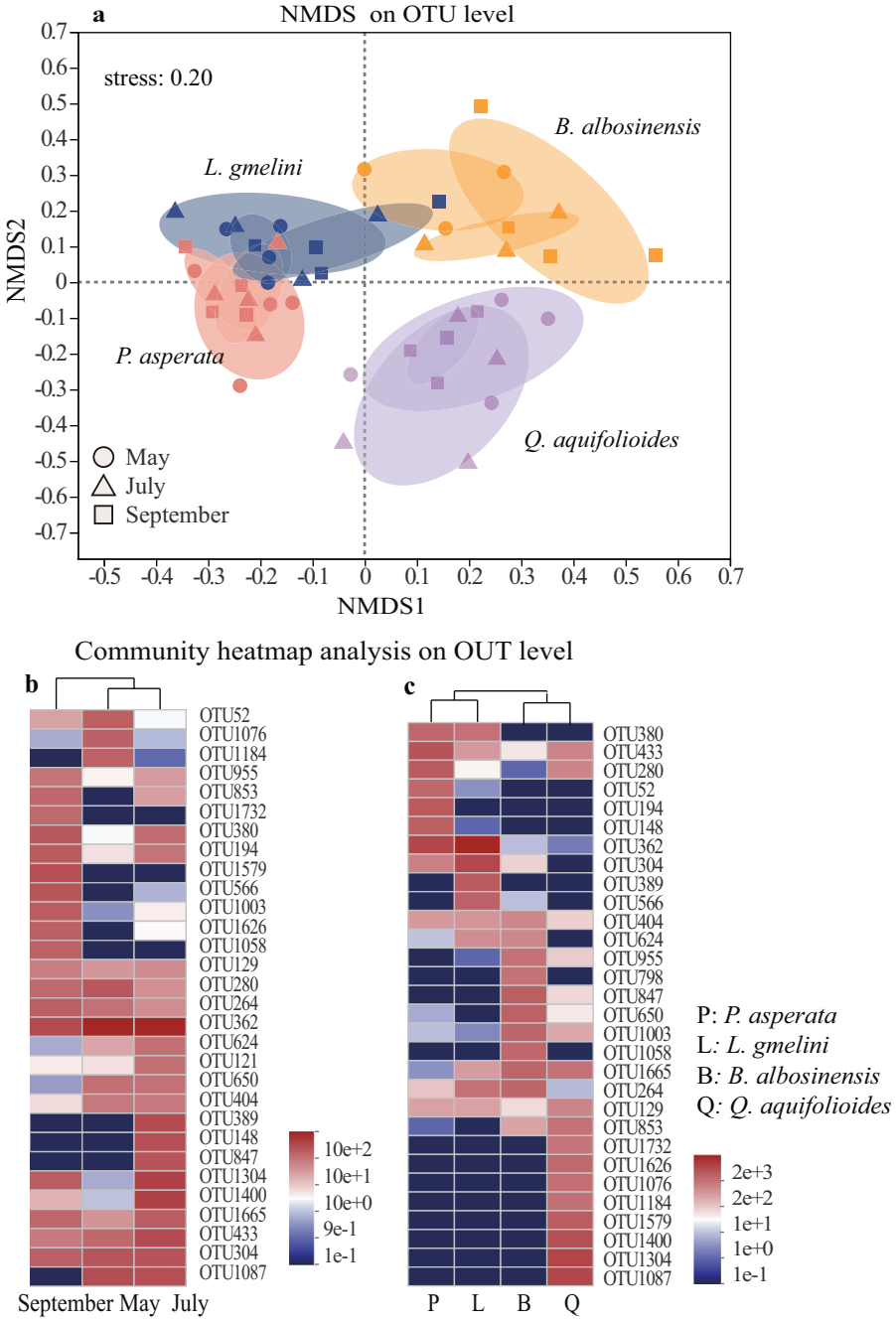

Fig. 5. Results of Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity (a) and the heatmap cluster analysis of soil fungal community on OTU level (b, c) across seasons and forest types.

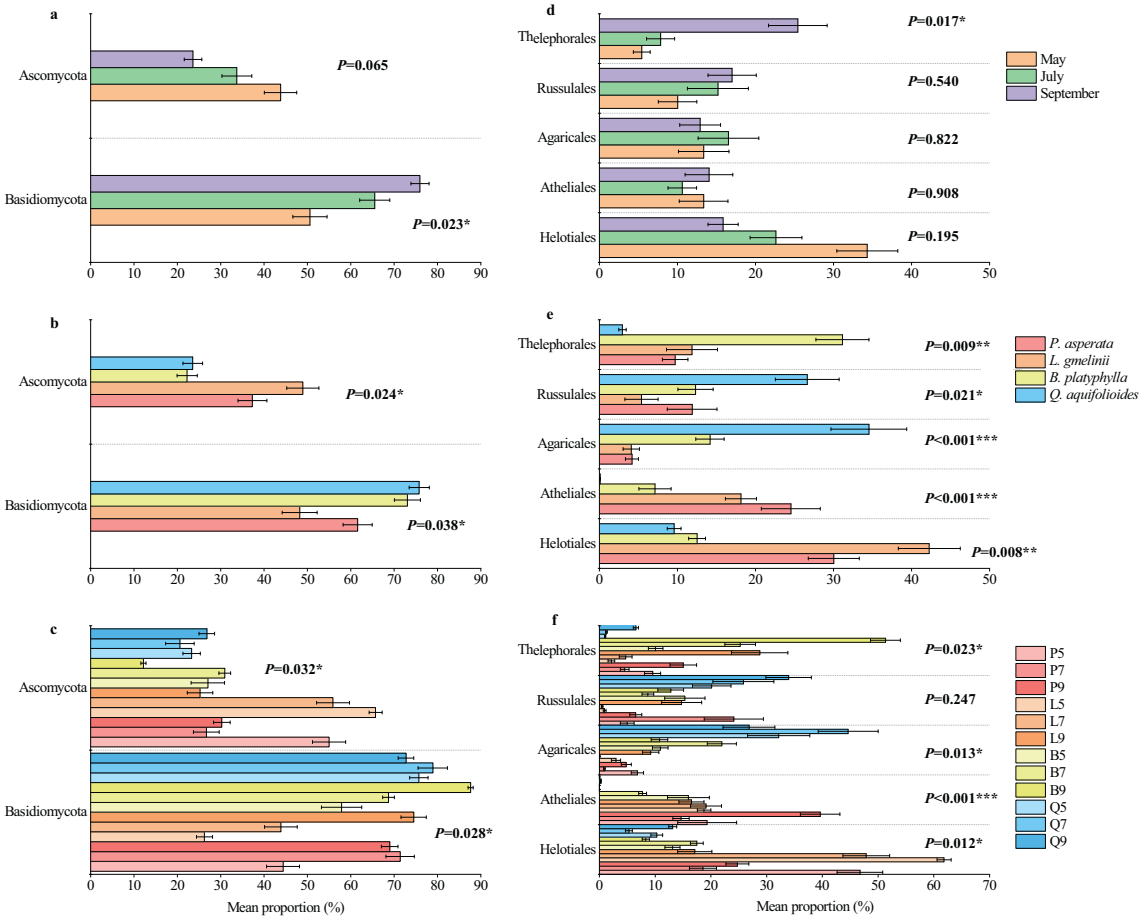

Fig. 6. Differences in the abundance proportions of the most abundant phyla (a, c, e) and orders (b, d, f) across seasons and forest types. Asterisks indicate significant differences among seasons and forest types (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$). P, L, B, Q indicate *Picea asperata*, *Larix gmelina*, *Betula platyphylla* and *Quercus aquifolioides*, respectively. 5, 7, 9 stand for sampling in May, July and September, respectively.

3.4. Relationships between fungal community and related variables

Our data showed that the first two axes in RDA explained 31.72 % of variance in relationships between soil fungal community alpha-diversity and soil properties and root variables (Fig. 7a, Table 3). The forward selection of variables in RDA showed that fungal community alpha-diversity was primarily affected by soil pH and NH_4^+-N (Table 3). These strong factors explained 10.7 % and 9.6 % of variations in fungal community diversity, respectively (Table 3). The first and second ordination axis explained 18.74 % and 7.33 % of total data variance in soil fungal community composition, respectively (Fig. 7b, Table 4). Soil moisture, SOC, NH_4^+-N and RTD exerted significant influences on fungal community composition and explained 8.7 %, 4.1 %, 6.75 % and 6.1 % of variations in fungal community composition, respectively (Table 4).

Furthermore, the effects of forest type and season on soil fungal community were identified by SEM analyses (Fig. 8). We identified significant SEM paths (goodness of fit = 0.89, $\chi^2/F = 1.87$, $P < 0.001$, RMSEA = 0.14 and AIC = 231.51), which indicated this model adequately explained soil fungal diversity. Also, we comprehensively identified indirect and direct pathway effects of forest type and season on soil fungal diversity. Forest type and season directly affected soil fungal diversity (0.33 and 0.24, respectively) and indirectly by soil properties and root variables, while a negative effect was observed for root variables and soil properties (their total effects were −0.03 and −0.24, respectively) (Fig. 8b).

4. Discussion

In this study, we analysed the diversity and composition of fungal community in coniferous and broadleaved forests using Illumina MiSeq sequencing. Data revealed that soil fungal community alpha-diversity varied with forest type and structural composition clearly differed across seasons and forest type. They can affect soil fungal community composition by changing soil properties and root variables in subalpine forests.

4.1. Differences in soil fungal community diversity across forest types

In this study, Sobs and ACE indices and phylogenetic diversity in broadleaved forests were higher than conifer forests (Table 2), which supported our first hypothesis. Many studies have reported variations in fungal community diversity across forest types (Siles and Margesin, 2017; Mommer et al., 2018; Põlme et al., 2018). These findings emphasised the significance of forest type on soil fungal community diversity, which were ascribed to microenvironmental changes (soil pH, organic carbon and other nutrients) caused by plant performance (Ushio et al., 2010; Krashevska et al., 2015; Chen et al., 2019). Leaf litters and roots of broadleaved forests contain more decomposable and lower concentrations of chemically complex compounds such as lignin than that of conifer forests (Augusto et al., 2015; Dawud et al., 2017) and provide more favourable microenvironments for soil fungi (Deng et al., 2019). In our study, soil fungal diversity and richness were explained by soil pH and NH_4^+-N (Table 3), while higher fungal diversity and richness were identified in soils with a

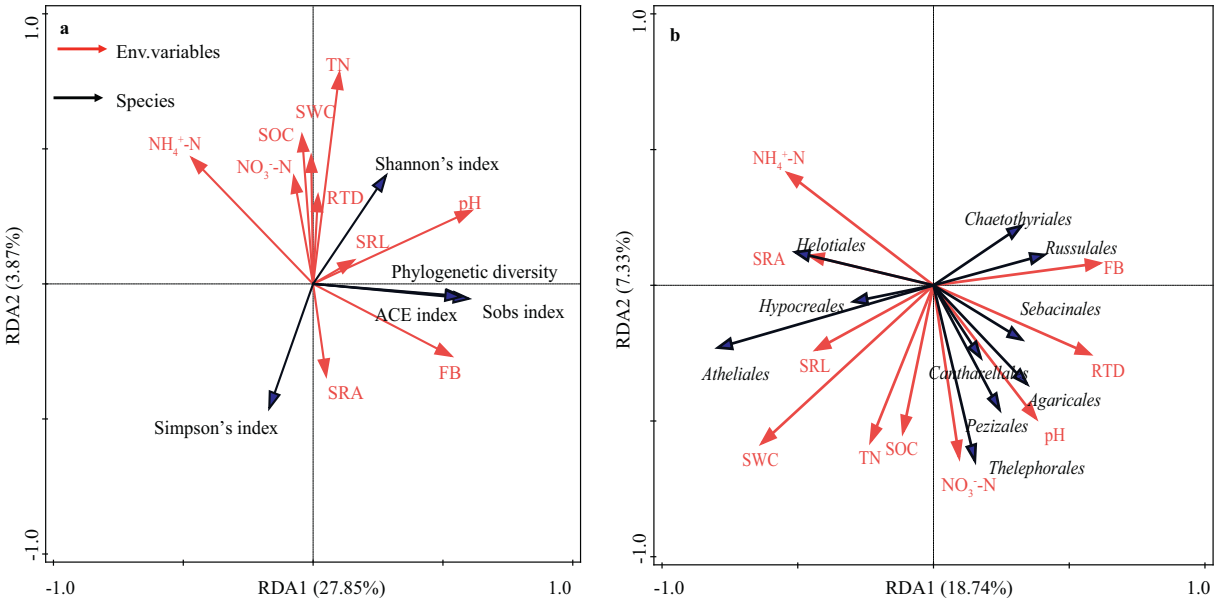

Fig. 7. Redundancy analysis illustrating relationships between soil fungal community diversity (a), composition (order-level) (b) and root variables and soil properties across seasons and forest types. Ordination diagrams present species scores and environmental factor scores (vectors) in the redundancy analysis. pH, soil pH; SWC, soil water content; NH_4^+-N, soil NH_4^+-N concentration; NO_3^--N, soil NO_3^--N concentration; SOC, soil organic carbon concentration; TN, soil total nitrogen concentration; FB, fine root biomass; RTD, root tissue density; SRL, specific root length; SRA, specific root surface area.

higher soil pH and lower NH_4^+-N (Table S3) (broadleaved forest soil characteristics are shown: Fig. 2a, d). It is accepted that soil pH and nitrogen availability are important factors shaping fungal community richness and diversity (Adamo et al., 2021; Siles and Margesin, 2016; Ren et al., 2018). Fierer and Jackson (2006) observed that soil pH accounted for >70 % variation in microbial diversity across different forest ecosystems and that lower pH was stressful for microbial taxa (Wang et al., 2015). Higher nitrogen availability is generally associated with lower soil fungal diversity and abundance (Bahram et al., 2012; Chen et al., 2018). In our study, some taxa, i.e. Agaricales, Cantharellales, Pezizales, Sebacinales and Thelephorales were dominant at high pH and low NH_4^+-N conditions (Fig. 7b), suggesting these species could not adapt to acidification pressures at low pH or high nitrogen conditions (Pellissier et al., 2014; Wang et al., 2015). In general, low pH was not conducive to microbial taxa, because it can support the maintenance but not for the prosperity of their community, e.g. acidic conditions accommodated a much reduced microbial taxa than normal conditions (Kuang et al., 2013; Wang et al., 2015).

Supporting our second hypothesis, significant fungal diversity variations were only identified in July, which was possibly related to seasonal climate differences (e.g. water and heat), plant traits (e.g. growth rate) or modified soil microenvironments (Tedersoo et al., 2014; Siles et al., 2016; Lwila et al., 2021). Generally, plants experience rapid growth rates and major expansion in the growing season, but experience smaller expansion under unfavourable water or heat conditions (Montagnoli et al., 2014). A similar tendency was found for fungal activity (Smith and Read, 2010; Ji et al., 2021). Therefore, we speculated that plants and fungi were vigorous in July under favourable hydrothermal conditions and responded rapidly to environmental changes. Ji et al. (2021) revealed that fungal community diversity, growth rates and metabolic activity of soil fungi in a warm July were higher than in May and September. Indeed, elevation differences between plantations may have affected the soil microclimate (e.g. temperature or humidity), which requires further study. In this present study, we mainly focused on the effects of season and forest type on soil fungal community with respect to root variables and soil properties (e.g. pH and

Table 3
Effects of the variables on soil fungal community alpha-diversity as determined by forward selection in redundancy analysis (RDA).

Variables	Explains %	Contribution %	pseudo-F	*P*
pH	10.7	32.7	5.3	**0.022**
SWC	1.7	5.1	0.9	0.324
SOC	0.2	0.7	0.1	0.924
NH_4^+-N	9.6	29.3	5.2	**0.001**
NO_3^--N	1.3	3.9	0.7	0.440
TN	2.0	6.0	1.1	0.308
FB	2.8	8.6	1.5	0.236
RTD	0.6	1.7	0.3	0.696
SRL	2.7	8.3	1.5	0.246
SRA	1.2	3.8	0.7	0.506

pH, soil pH; SWC, soil water content, SOC, soil organic carbon concentration; TN, soil total nitrogen concentration; NO_3^--N, soil NO_3^--N concentration; NH_4^+-N, soil NH_4^+-N concentration. FB, fine root biomass, RTD, root tissue density, SRL, specific root length, SRA, specific root surface area. Bold values indicate significant effects at $P < 0.05$.

Table 4
Effects of the variables on soil fungal community composition as determined by forward selection in redundancy analysis (RDA).

Variables	Explains %	Contribution %	pseudo-F	*P*
pH	3.0	7.9	1.7	0.138
SWC	8.7	22.8	4.2	**0.004**
SOC	4.1	10.8	2.3	**0.024**
NH_4^+-N	6.7	17.5	3.6	**0.002**
NO_3^--N	2.3	5.9	1.3	0.228
TN	2.8	7.3	1.6	0.136
FB	1.0	2.5	0.5	0.808
RTD	6.1	16.0	3.1	**0.012**
SRL	1.7	4.5	1.0	0.414
SRA	1.8	4.8	1.1	0.352

pH, soil pH; SWC, soil water content, SOC, soil organic carbon concentration; TN, soil total nitrogen concentration; NO_3^--N, soil NO_3^--N concentration; NH_4^+-N, soil NH_4^+-N concentration. FB, fine root biomass, RTD, root tissue density, SRL, specific root length, SRA, specific root surface area. Bold values indicate significant effects at $P < 0.05$.

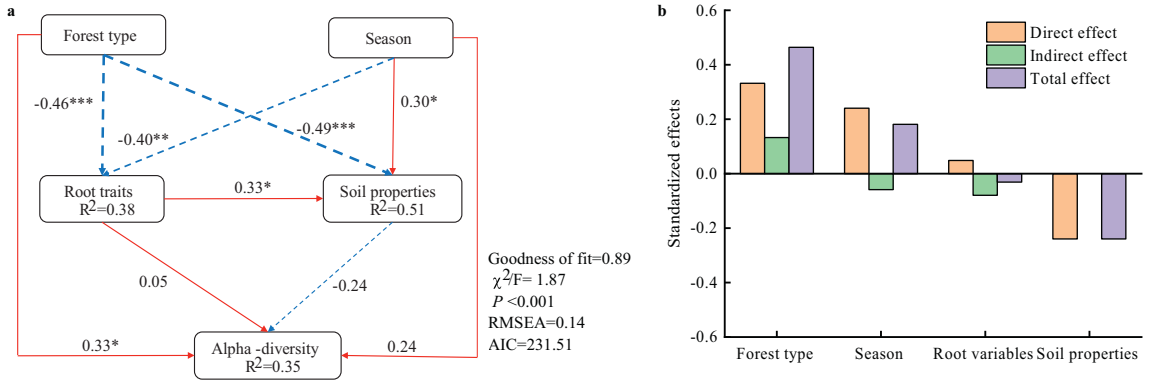

Fig. 8. Structural equation model (SEM) showing the pathway of forest type and season on soil fungal diversity (a). Numbers adjacent to arrows indicate the effect size of their relationship. Soil properties, root variables and alpha-diversity were grouped in the model for graphical simplicity due to they are independent observable variables. SEM considered all plausible pathways, and larger path coefficients are shown as wider arrows. Red and blue colors indicate positive and negative effects, respectively. Path coefficients and coefficients of determination (R^2) were calculated after 999 bootstraps, and represent the proportion of variance explained for each dependent variable in SEM. Significance levels are indicated by *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Standardized direct and indirect effects derived from the partial least-squares path models (b).

nutrients). This was in consistent with Han et al. (2021), who studied the effects of season and forest type although there were differences in elevation among stands.

4.2. Seasonal variations in soil fungal community composition across forests

In agreement with our third hypothesis, significant changes in soil fungal community composition occurred across seasons and forest types (Figs. 4–6). The dominant phyla (comprised primarily of Basidiomycota and Ascomycota) were consistent with forests on a global scale (Tedersoo et al., 2014). Ascomycota and Basidiomycota play essential roles in forest ecosystems, as they are the important decomposers in forest soils by degrading organic matters and regulating nutrient cycling (Lauber et al., 2008; Riley et al., 2014; Zeng et al., 2020). In our study, Ascomycota was abundant in coniferous forests, while Basidiomycota preferred broadleaved forests in September (Fig. 6a, b), which may have been due to their specific ecological functions. Coniferous litter exhibits higher recalcitrance (cellulose and lignin) and slower decomposition rates than in broadleaved forests (Kong, 2004; Li et al., 2009; Setälä et al., 2016), Ascomycota are more involved in early cellulose decomposition stages in forests (Stursova et al., 2012; Ma et al., 2013), but many Ascomycota species cannot metabolise structural carbon (Osono et al., 2003), and this may limit their competitiveness over time. However, Basidiomycota are more abundant in fertile forest soils thus reflecting their importance as wood and litter decay agents and decomposing litter (Osono et al., 2003; Li et al., 2015; Zeng et al., 2020). In forest ecosystems, plant litter decomposition is the main nutrient source for soil microorganisms, which may limit the abundance of some fungal taxa (Osono et al., 2003; Li et al., 2015). At the order level, conifer forests contained high Helotiales and Atheliales proportions (Fig. 6d), as these species could form mycorrhizal symbiotes to acquire nutrients, with thick and black cell walls to adapt to inclement environments (Fernandez and Koide, 2013; Clemmensen et al., 2015; Sulistyo et al., 2021). Conversely, Agaricales, Russulales and Thelephorales were abundant in broadleaved forests (Fig. 6d) as they exploited readily decomposable nutrients and tended to proliferate in nutrient-rich conditions (Li et al., 2015; Suz et al., 2017). We found that soil nutrients (SOC and NH_4^+-N) significantly influenced soil fungal community composition (Table 4). Zhang et al. (2016) reported that soil fungal community composition was affected by SOC, TN and total phosphorus. Thus, variations in soil fungal community composition across forest types and seasons were attributed to their functional adaptation, which were reflected by modified soil nutrients (SOC and NH_4^+-N).

4.3. Relationships between fungal community, soil properties and root variables

We observed clear correlations between soil fungal community, soil properties and root variables (Fig. 7, Tables 3, 4). These results illustrated that forest type and seasons, indirectly via soil properties (SWC, SOC and NH_4^+-N) and root variables (RTD) drove soil fungal community composition (Fig. 8). Mitchell et al. (2012) and Zeng et al. (2020) suggested that high soil moisture promoted litter decomposition, increased nutrients in forest soils and subsequently altered soil fungal community composition. Soil carbon and nitrogen availabilities were principal factors determining the composition of fungal community, as they explained 4.1 % and 6.7 % of the variations in fungal community, respectively (Table 4). This finding agreed with previous studies showing that soil nutrients greatly contributed to soil fungal communities (Adamo et al., 2021; Bayranvand et al., 2021). Also, fungal richness and diversity were negatively correlated with soil nitrogen availability (Table S3), and agreed with Bahram et al. (2012) and Yao et al. (2017b), which showed that higher nitrogen levels in soils decrease fungal activity and led to a decline in fungal diversity and abundance.

Furthermore, variations in soil fungal community were clarified by RTD (Table 4), in agreement with Yang et al. (2020) and Spitzer et al. (2020), who reported that fungal community composition changed with respect to root variables. Yahara et al. (2019) indicated that RTD was an important parameter for identifying different phylogenetic microbial association groups. Also, fine root biomass was positively correlated with fungal diversity and richness (Table S3), and was mainly due to its ability to alter the soil microclimate and produce litter and root exudates as energy sources for microbes (Prescott and Grayston, 2013; Mueller et al., 2014). Guo et al. (2016) also reported that fine root biomass greatly contributed to soil fungal community in forests.

5. Conclusion

This study comprehensively explored the combined effects of forest type and season on the diversity and composition of soil fungal community and determined the driving factors of variations in fungal community in subalpine forests. Forest type had a considerable role in shaping soil fungal community diversity and richness. Sobs and ACE indices and phylogenetic diversity in broadleaved forests were higher than in conifer forests. We observed distinct differences in fungal community composition across forest types (coniferous and broadleaved forests) and seasons (May and July, September). Soil pH and NH_4^+-N were the main factors driving soil fungal diversity and richness and soil moisture, SOC, NH_4^+-N and RTD

were the main factors shaping soil fungal community. Our conceptual framework revealed the combined effects of root and soil characteristics on soil fungal diversity and provided new insight on the important role of root and soil traits in shaping soil fungal community. In conclusion, this study identified the main fungal community drivers and revealed differences and complex responses of soil fungi to seasonal changes in subalpine forest ecosystems. Our study is important for analysing interactions in plant-soil-microbial communities in subalpine forests.

CRediT authorship contribution statement

Chunying Yin and Lulu Xie conceived the ideas and designed methodology; Lulu Xie collected and analyzed the data; Lulu Xie and Chunying Yin led the writing of the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157409.

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