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Quantifying Nature-based
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Key Points:

- Nitrogen addition increased symbiotrophic fungi, but decreased saprotrophic fungi
- Precipitation reduction altered soil overall and pathotrophic fungal community
- Fine-root diameter and specific root length shaped soil overall fungal community

Supporting Information:

Supporting Information may be found in the online version of this article.

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Responses of Soil Fungi to Long-Term Nitrogen-Water Interactions Depend on Fungal Guilds in a Mixed *Pinus koraiensis* Forest

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Abstract Different fungal guilds within the soil fungal community regulate forest ecosystem processes. Soil fungi are dictated by edaphic factors such as soil water and nutrient availabilities. However, how the total number of fungi and the composition of different fungal guilds in the soil vary under covarying pattern of nitrogen deposition and precipitation regime in temperate forests has not been well documented. In this study, we explored the effects of long-term nitrogen-water interactions on the diversity and composition of soil overall fungi and different fungal guilds, and their relationships with fine-root and soil fungal mycelial traits in a temperate forest. The diversity of the totality of soil fungi or any fungal guilds did not change, but the community composition of saprotrophic and symbiotrophic fungi was significantly changed by nitrogen addition (N), showing increased abundance of soil symbiotrophic fungi but decreased abundance of soil saprotrophic fungi. Precipitation reduction (W) significantly altered soil overall and pathotrophic fungal community. Precipitation reduction combined with nitrogen addition (WN) significantly changed soil overall, pathotrophic and symbiotrophic fungal community composition. Soil pathotrophic, saprotrophic and symbiotrophic fungal community composition was variously related to fine-root diameter, root tissue density, nitrogen and/or phosphorus concentration. Long-term nitrogen-water interactions decreased the complexity of soil fungal networks, reflected by the lower edges and average degree, but the higher average path distance and modularity. These findings of soil fungal guild-specific responses to nitrogen-water interactions will deepen our understanding of soil carbon sequestration potential and nutrient cycling in temperate forest ecosystems under future climate changes.

Plain Language Summary Many forest ecosystem processes such as decomposition of soil organic matter and nutrient cycling are regulated by soil fungi. The soil fungal community is affected by soil factors such as soil water and nutrient availabilities. However, we don't well know how soil fungi and their composition respond to soil nitrogen and water changes in temperate forests. In this study, we studied the effects of long-term nitrogen-water interactions on the diversity and community of soil fungi in a mixed *Pinus koraiensis* forest, in relation to fine-root and soil fungal mycelial traits. We found that nitrogen addition did not change the diversity of soil overall fungi or any fungal guild, but altered the community composition of soil overall, saprotroph and symbiotroph fungi significantly. Precipitation reduction (W) and precipitation reduction combined with nitrogen addition (WN) significantly decreased the diversity of soil saprotroph fungi. W and WN increased the abundance of soil saprotroph and pathotroph fungi, but decreased that of soil symbiotroph fungi. Soil overall fungal community was related with fine-root diameter and specific root length. Long-term nitrogen-water interactions simplified soil fungal networks. These findings have important implications for our better understanding of forest soil carbon sequestration potential and nutrient cycling under climate changes.

1. Introduction

Soil fungi are highly diverse and contribute extensively to a wide range of forest ecosystem processes such as decomposition of soil organic matter, nitrogen and phosphorus cycling, and carbon storage (Kyaschenko et al., 2017; Sweeney et al., 2021; Treseder & Lennon, 2015). A functional guild is a group that exploits the same type of resources in a similar manner (Nguyen, Williams, et al., 2016; Shigyo & Hirao, 2021). Generally, pathogenic, symbiotic and saprophytic fungi are the most abundant fungal guilds in forest soils (Liang et al., 2023;

Nguyen, Song, et al., 2016). Soil pathogenic fungi can colonize plant root systems to make use of living plant tissues or extract nutrients by killing plant tissues, influencing patterns of seedling mortality and tree distributions in temperate forest ecosystems (Laliberté et al., 2015; Packer & Clay, 2000). Symbionts with plants mainly formed by ectomycorrhizal (ECM) fungi and arbuscular mycorrhizal fungi can aid plant nutrient acquisition, and improve disease and drought resistance (Fitter & Garbaye, 1994; Gonthier et al., 2019; Sweeney et al., 2021). Soil saprophytic fungi are generally considered a primary engine of the forest debris (e.g., dead root and leaf litter) decomposition and mineralization processes, which, in turn, drive the saprophytic fungal community (Francioli et al., 2021; Nguyen, Song, et al., 2016; Zhou et al., 2020). Accordingly, based on trophic strategies rather than species identities, distinguishing fungal guilds helps to understand the changes more accurately in their composition and function since different fungal guilds respond diversely to the variations in biotic and abiotic constraints.

Soil microbial communities show a strong correlation with plant traits owing to their associations with forest ecosystem processes (Cline & Zak, 2015; Shigyo & Hirao, 2021). The abundance of different fungal guilds has been related to plant traits (Francioli et al., 2021; Sweeney et al., 2021). Whereas evidences from studies of soil microbial functioning indicate that plant root traits, especially root diameter (RD), root nitrogen and specific root length (SRL), are more important factors for shaping the soil fungal communities than aboveground leaf traits (Cantarel et al., 2015; Orwin et al., 2010). The root traits explained about 16% of the variation in root-associated saprophytic fungal community structure (Francioli et al., 2021). Differences in root architecture and exudation patterns are known to impact soil properties such as nutrient concentrations and pH, which directly affect the soil fungal community composition (Goldmann et al., 2015). A microcosm experiment demonstrated that fungal biomass increased significantly with increases in root biomass (RB) and the amount of root exudates, namely increases in the resource availability for soil fungal communities (Eisenhauer et al., 2017; Philippot et al., 2013). Meanwhile, the influence of plant root traits on soil fungal communities may be guild-specific. Given that saprophytic fungi are trophically independent and obtain their carbon from decaying organic matter, while symbiotic fungi are biotrophic depending on plant tissues, it is expected that traits of living fine roots have less influence on soil saprophytic fungi than on symbiotic fungi (Högberg et al., 2003; Leake et al., 2003). Furthermore, soil pathogens are one of the primary causes of fine-root death, longer-living fine roots must be better protected against soil pathogens (Eissenstat & Yanai, 1997; Laliberté et al., 2015). Generally, thinner roots would be efficient to acquire soil nutrients, but might lead to greater susceptibility to soil pathogens. By contrast, thicker roots that strongly depend on mycorrhizal fungi for nutrients acquisition should be less susceptible to soil pathogens (Laliberté et al., 2015; Newsham et al., 1995). In conclusion, these findings suggest the potential for fine-root traits to be used within predictive frameworks of plant-fungal relationships for different trophic groups in forest ecosystems.

Global climate change has the potential to fundamentally alter soil fungal community composition through nitrogen deposition and more variable precipitation regimes (Averill et al., 2016; Bui et al., 2020; Hawkes & Keitt, 2015). It has been observed that nutrient availabilities negatively affected mycorrhizal diversity and biomass, and could change the composition of mycorrhizal species (Ekblad et al., 2013; Kjølner et al., 2012; X. Zhang et al., 2022). In contrast, the abundances of saprotrophic and/or pathogenic fungi would increase with increasing soil fertility in forest ecosystems (Castaño et al., 2019; Dejene et al., 2017; Zheng et al., 2017). Soil fertility was positively related to the abundance of fungal saprotrophs, which may grow at the expense of ECM fungi (Kyaschenko et al., 2017; Zheng et al., 2017). Interactions among soil fungal guilds play a central role in regulating organic matter accumulation in relation to fertility in a coniferous forest ecosystems (Kyaschenko et al., 2017). When a moisture shift occurs, a reduction in the activity of dominant soil fungi adapted to previous moisture conditions could result in a reduction in competitiveness with other fungal taxa, which are then able to dominate the community at the new water status (Kaisermann et al., 2015; Lennon et al., 2012). A recent study suggests that long-term precipitation reduction greatly changed soil fungal communities in a species-rich grassland (Sayer et al., 2017). The complexity of soil fungal co-occurrence networks also declined with decreasing precipitation due to enhanced species competitiveness and niche differentiation (Feng et al., 2024). Furthermore, soil moisture fluctuation induced by altered precipitation patterns strongly influences nutrient diffusion in the soils, leading to change the dependence of host plants on mycorrhizal fungal mycelial uptake of soil nutrients and even of water, and alter the soil fungal community structure (Hawkes et al., 2011; Kaisermann et al., 2015; Lennon et al., 2012). In this case, soil water reduction may intensify the negative effects of high nitrogen input on soil fungi due to the close associations of water with nitrogen in forest soils (Huang et al., 2022). It is observed that about 42% variation on fungal community composition in the rhizosphere of *Alhagi sparsifolia* was explained by the nitrogen-water interactions (Z. Zhang et al., 2021). However, very little is known about the interactive

effects of nitrogen and water on soil fungal diversity and community composition (R. Wang et al., 2017). Thus, clarifying soil fungal responses to altered precipitation patterns and their interactions with elevated nitrogen deposition is important because they are key drivers of biogeochemical cycling and numerous important ecosystem processes such as decomposition (Xu et al., 2020).

In recent decades, the Changbai Mountain region are being subjected to increasing nitrogen deposition and decreasing precipitation (Dai et al., 2013; Lü & Tian, 2007), which have greatly influenced the structure and function of the temperate mixed mature *Pinus koraiensis* forest (C. Wang, 2006). Indeed, there are several studies addressing the individual effect of climate change factors such as elevated nitrogen deposition and decreased precipitation on associated-root fungal communities and diversity. For instance, our previous studies in this region have showed that nitrogen addition decreased the diversity of the ECM fungal community inhabiting roots of *Corylus mandshurica* and *Tilia amurensis*, altering their fungal community composition (J. Wang et al., 2022). However, there is very limited knowledge of the interactions among multiple drivers (i.e., nitrogen and water), which are expected to play simultaneously a critical role on the composition of soil fungal communities in temperate forests (Li et al., 2015; J. Wang et al., 2022). Accordingly, a field experiment under nitrogen addition and precipitation reduction treatments was conducted to characterize the diversity and community composition of soil fungi and their relationships with fine-root and fungal mycelial traits in this temperate forest. Three hypotheses will be tested: (a) total soil fungal diversity decreases with nitrogen addition and/or precipitation reduction, and the interactions between nitrogen and water have negative influences on soil fungi by altering their community composition, (b) soil fungi of different functional guilds exhibit contrasting response patterns to nitrogen-water interactions due to their diverging trophic strategies, and (c) fine root and fungal mycelial traits such as diameter are key factors driving the shifts in soil fungal communities, but the strength depends on the identity of soil fungal guilds.

2. Materials and Methods

2.1. Study Site

The study site (42°24'N, 127°47'E, 738 m above sea level) is located in a mixed mature *P. koraiensis* forest within the Changbai Mountain Nature Reserve (CMNR) in Jilin province, China. CMNR has a typical temperate and continental climate. In this region, the mean annual air temperature is 3.5°C, and the highest (20.5°C) and lowest (−16.5°C) monthly mean air temperatures appear in August and January, respectively. The precipitation of seven drought years (572 mm in 1985; 572 mm in 1997; 534 mm in 1999; 559 mm in 2001; 556 mm in 2003; 559 mm in 2008 and in 526 mm in 2014) is about 30% less than mean annual precipitation of 740 mm in the last 40 years. The soil developed from volcanic ash is classified as Eutric cambisol (FAO classification), with a high organic matter content in the surface layer. The temperate mixed mature forest is dominated by the coniferous species *P. koraiensis* and the broad-leaved tree species, such as *Acer mono*, *Fraxinus mandshurica*, *Quercus Mongolica* and *T. amurensis*. The main shrub species include *C. mandshurica*, *Philadelphus schrenkii*, *Euonymus alatus* and *Lonicera japonica*, and the main herb species are *Anemone raddeana*, *A. cathayensis*, *Cyperus microiria* and *Funaria officinalis* in the mixed mature forest.

2.2. Experiment Design

In July 2009, we selected six 50 m × 50 m plots with a 20 m buffer around from each other in the mixed mature *P. koraiensis* forest. Three of the plots were randomly selected, where 30% of the canopy throughfall (about 220 mm year^{−1}) was excluded using high-light-transmittance polycarbonate V-shaped panels supported at minimum 1 m above ground level (C. Wang et al., 2021). The other three of the plots received ambient growing season precipitation. Then we used iron sheets with the depth of 50 cm to subdivide each of the six 50 m × 50 m plots into two subplots of 25 m × 50 m. One of the subplots was treated with nitrogen addition (50 kg N ha^{−1} year^{−1}), which was applied evenly over six times from May to October during the growing season. When fertilizing, solid NH₄NO₃ was added to 40 L water and stirred until it dissolved. Then the NH₄NO₃ solution was sprayed onto nitrogen addition subplots using a backpack sprayer. The same amount of water (40 L) was sprayed onto the subplots without nitrogen treatments to eliminate the influence of the nitrogen addition treatment on soil moisture. Accordingly, the 12 subplots were designed into four treatment groups in total (each treatment was applied on three subplots): control (CK, ambient precipitation without nitrogen addition), nitrogen addition with ambient

precipitation (N), precipitation reduction without nitrogen addition (W) and precipitation reduction combined with nitrogen addition (WN).

2.3. Sample Collection and Trait Measurement

We applied modified double-layer (outer and inner) ingrowth bags to collect fine roots (<2 mm in diameter) and fungal mycelium, respectively (Persson, 1983; Wallander et al., 2004; C. Wang et al., 2021). The outer-layer polyethylene bags with mesh size of 2 mm were 30 cm length and 6 cm diameter, and filled with root-free soils from the study site to select for the root ingrowth. The inner-layer polyethylene bags with 25 μm mesh size were 30 cm length and 2 cm diameter, and filled with acid-washed quartz sand (0.3–2.0 mm, Sinopharm, Shanghai, China) to select for the mycelial ingrowth. In October 2015, we randomly installed five double-layer ingrowth bags in each plot, keeping a minimum distance of 5 m to each other and the plot edges. In October 2018, we collected the double-layer ingrowth bags, and the roots outside the bags were carefully cut off with knives and/or scissors (Figure S1 in Supporting Information S1). The bags were immediately packed into a cooler containing ice. In the laboratory, the double-layer ingrowth bags were further subdivided into three soil layers: 0–10 cm, 10–20 cm and 20–30 cm. The ingrowth cores of the same soil layer from each plot were pooled as a composite sample. The living fine roots were carefully excavated from soils of outer-layer root ingrowth bags with tweezers. The soil particles attached to the roots were gently washed with deionized water. The collected fine roots were placed into a freezer at 4°C until morphological analysis. Meanwhile, about 50 g soil was collected and stored at –20°C in a freezer for soil fungal community composition analysis.

For fine-root morphological analysis, roots were scanned and then analyzed using the WinRHIZO software package (Regent Instruments Inc., Quebec, Canada) to assess morphological traits such as length, diameter and number of tips. Fine roots were then dried at 65°C until reaching a constant weight, weighted and ground to a fine powder using a ball mill. Nitrogen, phosphorus and potassium concentrations of fine roots were determined with Kjeldahl method (Kjeltec 8400, FOSS, Hilleroed, Denmark), vanadium molybdate blue colorimetric method and flame spectrophotometry, respectively. We measured fine-root biomass (RB, standing biomass of fine roots per unit soil surface area, mg cm^{-2}), fine-root length density (RLD, length of fine roots per unit soil volume, mm cm^{-3}), fine-root area density (RAD, area of fine roots per unit soil surface area, $\text{cm}^2 \text{cm}^{-2}$), fine-root average diameter (RD, mm), SRL (length of fine roots per unit fine-root mass, m g^{-1}), fine-root tissue density (RTD, fine-root mass per unit fine-root volume, g cm^{-3}) and fine-root branching intensity (BI, number of first-order roots per centimeter of second-order roots, number cm^{-1}). In this study, we defined the most distal roots with no branches as first-order roots, and the roots that contained only first-order roots comprised the second-order roots (Pregitzer et al., 2002).

For fungal mycelial morphological analysis, the incubated sand weighting 15 g was homogenized in 100 ml distilled water. Then a suspension subsample was filtered through a 0.45 μm mesh size, 25 mm diameter cellulose nitrate membrane (Whatman, UK). When the sample had been filtered, the membrane was stained with pure blue ink (HERO 203, Shanghai Ink Factory, Shanghai, China). The membrane was placed on a glass slide and observed under a light microscope (Axio Lab. A1, Zeiss, Jena, Germany). Microscope fields were selected randomly and up to 30 fields were observed per membrane filter. We assessed fungal mycelial length and diameter by counting the intersections between the grid and the mycelium, using an eyepiece grid (Wallander et al., 2004), and quantified the length of mycelium per unit sand (mm g^{-1} sand) to present fungal mycelial length density.

2.4. Determination of Soil Fungal Community

Soil genomic DNA was extracted using an E.Z.N.A.® stool DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manual. In this study, the fungal ITS1 region was amplified with primers ITS1F (CTTGGTCAT-TTAGAGGAAGTAA) for forward and ITS2R (GCTGCGTTCCTCATCGATGC) for reverse directions (Bokulich & Mills, 2013). PCR reactions were performed in 20 μl mixtures containing 10 ng template DNA, 4 μl 5 \times FastPfu Buffer, 2 μl 2.5 mM dNTPs, 0.8 μl Forward Primer (5 μM), 0.8 μl Reverse Primer (5 μM), 0.4 μl FastPfu Polymerase and 0.2 μl BSA, and finally ddH_2O up to 20 μl . The thermocycling conditions were as follows: (a) initial denaturation at 95°C for 3 min; (b) 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s; (c) an extension at 72°C for 10 min. The qualified PCR products were extracted from 2% agarose gels, and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's

instructions and then quantitated using QuantiFluor™-ST (Promega, Madison, WI, USA). Purified amplicons were subjected to Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) for sequencing using the Illumina MiSeq system (Illumina, San Diego, CA, USA). Low-quality sequences <50 bp in length under an average quality score lower than 20 were removed according to Prinseq version 0.20.4 (Bokulich et al., 2013), available under the GNU General Public License at <http://prinseq.sourceforge.net>. The sequences were trimmed using Mothur (Schloss et al., 2009), available under the GNU General Public License at <https://www.mothur.org>. Chimeric sequences were removed from further analysis according to the USEARCH algorithm (Edgar, 2018), licensed to use 32-bit USEARCH at <http://www.drive5.com/usearch/>. Accordingly, the high-quality sequences with a 97% identity threshold were classified as operational taxonomic units (OTUs) by UPARSE. Taxonomic assignments were conducted to assign different trophic modes based on the FUNGuild database FUNGuild v1.0 (Nguyen, Song, et al., 2016), available under the GNU General Public License on GitHub at <https://github.com/UMNFuN/FUNGuild>. The soil overall fungi in this study were divided into three trophic mode subsets: pathotrophic, saprotrophic and symbiotrophic fungi.

2.5. Statistical Analysis

Effects of treatments and soil layers on the diversity (Shannon index) of soil overall fungi and different trophic mode fungi were determined using one-way non-parametric Kruskal-Wallis test in R version 4.1.2 (R Core Team, 2021). The similarity in soil fungal community composition between CK plots and N, W and WN plots was tested using non-metric multidimensional scaling (NMDS) via “metaMDS” function of *vegan* package version 2.5–7 (Jari et al., 2020). The Bray-Curtis dissimilarity of square-root-transformed data was used to construct the fungal community dissimilarity matrix. Redundancy analysis (RDA) combined with a Hellinger transformation was applied to visualize differences in the community composition of soil overall fungi and different functional guild fungi among different treatment plots based on the relative abundance of OTUs using the ‘rda’ function in the *vegan* package version 2.5–7 (Jari et al., 2020). The ‘envfit’ function in the *vegan* package version 2.5–7 (Jari et al., 2020) was used to determine the fine-root and fungal mycelial traits, which were significantly correlated with the community composition of soil overall fungi and different functional guild fungi. Co-occurrence network analyses were performed with *psych* version 2.3.3 (William, 2023), *igraph* (Gabor & Tamas, 2006) and *Hmisc* packages version 5.1–0 (Frank & Harrell, 2023) according to the Spearman correlation matrix (correlation coefficient >0.6) and $p < 0.05$ and graphed using Gephi version 0.10 (Bastian et al., 2009), available under the dual license CDDL 1.0 and the GNU General Public License v3 at <https://gephi.org/users/download/>. The fungal network based on all soil layers mixed was constructed by nodes, edges, linkage density, average clustering coefficient, average path distance, modularity and average degree. We applied Linear Discriminant Analysis (LDA) Effect size (LEfSe) analysis to identify the soil fungal biomarkers across different treatments. LEfSe analysis was performed using the online LEfSe project (Galaxy version 1.0) with the default settings (Segata et al., 2011), available without account creation on Galaxy Hutlab Galaxy at <http://huttenhower.sph.harvard.edu/galaxy/>. We reported indicator species with an LDA score >2 and $p < 0.05$ in this study.

3. Results

The responses of the soil overall fungal diversity to nitrogen-water treatments were different across soil layers (Figure 1a). Specifically, different treatments did not change the soil overall fungal diversity in 0–10 cm soil layer. However, for 10–20 cm and 20–30 cm soil layers, soil overall fungal diversity was significantly higher in precipitation reduction combined with nitrogen addition (WN) plots than in ambient precipitation without nitrogen addition (CK) plots and/or nitrogen addition with ambient precipitation (N) plots. Moreover, soil overall fungal diversity consistently decreased with increasing soil layers in CK and N plots, but the decreased trend was not observed in precipitation reduction without nitrogen addition (W) and WN plots (Figure 1a).

We did not find that the effects of nitrogen-water treatments on the diversity of soil pathotrophic fungi in 0–10 cm and 20–30 cm soil layers, but for 10–20 cm soil layer, WN plots showed the lower diversity than W plots. Moreover, the lowest and highest Shannon index in WN plots appeared in 10–20 cm and 20–30 cm soil layer, respectively (Figure 1b). For 0–10 cm soil layer, the diversity of soil saprotrophic fungi was significantly lower in WN plots than in CK, N and W plots. CK, N and W plots had the similar Shannon index. Moreover, for 20–30 cm soil layer, the diversity of soil saprotrophic fungi was lower in W and WN plots than in CK plots (Figure 1c). By contrast, for 0–10 cm soil layer, W plots showed the greater diversity of soil symbiotrophic fungi than WN plots.

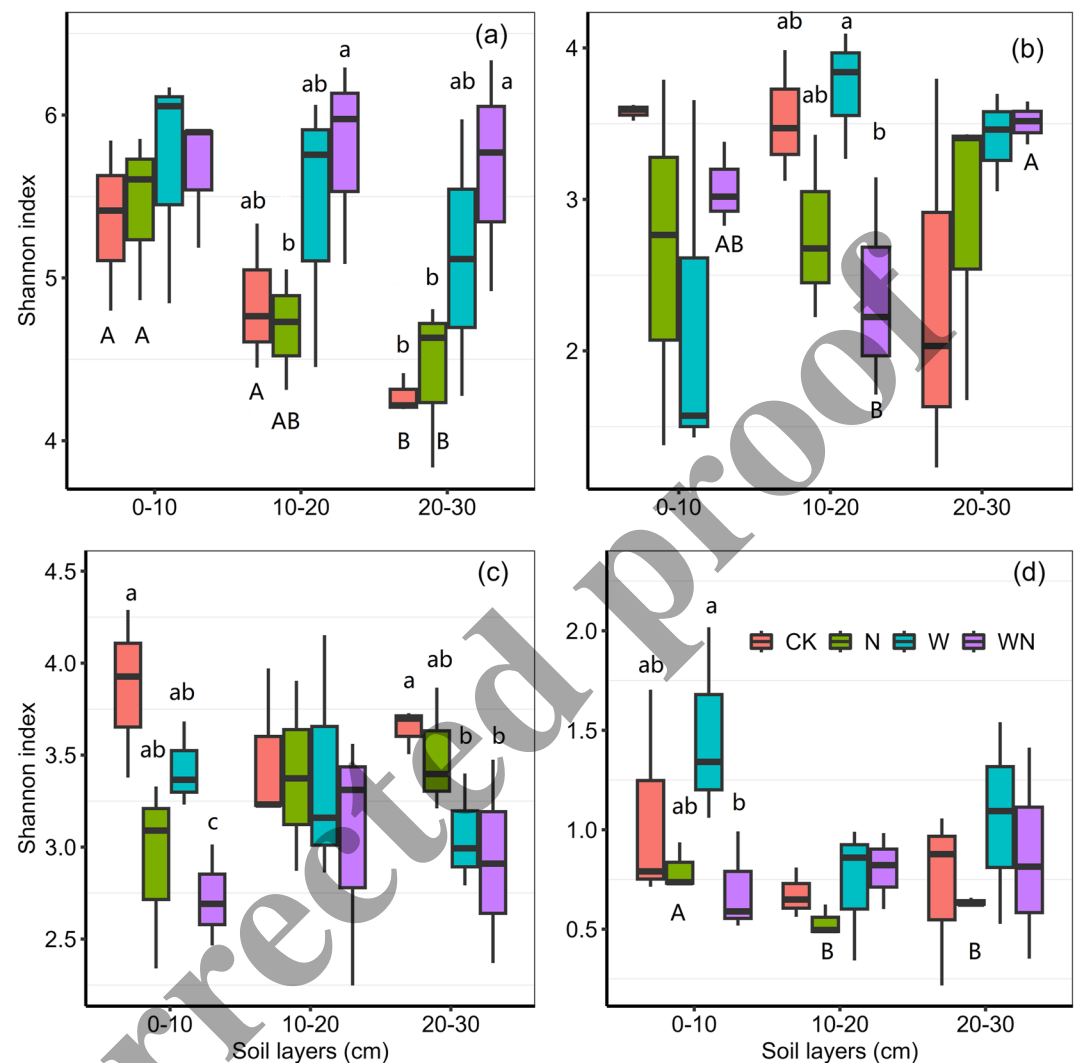


Figure 1. The Shannon indices of soil overall (a), pathotrophic (b), saprotrophic (c) and symbiotrophic (d) fungi for 0–10 cm, 10–20 cm and 20–30 cm soil layers in ambient precipitation without nitrogen addition (CK), nitrogen addition with ambient precipitation (N), precipitation reduction without nitrogen addition (W) and precipitation reduction combined with nitrogen addition (WN) plots. The lower case letters indicate the statistical significance among different treatments for each soil layer. The capital letters indicate the statistical significance among different soil layers for each treatment. The statistical significance with $p < 0.05$ is only given.

WN plots had the same Shannon index with CK and N plots. The diversity of soil symbiotrophic fungi was not affected by the treatments for the other soil layers (Figure 1d).

While the relative abundance of different fungal guilds varied among soil layers (Figure S2 in Supporting Information S1), saprotrophic fungi (42%) were the most abundant fungi of the three fungal guilds, followed by symbiotrophic (37%) and pathotrophic fungi (21%; Figure 2). We found that N plots had more soil symbiotrophic fungi, but the less soil saprotrophic fungi than CK plots. By contrast, W and WN plots had more soil sapro- and pathotrophic fungi, but the less symbiotrophic fungi than the CK plots (Figure 2).

Non-metric multidimensional scaling (NMDS) showed that the composition of soil overall fungi in N, W and WN plots was significantly different from that in CK plots along the second axis (Figure 3a). For different fungal guilds, the composition of soil pathotrophic fungi in W and WN plots was significantly different from that in CK plots along the first axis (Figure 3b), while N plots had different composition of soil saprotrophic fungi compared to CK plots along the first axis (Figure 3c). Moreover, N and WN plots showed different composition of soil symbiotrophic fungi from CK plots along the second axis (Figure 3d). In total, 62 OTU were determined to be

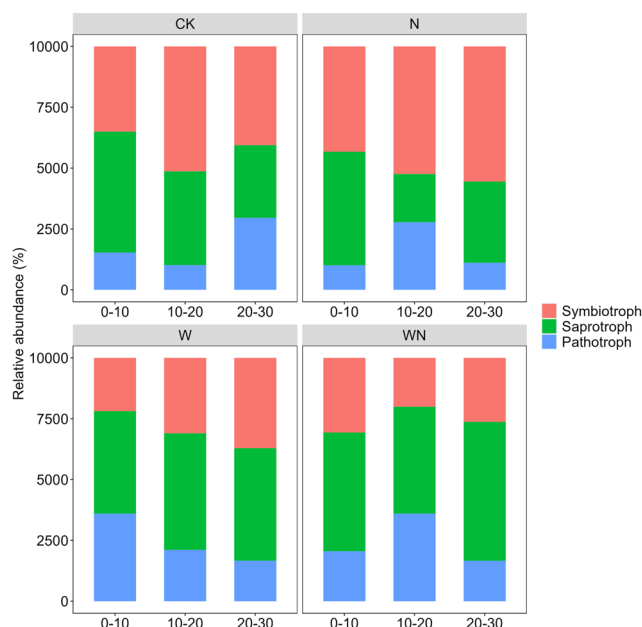


Figure 2. The relative abundance of soil pathotrophic, saprotrophic and symbiotrophic fungi for 0–10 cm, 10–20 cm and 20–30 cm soil layers in ambient precipitation without nitrogen addition (CK), nitrogen addition with ambient precipitation (N), precipitation reduction without nitrogen addition (W) and precipitation reduction combined with nitrogen addition (WN) plots.

used as the indicator species (LDA score >2.0 and $p < 0.05$) for the three fungal guilds, including 19 indicators for pathotrophic fungi, 24 indicators for saprotroph fungi, 19 indicators for symbiotrophic fungi (Figure S3 in Supporting Information S1).

Co-occurrence network analyses indicated that N, W and WN plots showed the lower nodes, edges and average degree, but the higher average path distance and modularity than CK plots (Figure 4). Moreover, W plots showed the highest average path distance and the lower average clustering coefficient, while WN plots showed lowest nodes, edges and average degree among all the treatments (Figure 4).

Redundancy analyses indicated that fine-root diameter (RD) and SRL were the most important factors relating the soil overall fungal community structure (Figure 5a, Figure S4 in Supporting Information S1). With respect to different fungal guilds, soil pathotrophic fungi were related to fine-root nitrogen concentration (RN) and root tissue density (RTD; Figure 5b, Figure S4 in Supporting Information S1). Soil saprotrophic fungi were associated with RD and RTD (Figure 5c, Figure S4 in Supporting Information S1), while soil symbiotrophic fungi with RN and fine-root phosphorus concentration (RP; Figure 5d, Figure S4 in Supporting Information S1).

4. Discussion

4.1. Effects of Nitrogen Addition on Soil Fungal Community

In this study, we found no response of the diversity of soil overall fungi or any fungal guild to long-term nitrogen addition (N; Figure 1), which was inconsistent with our first hypothesis. This might be related to the high background total nitrogen deposition rate in this temperate forest, where the high nitrogen deposition ($23 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) had exceeded the critical load for most soil fungi (Lilleskov et al., 2019; Lü & Tian, 2007). Consequently, some nitrogen-sensitive genera eliminated by high nitrogen deposition likely restricted ability to detect changes in the species diversity of the soil fungal communities in this temperate forest (Hay et al., 2015; Lilleskov et al., 2011). Although the long-term nitrogen addition had no effect on diversity, the composition of the fungal community in the soil changed greatly overall. At the same time, the responses of the community composition to long-term nitrogen addition varied across different fungal guilds (Figure 3). In accordance with our findings, the previous studies conducted in coniferous forests showed that the contrasting patterns of symbio- and saprotrophic fungal communities and the variations observed in symbiotrophic fungal diversity can largely be explained by nitrogen supply (N. Yang et al., 2022). Therefore, further classification of complex soil fungal species into functional guilds with different ecological functions will be beneficial to explore the response processes of soil fungi under increasing nitrogen deposition.

It has been showed that interactions between fungal guilds can arise in particular through changes in nitrogen availability in the soil (Fernandez & Kennedy, 2016). The most important interactions are negative impacts between ECM and saprotrophic fungi (Fernandez & Kennedy, 2016; Gadgil & Gadgil, 1971). In this study, we found that long-term nitrogen addition tended to increase the abundance of soil symbiotrophic fungi but decreased the abundance of soil saprotrophic fungi (Figure 2), supporting our second hypothesis. However, it should be emphasized that the positive relationships between saprotrophic fungal abundance and soil fertility have also been found in some tropical and temperate forest ecosystems (Castaño et al., 2019; Dejene et al., 2017; Zheng et al., 2017). We speculate that the associations between saprotrophic soil fungi and soil fertility are context-dependent and vary with environmental factors such as soil temperature and moisture as well as biotic factors such as tree age and species (Castaño et al., 2019; Kyaschenko et al., 2017; Zheng et al., 2017). It was observed that the suppression of saprotrophs by ECM fungi was enhanced under high levels of nitrogen deposition in a temperate conifer plantation (Liang et al., 2022). Besides the suppression of soil saprotrophic fungi by soil symbiotrophic fungi, nitrogen application had a direct inhibitory effect on the soil saprotrophic fungi through the repression of enzyme activity and the buildup of recalcitrant and toxic compounds (Bardgett et al., 1999). The higher nutrient availability provided by the mycorrhizal symbiosis can stimulate plant carbon sequestration and

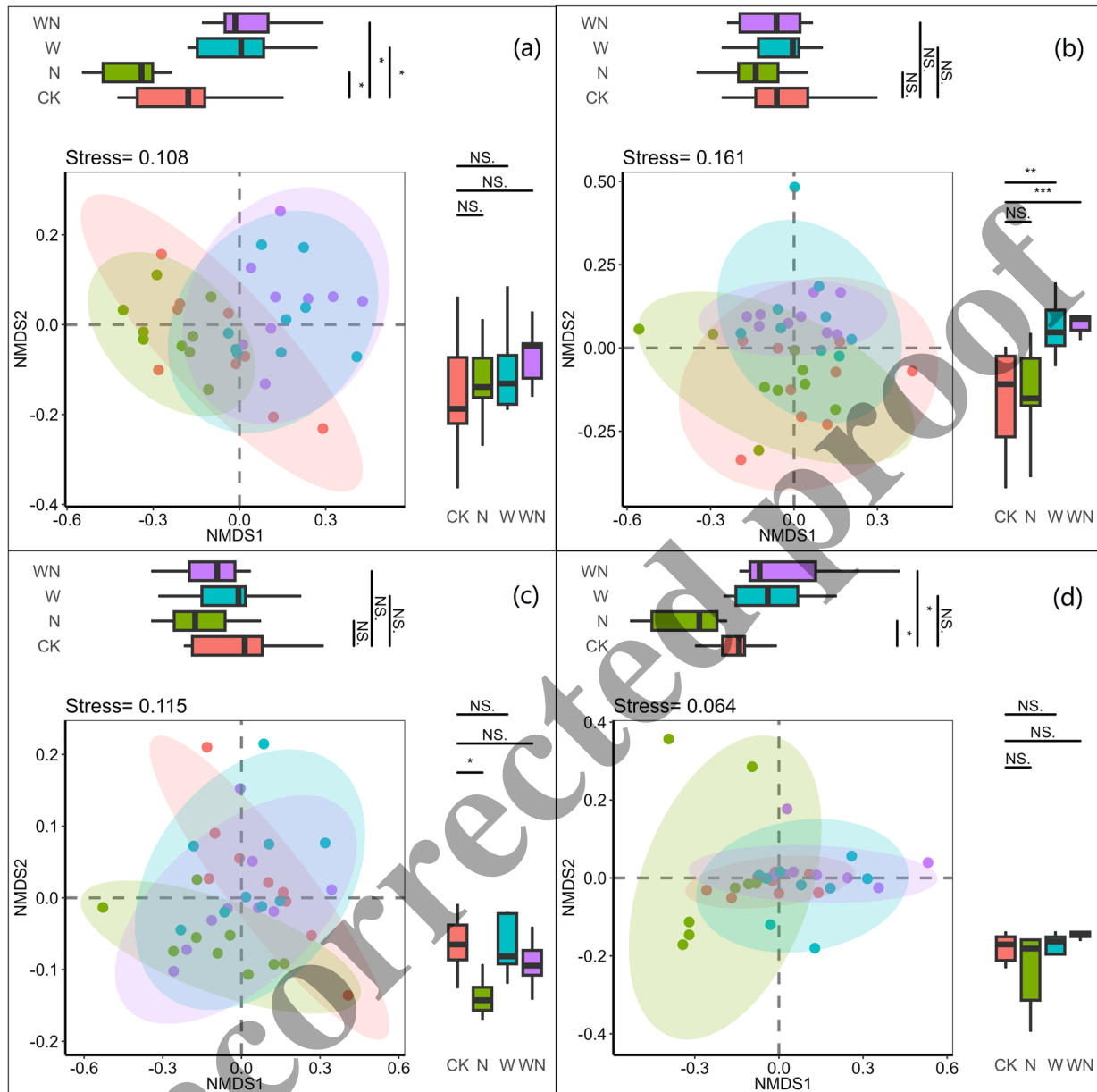


Figure 3. Non-metric multidimensional scaling showing changes in community composition of soil overall (a), pathotrophic (b), saprotrophic (c) and symbiotrophic (d) fungi among ambient precipitation without nitrogen addition (CK), nitrogen addition with ambient precipitation (N), precipitation reduction without nitrogen addition (W) and precipitation reduction combined with nitrogen addition (WN) plots. NS. indicates no difference; * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$.

subsequent carbon inputs to the soil (Zhou et al., 2020). At the same time, the low abundance of soil saprotrophic fungi may impede soil organic matter decomposition under long-term nitrogen addition (S. Frey et al., 2014; Zak et al., 2016). Thus, our findings might consolidate the conclusion that nitrogen deposition accelerated carbon sequestration in temperate forest soils (Janssens et al., 2010; Liang et al., 2022).

4.2. Effects of Precipitation Reduction on Soil Fungal Community

We found that long-term precipitation reduction (W) significantly changed the composition of the overall and the pathotrophic fungal community (Figures 3a and 3b) in this temperate forest soil, partly supporting our first hypothesis. A recent study of natural seasonality with 14-year of 50% canopy throughfall exclusion showed that

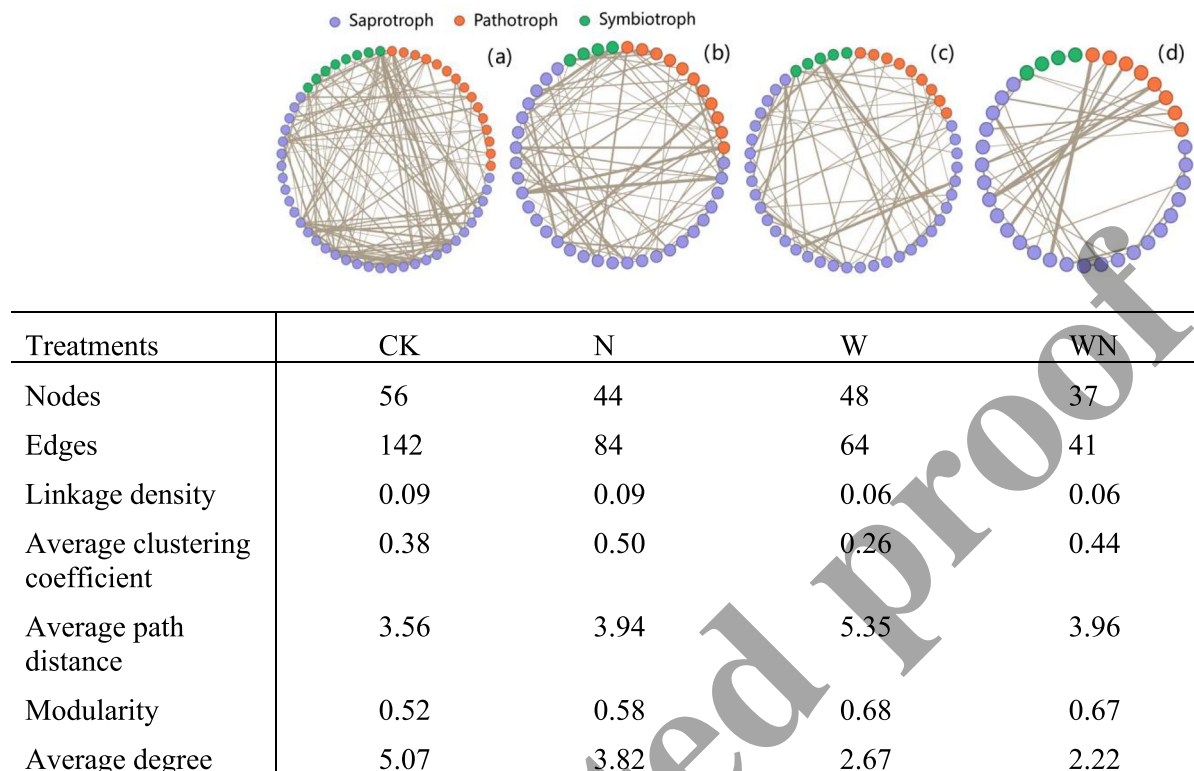


Figure 4. Co-occurrence network analyses on soil pathotrophic, saprotrophic and symbiotrophic fungi in ambient precipitation without nitrogen addition (CK; a), nitrogen addition with ambient precipitation (N; b), precipitation reduction without nitrogen addition (W; c) and precipitation reduction combined with nitrogen addition (WN; d) plots.

large changes in soil moisture could shape soil fungal communities with a conspicuous increase in their functional diversity (Buscardo et al., 2021). The occurrence of compositional shifts within the soil fungal community was correlated to changes in soil water status in boreal peatland (Asemaninejad et al., 2017). At the global level, soil fungal diversity could be also largely explained by mean annual precipitation across various ecosystems (Tedersoo et al., 2014). Similarly, this study showed that long-term precipitation reduction significantly increased the relative abundance of soil saprotrophic fungi but decreased the relative abundance of soil symbiotrophic fungi (Figure 2). Our results of distinct soil fungal guild-specific responses to reduced precipitation indicated that climate-driven soil water changes favor saprophytic fungi that benefit from drier conditions in temperate forest ecosystems. It has been observed that a reduction in soil moisture due to decreased precipitation can facilitate the cellular energy metabolism through improved aerobic conditions favored by some soil aerobic fungi, especially saprotrophs (Allison & Treseder, 2011; Asemaninejad et al., 2017). As discussed above, saprophytic fungi are generally considered a primary engine of the decomposition process (Francioli et al., 2021). The ability to degrade recalcitrant carbon of saprophytic organisms is an important trait for survival and growth in water-limitation forest ecosystems (Hartmann et al., 2017). Accordingly, possible changes in soil fungal community composition due to altered precipitation patterns could favor saprotrophic decomposers at the expense of other fungal groups (e.g., soil symbiotrophic fungi), with dramatic and crucial implications for the carbon sequestration pattern in temperate forests, potentially releasing previously sequestered carbon and exacerbating climate change.

4.3. Effects of Precipitation Reduction Combined With Nitrogen Addition on Soil Fungal Community

Soil water has a strong influence on soil nutrient supply, so reducing precipitation in combination with nitrogen addition (WN) had synergistic effects on fine root-inhabiting fungal communities of this mixed *P. koraiensis* forest (C. Wang et al., 2021; J. Wang et al., 2022). Clearly, the strong interactions between water and nitrogen are very important in this temperate forest where multiple resources interact. Here, we found that combined effects of nitrogen and water were greater than their individual effects on saprotrophic soil fungi, which was

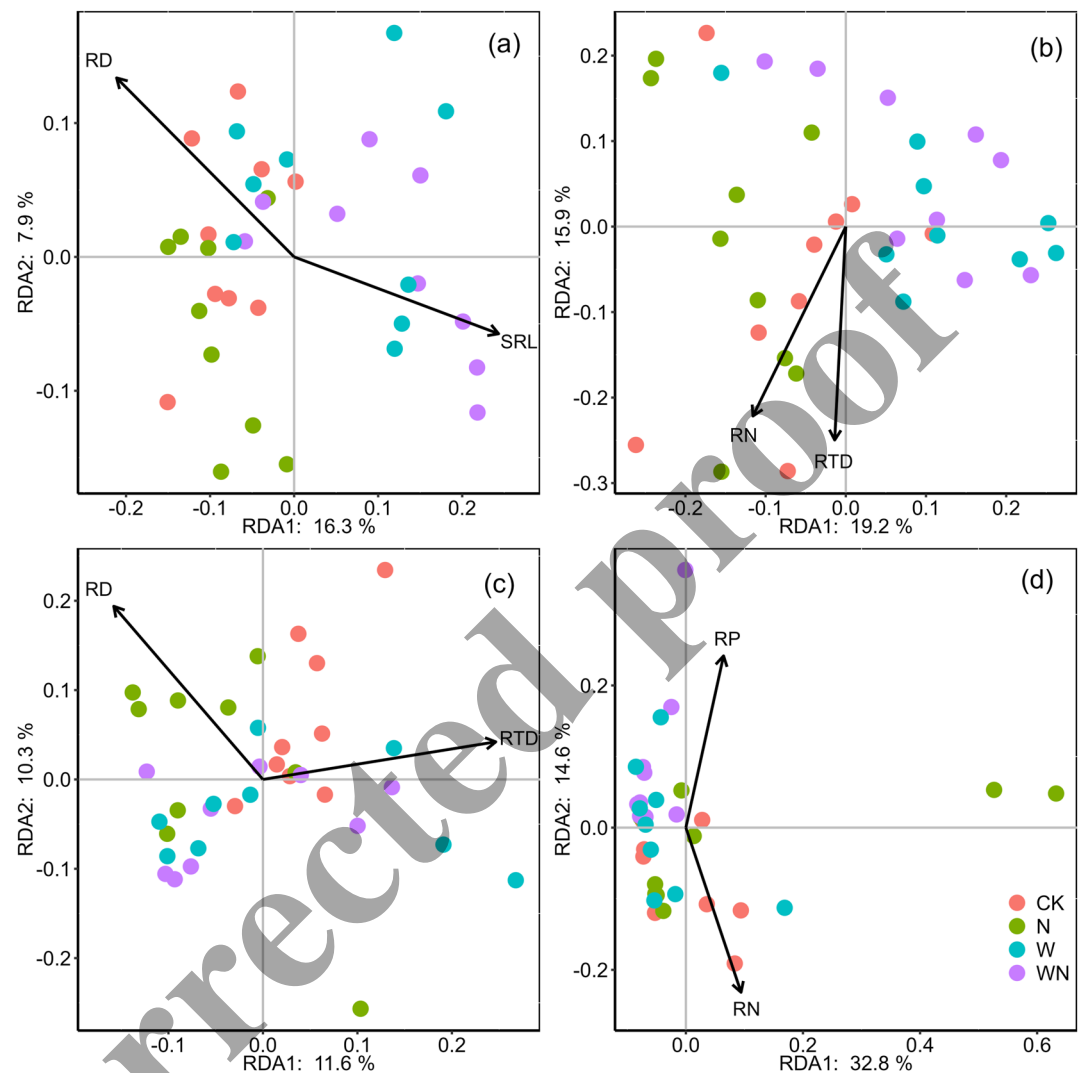


Figure 5. Redundancy analyses (RDA) to identify fine-root and soil fungal mycelial variables significantly (variables with $p < 0.05$ are given) influencing community composition of soil overall (a), pathotrophic (b), saprotrophic (c) and symbiotrophic (d) fungi among ambient precipitation without nitrogen addition (CK), nitrogen addition with ambient precipitation (N), precipitation reduction without nitrogen addition (W) and precipitation reduction combined with nitrogen addition (WN) plots. Root diameter, fine-root diameter; SRL, specific root length; RN, fine-root nitrogen concentration; RP, fine-root phosphorus concentration; root tissue density, fine-root tissue density.

verified by the lowest soil saprotrophic fungal diversity in 0–10 cm and 20–30 cm soil layers of WN plots (Figure 1c), which also supports our first hypothesis. However, the higher abundance of soil saprotrophic fungi in WN plots indicated that the decomposition of soil organic matter was potentially enhanced in the lower soil water but the higher soil nitrogen environment. While decreased precipitation and increased nitrogen deposition can impact soil fungal community composition, the direction and strength of these effects may be guild-specific (Asemaninejad et al., 2018; Geml et al., 2016). As expected in our second hypothesis, we only found that WN significantly changed the composition of soil patho- and symbiotrophic fungi, but not for soil saprotrophic fungi (Figures 3b–3d). Thus, our findings indicated that nitrogen-water interactions did not seem to have dramatic effects on saprotrophic soil fungal community in this temperate forest. At the same time, the relative abundance of soil pathotrophic fungi was higher and the relative abundance of soil symbiotrophic fungi was lower in WN plots than in CK plots (Figure 2). Therefore, the long-term nitrogen-water change might modify the interactions of plants with soil patho- and symbiotrophic fungi in this temperate forest. Soil pathogens can lead to seedling mortality pattern changes of some trees (Packer & Clay, 2000). The previous study conducted in this mixed *P. koraiensis* forest indicated the key role of soil pathogens in mediating plant species coexistence (Qin et al., 2021).

Mycorrhizal fungi can protect plants against root pathogens via mechanisms such as forming a physical barrier around (hyphal mantle) and within (Hartig net) the roots, which restrict the pathogens access to infection sites and photosynthates (Ghorbanpour et al., 2018). The protective effects of mycorrhizal symbioses against soil pathogenic fungi are very vital for the plant community composition in natural forest ecosystems (Bagchi et al., 2011; Fitter & Garbaye, 1994; Qin et al., 2021). The variations we found in the community composition and the abundance of soil patho- and symbiotrophic fungi suggest that long-term nitrogen-water interactions not only change the composition of the fungal community in the soil, but potentially also the coexistence of plants in temperate forests.

Due to the vertical distribution of carbon and nutrients along with soil layers, soil fungi have the various community diversity and composition, and distinct responses to soil environmental changes in different soil horizons (B. Frey et al., 2021). Compared with surface soils, subsurface soils may harbor novel soil fungi, which are responsible for keystone functions in deeper soil layers (Baldrian, 2019; Fierer, 2017; B. Frey et al., 2021). In this study, we also found that WN significantly increased the overall fungal diversity in the 20–30 cm soil layer (Figure 1a), leading to a homogenization of soil fungal diversity across the different soil layers. When soil fungi were grouped by different ecological functional guilds, WN significantly decreased soil saprotrophic fungal diversity in the 0–10 cm and 20–30 cm soil layers (Figure 1c). Moreover, WN drove compositional shifts in the diversity pattern of soil pathotrophic fungi across the soil layers, with the highest Shannon index appearing in the 20–30 cm soil layer (Figure 1b). The highly depth-dependent results suggest that examining the combined effects of multiple drivers of climate change on the fungal communities, particularly in the lower soil layers, will provide greater insight into future carbon dynamics in temperate forest ecosystems.

In this study, we found that nitrogen-water treatments decreased the nodes and edges, but increased the average path distance and modularity of soil fungal co-occurrence networks. Additionally, the soil fungal co-occurrence networks in WN plots were the weakest among the four treatments (Figure 4). These findings indicated that the complexity of soil fungal networks was reduced by elevated nitrogen and/or reduced precipitation, which were partly supported by the previous studies reporting that nitrogen fertilization decreased the complexity of fungal networks (Lekberg et al., 2021). It has previously been showed that disturbances can reduce network complexity of soil microbial communities (Karimi et al., 2017; Morriën et al., 2017). In this temperate forest, changes in soil nitrogen and/or water availabilities as a disturbance would greatly alter interactions within and among different fungal guilds. A field experiment with nitrogen-water addition in a grassland indicated that soil fungal networks are less interconnected under lower water status through modified soil acidification and substrate availability for soil fungi (Chen et al., 2017, 2020; Sorensen et al., 2013). The reduced complexity of soil fungal network with increased nitrogen input and decreased precipitation leads to greater instability of soil fungal communities and weaker resistance to environmental disturbance, thereby decreasing forest ecosystem functionality such as carbon sequestration. In fact, a previous study in this temperate forest has found that nitrogen-water interactions enhanced the inhibition of the soil organic carbon pool (glomalin-related soil protein) because decreased precipitation aggravated soil acidification through elevated nitrogen deposition (Huang et al., 2022). Considering that more connected soil fungal networks are more efficient in carbon uptake and nutrient cycling (Lekberg et al., 2021; Morriën et al., 2017; Yuan et al., 2021), further research is needed to disentangle the causes of these changing patterns in soil fungal networks and their functional implications for carbon sequestration in temperate forests.

4.4. Relationships of Soil Fungal Community With Fine-Root Traits

Here, we found that the overall fungal community composition in the soil was principally determined by fine-root diameter and SRL in this temperate forest (Figure 5). These findings were consolidated by the previous study conducted in a temperate broadleaf deciduous forest, which stated that SRL had the strongest influence on the soil fungal community assembly (Y. Wang et al., 2022). Furthermore, we found that fine-root diameter, fine-root nitrogen and phosphorus concentration, and RTD variously regulated the community structure of soil patho-, sapro- and symbiotrophic fungi, supporting our third hypothesis. Generally, soil patho- and symbiotrophic fungi are part of the biotrophic guilds that depend on living plants to survive, while soil saprotrophic fungi are non-biotrophic, meaning they acquire all their nutrients from non-living plant material or surrounding soil organic matter (Liang et al., 2023). Due to their ecological and functional dissimilarities, these fungal guild communities of biotrophic and non-biotrophic guilds might be shaped by different determinants associating soil and plant traits (Nguyen,

Williams, et al., 2016; T. Yang et al., 2019). For example, symbiotrophic fungal community richness responded more strongly to plant community changes than that of saprotrophic fungi (Peay et al., 2013). In this study, soil symbio- and pathotrophic fungal communities were mainly related to fine-root chemical traits such as phosphorus and/or nitrogen concentration (Figure 5). For symbiotrophic fungi, it is well known that they play crucial roles in promoting the uptake of nutrients, especially phosphorus and nitrogen by host plants (Laliberté et al., 2015; Wallander et al., 2004). However, pathotrophic fungi generally require carbon, nitrogen and phosphorus sources from the hosts (Johns et al., 2021; Laliberté et al., 2015). By contrast, the structure of soil saprotrophic fungal community was associated significantly with fine-root morphological traits such as fine-root diameter. This result may be related with the pivotal role of soil saprophytic fungi in the decomposition process of dying and decaying roots (Francioli et al., 2021), which are greatly dependent on RD (Li et al., 2020). Accordingly, our results suggests that the contrasting roles of various fine-root traits in shaping soil fungal communities clearly depended on fungal ecological guilds in this temperate forest.

5. Conclusions

In this study, we investigate the effects of 9-year nitrogen addition, precipitation reduction and their interactions on soil fungal community in a temperate forest, based on combined assessment of the observed fungal taxa and their corresponding trophic mode function. We found that the direction and strength of nitrogen-water interactions were variable across soil fungal guilds due to their diverse ecological attributes in forest soils. Changes in the composition of the fungal communities of different functional guilds and their shifts in relative abundance would have substantial implications for soil nutrient cycling and carbon sequestration potential in the temperate forest. The long-term nitrogen addition changed the interactions between soil symbio- and saprotrophic fungi, with the higher abundance of soil symbiotrophic fungi and the lower abundance of soil saprotrophic fungi, indicating that nitrogen deposition may accelerate carbon sequestration in temperate forest soils. However, the overall transition in soil fungal communities in favor of saprotrophs at the expense of mycorrhizal root-associated fungi suggests that temperate forests potentially lose more soil carbon due to the accelerated rate of decomposition by saprotrophic soil fungi under the long-term reduced precipitation condition. Given the key role of soil pathogens and mycorrhizal fungi in mediating plant species coexistence, variations in community composition and abundance of patho- and symbiotrophic soil fungi suggest that long-term nitrogen-water interactions may potentially alter plant community assembly in temperate forest ecosystems. Accordingly, our study indicated the importance of accounting functional groups of soil fungi to gain a deeper understanding of the overall fungal community in soil and the responses and variabilities of functional models of soil fungi in temperate forest ecosystems under future climate changes.

Data Availability Statement

All data on soil fungi and mycelial/fine-root traits used in this study are available from <https://datadryad.org/stash/share/CvFTKpn8-ZaQnI7rz09Cw-xYT4y6EEu5vTQgjsAemFA> (Guo et al., 2023).

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