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Long-term Consequences on Soil Fungal Community Structure: Monoculture Planting and Natural Regeneration

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Abstract

Understanding the regeneration and succession of belowground communities, particularly in forests, is vital for maintaining ecosystem health. Despite its importance, there is limited knowledge regarding how fungal communities change over time during ecosystem development, especially under different forest restoration strategies. In this study, we focused on two restoration methods used in northern Japan: monoculture planting and natural regeneration. We examined the responses of the fungal community to monoculture plantations (active tree planting) and naturally regenerated (passive regeneration) forests over a 50-year chronosequence, using natural forests as a reference. Based on DNA metabarcoding, we assessed the richness of fungal Operational Taxonomic Units (OTUs) and their dissimilarity. Our findings revealed that soil fungal richness remained stable after natural regeneration but declined in monoculture plantations, from 354 to 247 OTUs. While the compositional dissimilarity of fungal assemblages between monoculture plantations and natural forests remained consistent regardless of the time since tree planting, it significantly decreased after natural regeneration, suggesting recovery to a state close to the reference level. Notably, the composition of key functional fungal groups—saprotrophic and ectomycorrhizal— has increasingly mirrored that of natural forests over time following passive natural regeneration. In summary, our study suggests that monoculture plantations may not be effective for long-term ecosystem function and service recovery because of their limited support for soil fungal diversity. These results underscore the importance of natural regeneration in forest restoration and management strategies.

Keywords Chronosequence · DNA metabarcoding · Forest restoration · Monoculture plantation · Natural regeneration · Fungal community

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Introduction

Forest restoration initiatives have expanded in response to the rapid decrease in natural forests worldwide (Lewis et al. 2019). Additionally, there is growing interest in restoring native ecosystems as well as increasing forest areas, as many studies have demonstrated that natural forests maintain higher biodiversity and ecosystem functioning (Gibson et al. 2011; Mori et al. 2021). In particular, while aboveground restoration has attracted considerable interest (Latawiec et al. 2016; Lewis et al. 2019), the importance of belowground ecosystems and their diversity has also recently been recognized (Lehmann et al. 2020; van der Putten et al. 2023). For example, the European Commission's New EU Forest Strategy for 2030 (2021) mentions the importance of knowledge accumulation associated with sound and site-adapted forest soil restoration. In recent years, recovery of belowground ecosystems has become the next step in forest restoration worldwide.

The development of high-throughput DNA sequencing has enabled the determination of soil microbial diversity. In particular, fungi are key components of belowground ecosystems that occupy a large portion of soil microbial biomass and play important roles in regulating multiple ecological processes, such as decomposition, nutrient transformation, and carbon storage (Bardgett and Wardle 2010; Bardgett and Van Der Putten 2014; Shi et al. 2021). For example, the mycorrhizal network is key to maintaining healthy forests by enabling trees to share nutrients, water, and defense signals (Selosse et al. 2006). Moreover, soil fungi also have strong interactions with plants, which are the main components of forests, such as symbiosis in the case of ectomycorrhizal fungi in the rhizosphere and decomposition in the case of saprotrophic fungi in leaf litter (Osono 2007; Teste et al. 2017). Therefore, understanding the diversity and composition of soil fungal communities is essential for restoring natural forests (Mori et al. 2016; Shi et al. 2021).

In many parts of the world, natural forests have been converted to monoculture plantations (active tree planting), such as Norway spruce (*Picea abies*) in Europe, Japanese cedar (*Cryptomeria japonica*) in Japan and Douglas fir (*Pseudotsuga menziesii*) in North America (FAO 2016; Liu et al. 2018). These artificial forests are effective in producing timber at the expense of biodiversity and other important ecosystem functions (Kelty 2006; Wingfield et al. 2015; Hua et al. 2022). This kind of environmental homogenization can simplify ecological communities, including soil fungi (Tatsumi et al. 2021). On the other hand, natural regeneration (passive regeneration) is gaining attention because it is effective in restoring the plant diversity and vital functionality of forests (Poorter et al. 2021). Although soil fungi play important roles in forest ecosystem functioning, a knowledge gap still exists regarding soil fungal communities as a consequence of implementing these different reforestation activities. One of the main reasons for this knowledge gap is the lack of long-term data (Matsuoka et al. 2016) — high-throughput DNA sequencing has only been available for the past 20 years.

In the present study, we addressed this knowledge gap using the chronosequence approach, which allows us to estimate long-term changes in fungal communities during forest growth. Specifically, we compared the long-term succession of the fungal community between monoculture conifer plantations and naturally regenerated forests in northern Japan using this approach, and evaluated the recovery of natural forests. We hypothesized that tree planting would result in soil fungal diversity and community composition that would differ from those of natural forests. Conversely, we speculated that with natural regeneration, the soil fungal diversity and community composition could be restored to a pre-disturbed state. Furthermore, we assumed that the effects of restoration schemes would be reflected in the fungal functional guilds of the forest ecosystems. Such an assessment based on a temporal approach will aid in the development of better methods for forest restoration, including that of belowground ecosystems.

Materials and methods

Study sites and soil sampling

This study was conducted in the Teshio Experimental Forest of Hokkaido University (44°54′–45°06′N, 141°56′–142°10′E; area: 22,517 ha) in the northern part of Hokkaido, Japan. The mean monthly temperature ranges from −5.4 to 18.1 °C (January and July, respectively), and the annual precipitation is approximately 1000 mm. The study sites were located in the transition zone between cool-temperate and subboreal forest ecosystems. The soil in this region is classified as Cambisol (Food and Agriculture Organization soil taxonomy), with a pH value of 5.0, and the content of total nitrogen (N), exchangeable magnesium (Mg), calcium (Ca) and potassium (K) at 16.00, 0.40, 1.30 and 0.36 g kg^{-1}, respectively. The average depth of the FH and mineral horizons are 2.7 cm and 5.3 cm, respectively (Makoto et al. 2012).

Approximately 90% of the forest area is covered by natural forests and naturally regenerated forests, mainly consisting of deciduous broad-leaved trees (e.g., *Quercus crispula* and *Betula ermanii*) and evergreen conifer trees (e.g., *Picea glehnii* and *Abies sachalinensis*) (Takagi et al. 2015). The remaining 10% of forests have been replaced by conifer plantations. Soil scarification was conducted at the beginning of restoration to remove dwarf bamboo (*Sasa*

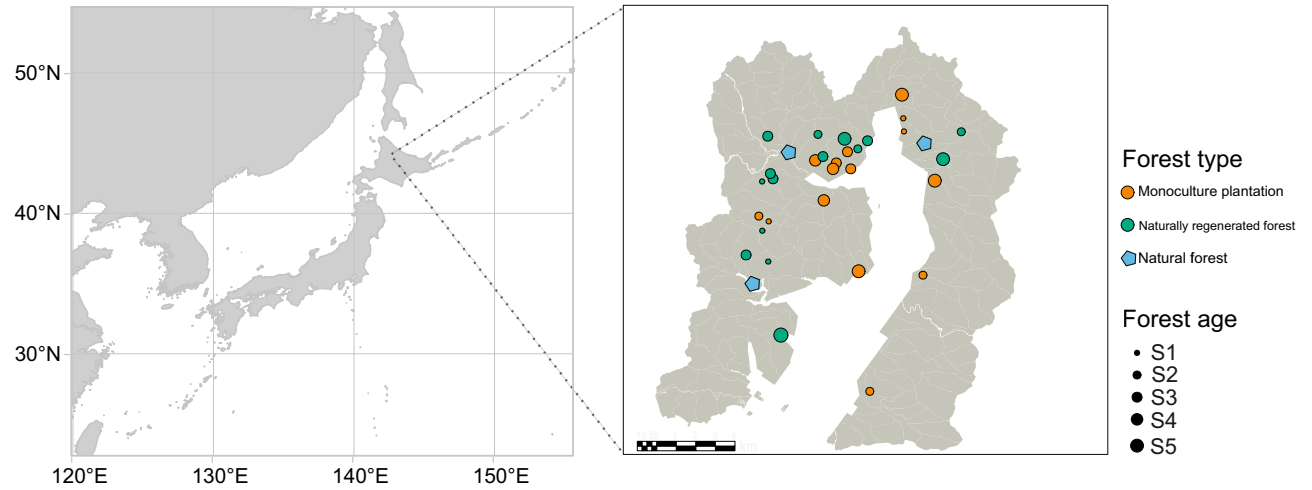

Fig. 1 Study site in the Teshio Experimental Forest of Hokkaido University. We conducted our research at 33 forest sites. The colors of the points denote the three types of forest: orange, monoculture plantation; green, naturally regenerated forest; and light blue, natural forest. The size of the points denotes the age of the forest: S1, 10-year-old stand; S2, 20-year-old stand; S3, 30-year-old stand; S4, 40-year-old stand; and S5, 50-year-old stand

kurilersis), which promoted tree establishment (Yoshida et al. 2005). Coniferous species such as *P. glehnii* and *A. sachalinensis* were selected for monoculture planting. In this study, natural regeneration involved spontaneous recovery with human intervention, such as soil scarification, which is necessary to facilitate this process, and is increasingly referred to as assisted natural regeneration (Latawiec et al. 2016; Crouzeilles et al. 2017; Lewis et al. 2019; Kumar et al. 2006; Shono et al. 2007). Naturally regenerated forests resulting from natural regeneration are dominated by *Betula* spp. (*B. ermanii, B. platyphylla*, and *B. maximowicziana*), which are the pioneer species in this region. Forests undergoing restoration are distributed in patches with different stand ages, ranging from 0 to 50 years (Katayama et al. 2020), which makes them ideal for applying a chronosequence approach to study fungal community succession after two forest restoration schemes.

Five successional stages were determined based on the standard age of the forests undergoing restoration for each decade as one stage (Table S1). Three *P. glehnii* and naturally regenerated forests at each successional stage and three natural forests aged > 70 years were selected (33 forests in total) as study sites to estimate the restoration of fungal communities by monoculture planting and natural regeneration (Fig. 1 and Table S1). The site size ranges from 0.22 to 16.77 m^2 (Table S1).

Soil samples were collected in August 2021. At each site, three plots (1 × 1 m) were arranged on a line transect, excluding the forest edge. In each plot, after removing the litter layer, we collected three soil samples (50 cm apart) using a sterile soil core (2.5 cm diameter, 5 cm depth), and mixed them as a single sample (three soil samples for each site and 99 in total). Soil samples were placed in sterile plastic bags, passed through a 2 mm sieve, and stored at −20 °C for analysis.

DNA Extraction, Polymerase Chain Reaction (PCR), and DNA Sequencing

According to the manufacturer's instructions, soil genomic DNA was extracted from fresh soil (0.5 g) using the Fast DNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The primer sets ITS1Fkyo2 (5'-*TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG* TAG AGG AAG TAA AAG TCG TAA-3') and ITS2-kyo2 (5'-*GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G*TT YRC TRC GTT CTT CAT C-3') were used to amplify the internal transcribed spacer region in the fungal genomes by PCR. Italics and normal letters represent the fungus-specific and MiSeq sequencing primers, respectively. PCR was performed in a reaction volume of 25.0 µl with the buffer system of Ex Taq HS system (TAKARA Bio, Kusatsu, Japan), which contained 1.0 µl of template DNA, 0.2 µl of Takara Ex Taq Hot Start Version, 2.0 µl of 10 × Ex-buffer, 1.6 µl of dNTP, 0.8 µl each of the two primers (5 µM), and 18.6 µl of nuclease-free water. The PCR conditions were as follows: initial denaturation for 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, annealing for 30 s at 50 °C, 60 s at 72 °C, and a final extension for 5 min at 72 °C. The PCR products were purified using Agencourt AMPure XP (PCR product: AMPure XP beads = 1:0.8; Beckman Coulter, Brea, CA, USA) prior to the supplemental PCR. Subsequently, supplemental PCR was performed using the primer set Nextera XT Index Primer1 (N7xx) / Nextera XT Index Primer2 (S5xx) to add Illumina sequencing adaptors to soil samples. The PCR was performed in a 50 µl reaction

volume containing 5.0 µl of template, 25.0 µl of 2 × KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, WA, USA), 5.0 µl of each primer, and 10.0 µl of PCR grade water. The PCR conditions were as follows: initial incubation for 3 min at 95 °C, followed by eight cycles of 30 s at 95 °C, 30 s at 55 °C for annealing, and 30 s at 72 °C, and final extension for 5 min at 72 °C. PCR products were purified using AMPure XP beads (Beckman Coulter), mixed with PhiX control DNA at a ratio of 75:25, and paired-end sequencing (2 × 300 bp) was performed using the MiSeq Reagent Kit v3 on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Sequence data were deposited in the Sequence Read Archive of the DNA Data Bank of Japan (accession number: DRR444239-DRR444337).

Bioinformatics

Bioinformatic analyses were performed as described by Matsuoka et al. 2021. For the raw FASTQ files, paired-end reads were merged using commands implemented in the Claident pipeline (Tanabe and Toju 2013; software available online: https://www.claident.org/). Chimeric reads and sequencing errors were removed using UCHIME v4.2.40 (Edgar et al. 2011) and CD-HIT-OTU (Li et al. 2012), respectively. The remaining reads were assembled into operational taxonomic units (OTUs) at a 97% similarity threshold. OTU taxonomic identity was determined based on the query-centric auto-k-nearest-neighbor method (Tanabe and Toju 2013) using the NCBI database and the lowest common ancestor algorithm (Huson et al. 2007) in Claident. Functional guilds were determined based on the Fungal Traits database for each fungal OTU in which the family and genus could be identified (Põlme et al. 2020). We successfully sequenced 98 (from 45 naturally regenerated, 45 plantation and eight natural forests) of the 99 samples and performed fungal community analysis ($n = 98$).

Statistical Analyses

All statistical analyses were performed using R version 4.2.0 (R Core Team, 2022). We calculated OTU richness to estimate α diversity. In this study, we used the Jaccard dissimilarity distance between communities in restored and natural forests (hereafter, the Jaccard dissimilarity index) to represent the restoration rate of fungal community composition. A lower Jaccard dissimilarity index indicates a community composition more similar to that of natural forests, indicating a higher fungal restoration rate (Fang et al. 2023). In each restoration scheme, linear regression models were used to explore the changes in OTU richness and Jaccard dissimilarity index with stand age. Moreover, the community composition of soil fungi in different forest types was visualized by principal coordinate analysis (PCoA) based on Jaccard dissimilarity matrices using the 'vegan' package (Oksanen et al. 2022). We then calculated the Jaccard dissimilarity index of the top two functional guilds with the highest relative OTU numbers: saprotrophic and ectomycorrhizal (ECM) fungi (23 and 7.8% on average, respectively). To assess the restoration of these two important functional guilds, the richness of shared OTU (observed in both restoration and natural forest) and unshared OTU (observed in restoration but not in natural forest) was calculated.

Results

A total of 5264 fungal OTUs were obtained from 98 soil samples (45 naturally regenerated forests, 45 monoculture plantations, and eight natural forests) of 99 soil samples (Fig. S1). Of the obtained OTUs, 2695 (51% of the total number of OTUs) were from Ascomycota, 1710 (32%) from Basidiomycota, 430 (8%) from Mucoromycota, 85 (2%) from Chytridiomycota, 57 (1%) from Zoopagomycota, 3 (0.1%) from Cryptomycota, 2 (< 0.1%) from Blastocladiomycota, and 282 (5%) could not be identified at the phylum level.

Fungal OTU richness decreased significantly with forest age in monoculture plantations (from 354 in S1 to 247 in S5) but not in naturally regenerated forests (plantation forest: $R^2 = 0.25$, $P < 0.001$; naturally regenerated forest: $R^2 = 0.04$, $P > 0.05$) (Fig. 2). In addition, the Jaccard dissimilarity index between the fungal communities in the naturally regenerated forests decreased significantly with forest age ($R^2 = 0.27$, $P < 0.001$) (Fig. 3), indicating that the fungal community composition in the naturally regenerated forest became increasingly similar to that in the natural forest over time. However, no significant relationship was observed in the monoculture plantation ($R^2 < 0.00$, $P > 0.05$). The PCoA ordination plot showed that the composition of the fungal community in plantations did not resemble that of the natural forest, even as the forest aged. By contrast, in naturally regenerated forests, it converged to that of natural forests with increasing age (Fig. 4).

We used the Fungal Traits database to annotate fungi and saprotrophic fungi, ECM fungi, parasitic fungi, and pathogenic fungi, accounting for 1213 (23.0%), 412 (7.8%), 151 (2.9%), and 144 (2.7%) of the total number of OTUs, respectively. At all successional stages and forest types, saprotrophic fungi had the highest proportion of OTUs, followed by ECM fungi (Fig. S2). The Jaccard dissimilarity index of these two functional guilds in the naturally regenerated forest significantly decreased with forest age (saprotrophic fungi: $R^2 = 0.14$, $P < 0.001$; ECM fungi: $R^2 = 0.09$, $P < 0.001$) (Fig. 5a, b), whereas no trend was

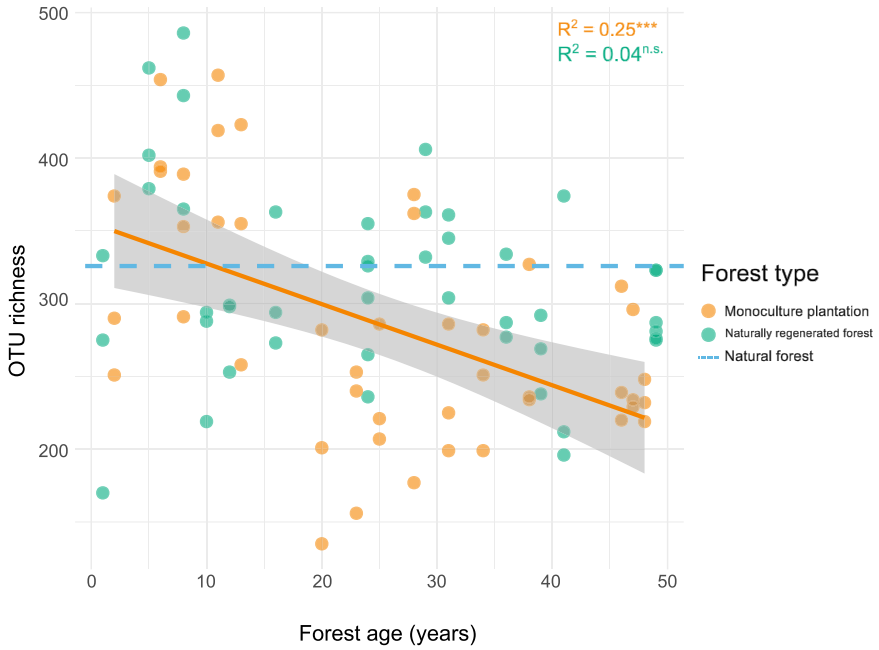

Fig. 2 Operational taxonomic unit (OTU) richness over time for each treatment. A trend line and 95% credible intervals are shown in cases where linear regression with forest age appeared significant at $P < 0.05$. The R^2 values in the upper right indicate the variance in the Jaccard dissimilarity index explained by forest age; ***$P < 0.001$; ns. $P \geq 0.05$

observed in the monoculture plantation (saprotrophic fungi: $R^2 = 0.00$, $P > 0.05$; ECM fungi: $R^2 < 0.00$, $P > 0.05$) (Fig. 5a, b). Differences were also observed between the schemes in terms of the richness of the shared and unshared OTUs. In the monoculture plantations, there was no correlation between shared OTU richness and forest age (saprotrophic fungi: $R^2 = 0.00$, $P > 0.05$; ECM fungi: $R^2 = 0.00$, $P > 0.05$) (Fig. 5c, d). The richness of the unshared OTUs of saprotrophic fungi significantly decreased with forest age, whereas that of ECM fungi significantly increased (saprotrophic fungi: $R^2 = 0.163$, $P < 0.001$; ECM fungi: $R^2 = 0.162$, $P < 0.001$) (Fig. 5e, f). In the naturally regenerated forest, the richness of shared OTUs increased for both saprotrophic and ECM fungi (saprotrophic fungi: $R^2 = 0.074$, $P < 0.001$; ECM fungi: $R^2 = 0.161$, $P < 0.001$) (Fig. 5c, d). However, the dynamics of unshared OTU richness in the naturally regenerated forest were consistent with those observed in the monoculture plantations (saprotrophic fungi: $R^2 = 0.027$, $P < 0.001$; ECM fungi: $R^2 = 0.231$, $P < 0.001$) (Fig. 5e, f).

Discussion

Impact of Forest Management Practices on Fungal Communities

Tree planting and natural regeneration are two major ways to restore forests. We focused on soil fungal communities and evaluated the effects of these forest regeneration approaches on soil fungi by comparing 50 years of community succession between monoculture plantations and naturally regenerated forests. Information regarding succession is fundamentally and practically required for forest restoration.

We found that natural regeneration maintained fungal richness, whereas monoculture planting reduced it. Single-species tree planting has been reported to decrease fungal richness because of environmental homogenization and host-plant specificities (Vitali et al. 2016; Lan et al. 2017; Guo et al. 2022). In particular, conifer plantations often have a lower fungal diversity (Garau et al. 2019a, 2019b; Guo et al. 2022) because their leaves are characterized by high lignin content and low pH, which has a significant impact on fungal host specificity (Guo et al. 2022; Marčiulynas et al. 2022). The negative correlation between OTU richness and stand age in monoculture plantations may be mainly ascribed to the reduced enzymatic activity, soil nutrient availability, and litter decomposition rate caused by *Picea* litter accumulation (Šťursová et al. 2012; Wang et al. 2022). Additionally, monoculture plantations cause biological homogenization, which is a process of biodiversity loss. Tatsumi et al. 2021 reported that fungal alpha diversity in monoculture plantations is higher, but beta diversity is significantly lower compared to natural forests in Hokkaido. Our study indicates that extreme biological homogenization in monoculture plantations may ultimately affect the decline in local alpha diversity by comparing 50 years of fungal succession. In recent years, the biodiversity of naturally regenerated forests has been investigated, particularly in the aboveground areas. Crouzeilles et al. (2017) reported that the diversity of plants, birds, and invertebrates in these areas was higher than that in plantations. We showed that natural regeneration is an

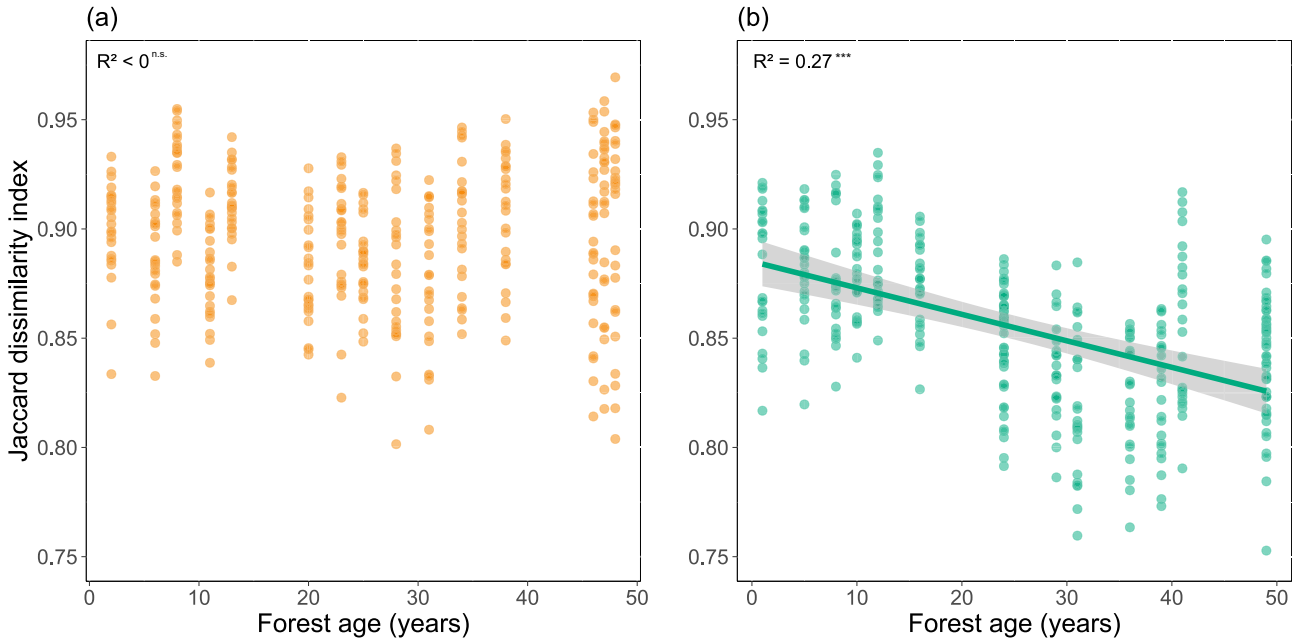

Fig. 3 Changes over time in the dissimilarity of fungal communities compared with natural forests. The Jaccard dissimilarity index was calculated for all plots of (**a**) monoculture plantations and (**b**) naturally regenerated forests. A trend line and 95% credible intervals are shown in cases where linear regression with forest age appears significant at $P < 0.05$. The R^2 values in the upper left indicate the variance in the Jaccard dissimilarity index explained by forest age; ***$P < 0.001$; ns. $P \geq 0.05$

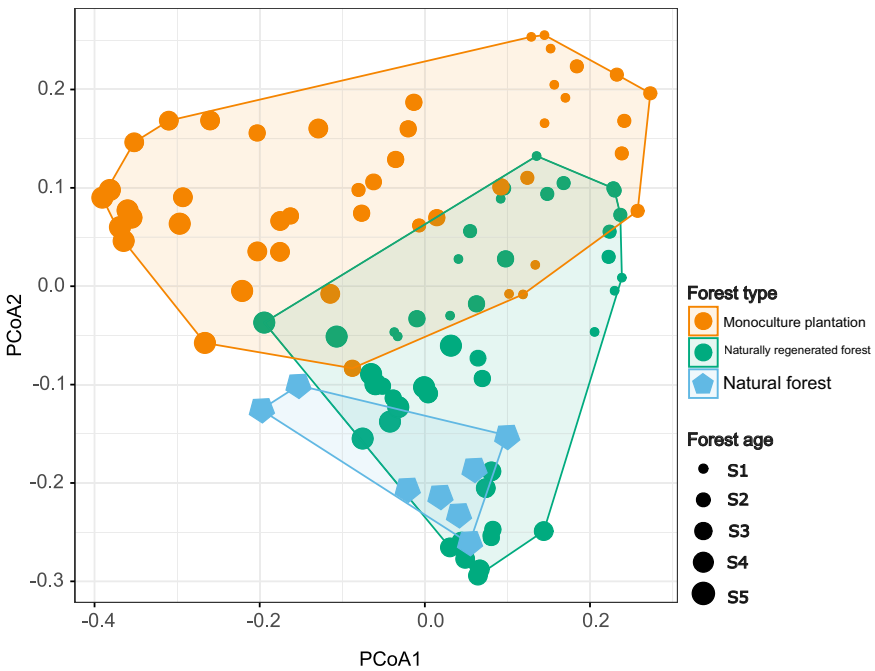

Fig. 4 Organization of fungal communities, highlighting forest types and their transitions. Principal coordinate analysis (PCoA) was performed using the Jaccard dissimilarity index for all the 98 plots. The size of the points denotes the age stage of the forest: S1, 10-year-old stand; S2, 20-year-old stand; S3, 30-year-old stand; S4, 40-year-old stand; and S5, 50-year-old stand

effective way to maintain the richness of soil fungi, which is one of the most important groups in belowground ecosystems.

PCoA showed that the fungal community composition changed at different successional stages in both monoculture plantations and naturally regenerated forests. This suggests that the time elapsed after the onset of community assembly can significantly influence fungal community patterns observed in the field (Matsuoka et al. 2016). Moreover, the fungal community assembly may differ between monocultures and naturally regenerated forests. It is widely accepted that changes in vegetation formation type strongly affect fungal community composition by altering soil properties, such as water content, pH, and nutrient

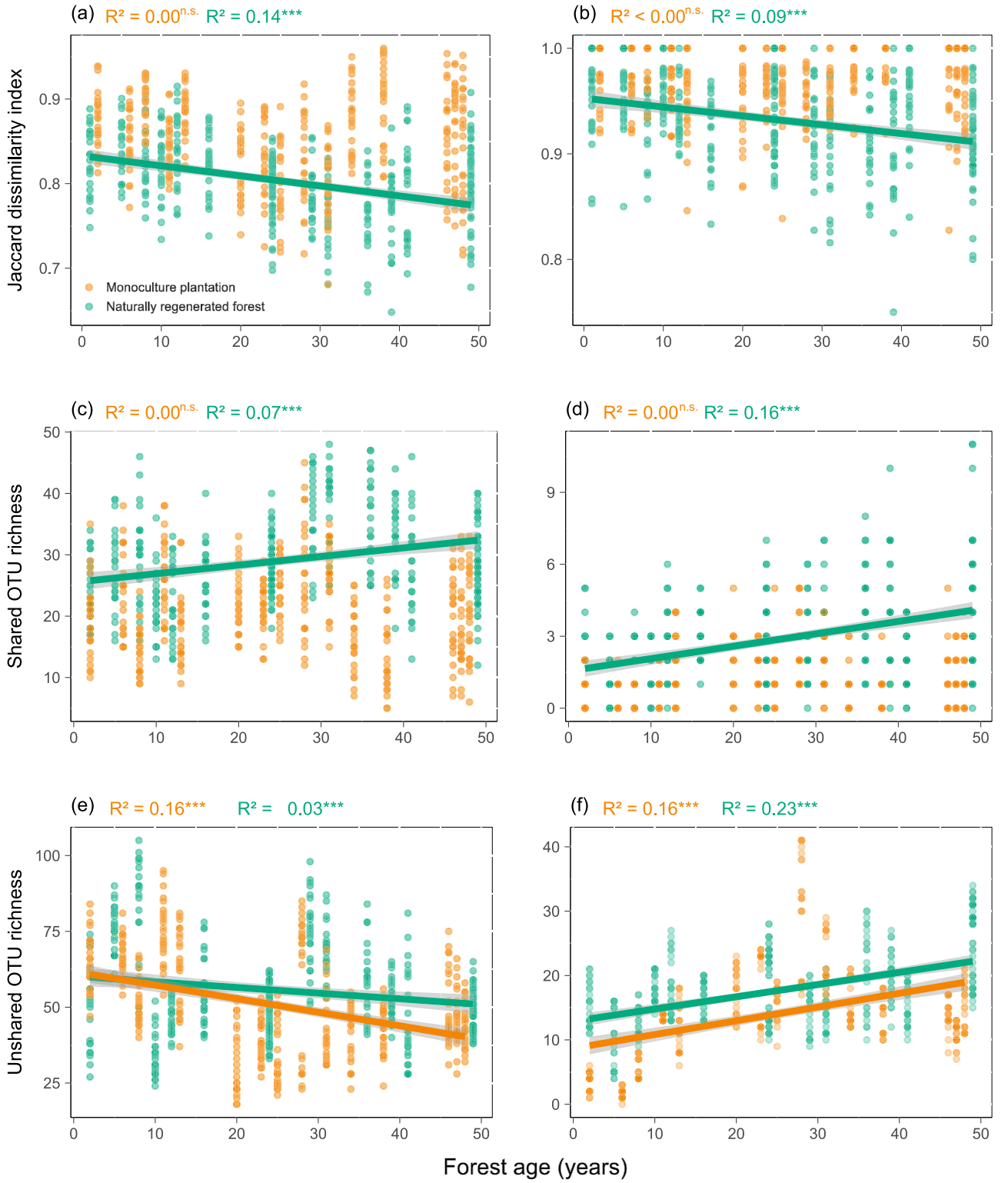

Fig. 5 Dynamics of the two major fungal functional guilds with forest age. The Jaccard dissimilarity index was calculated for (**a**) saprotrophic fungi and (**b**) ectomycorrhizal fungi. Shared operational taxonomic unit (OTU) richness of (**c**) saprotrophic fungi and (**d**) ectomycorrhizal fungi with natural forests over time. Unshared OTU richness of (**e**) saprotrophic fungi and (**f**) ectomycorrhizal fungi over time. A trend line and 95% credible intervals are shown in cases where linear regression with forest age appears significant at $P < 0.05$. R^2 values in the upper left indicate the variance in the variables explained by forest age; ***$P < 0.001$; ns. $P \geq 0.05$

content (McGuire et al. 2014; Guo et al. 2022). For example, Fang et al. (2023) found that total soil phosphorus explained the fungal community variation during the growth of subalpine *Picea asperata* monoculture plantations. In contrast, Jiang et al. 2021 demonstrated that total carbon is the most important soil factor affecting the fungal community composition during natural regeneration in boreal forests. The different consequences of fungal community assembly between monoculture plantations and naturally regenerated forests imply that different soil factors may drive soil fungal community construction, shifting, and assembly in these two forests. Additional studies are required to determine the differences in the driving forces of community assembly between the two reforestation schemes.

Previous studies have demonstrated that the composition of fungal communities is highly heterogeneous compared with that of other microbes (Yan et al. 2021). Nonetheless, we found that the fungal community composition in naturally regenerated forests converged with that in natural forests. Our findings imply that temporal aboveground-belowground linkages generate completely different compositions in monoculture plantations and naturally regenerated forests, which has been difficult to achieve in previous studies based on data from a single point in time (McGuire et al. 2014). However, distinct differences in fungal communities between naturally regenerated and natural forests were observed even at the late successional stage, which is consistent with the results of Adamo et al. (2021). This can be attributed to the incomplete recovery of forest vegetation during the 50 years of forest growth (Poorter et al. 2021), and the absence of keystone fungi in natural forests may also influence the community composition (Banerjee et al. 2018; Wall et al. 2020). Additional studies are needed to identify the essential taxa in fungal networks and reveal the mechanisms leading to their establishment, including ecological selection and dispersal effects.

Further classification of complex fungal species into guilds with different ecological functions would be beneficial for exploring ecological processes driven by fungi. Ectomycorrhizal and saprotrophic fungi are the most common fungal functional groups, and have different life strategies associated with litter decomposition and nutrient cycles (Wang et al. 2019; Li et al. 2020). Although these functional guilds are often filtered in different environments (Mooshammer et al. 2014; Shigyo et al. 2019), we found that the community composition of these two functional guilds in the naturally regenerated forest was recovered to the natural forest consistently. In contrast, no such trend was observed in the monoculture plantation. Indeed, these functional guilds have been shown to have host preferences (Osono 2007; Matsuoka et al. 2020), and the change in fungi may cause a shift in the nutrient cycle during forest growth based on their relationship with plants, such as *Picea* in monoculture plantations and *Betula* in naturally regenerated forests (Matsuoka et al. 2020).

We found some specific fungi that characterized the specific environment of the *Picea glehnii* plantation. For example, *Coccomyces*, observed in later successional stages, are saprotrophic fungi known to be associated with bleached leaf litter and have vigorous ligninolytic abilities (Osono 2007). The saprotrophic fungal community may be dominated by a few host-specific fungi owing to the accumulation of conifer litter with a high lignin content, resulting in a decrease in unshared species in monoculture plantations. However, a decrease in unshared species richness was also observed in naturally regenerated forests. Oligotrophic saprotrophic fungi may be gradually replaced by eutrophic saprotrophic fungi along a gradient of increasing soil nutrients (Sterkenburg et al. 2015; Ning et al. 2021), and a decrease in unshared richness in monoculture plantations may reflect a general trend of change from saprotrophic fungi of grassland origin to those of forest origin as well. In contrast, leaves of *Betulat* dominat in naturally regenerated forests can facilitate community replacement in the natural state, as broad-leaved litter decomposes more easily than conifer needles and has the potential to host a variety of fungi (Osono 2007, 2011). Saprotrophs are capable of decomposing complex polymers, such as cellulose and chitin, although both the substrates that they decompose and the enzymatic pathways that they use vary considerably in individual species (Peay et al. 2016). In naturally regenerated forests, the increase in shared fungal richness with natural forests strongly indicated the recovery of the saprotrophic fungal community and fungi-mediated nutrient cycling to those of natural forests.

For ECM fungi, we confirmed the presence of *Wilcoxina mikolae* and *tomentella*, which are symbiotic with *Picea* (Ingeborg HAUG 2002; Trocha et al. 2006), as unshared species in monoculture plantations. As reported in a previous study, ECM fungal diversity increases with forest growth, and root growth of host species might create new niches for ECM fungi that prefer the host tree (Twieg et al. 2007; Marčiulynas et al. 2022), which may explain the increase in unshared ECM fungi in monoculture plantations. In addition, we found that fungi were linked to ecosystem functioning in plantations. For example, *Cortinarius acutus*, observed only in plantations, negatively affects soil carbon accumulation (Lindahl et al. 2021). Changes in ECM fungi in plantations can be major drivers that make forest ecosystem functions significantly different from those of natural forests (Guo et al. 2022). In naturally regenerated forests, other ECM tree fungal species such as *Quercus* and *Abies* were observed during later successional stages of the

naturally regenerated forests. Therefore, an increase in shared ECM fungi may also be promoted by plant species other than *Betula* spp. However, the increase in unshared ECM fungi could explain why it is difficult to fully recover natural forest fungal communities. It is known that the priority effect drives community assembly in ECM fungi (Kennedy and Bruns 2005; Kennedy et al. 2009), suggesting that establishing fungi from natural forests would be difficult. Overall, we conclude that the convalescence of fungi in the naturally regenerated forest may have caused the recovery of the nutrient cycle during succession in the natural forest.

Management Implications

Our findings demonstrate the effectiveness of natural regeneration in recovering soil fungal communities to a natural state compared to monoculture *Picea glehnii* plantations, which form fungal communities with different structures. This is the first study to show differences in fungal community succession among forest restoration practices, and it provides a significant understanding of the consequences of fungal community structure between forestry schemes, as the main components of restoration (planning, implementation, and evaluation) are linked to succession and community assembly (Walker et al. 2007). Moreover, we found that microbiological recovery in naturally regenerated forests might take a long time. This may be due to plant-fungal interactions associated with difficulties in facilitating full vegetation recovery (Chen et al. 2019; Matsuoka et al. 2020; Poorter et al. 2021). Additional studies are required to determine the effects of fungal community assembly on forest ecosystem functioning (van der Putten et al. 2023), to provide a better understanding of the impacts of restoration practices.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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