Factors associated with seedling establishment on logs of different fungal decay types – a seed sowing experiment

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Abstract

1. Activities of wood decomposer fungi alter abiotic and biotic properties of deadwood, which are important for tree seedling regeneration on nurse logs. However, the effects were seldom evaluated experimentally. 2. In this study, we examined germination, growth, and survival of six arbuscular mycorrhizal and six ectomycorrhizal tree species on three substrates (brown rot logs, white rot logs, and soil) by seed sowing experiments in a mixed forest dominated by Pinus densiflora and Quercus serrata. We also analyzed fungal communities in these substrates by rDNA ITS1 sequencing. 3. Some significant substrate effects were found on seedling performance when comparing wood decay types, but these were not clearly consistent across mycorrhizal status of the seedlings. Nevertheless, seedlings of arbuscular mycorrhizal trees tended to show better growth on brown rot logs than on white rot logs, whereas ectomycorrhizal tree seedlings tended to survive better on white rot logs and soil compared to brown rot logs. 4. The fungal community was significantly different across three substrates. Richness of Operational Taxonomic Units (OTUs) of arbuscular mycorrhizal fungi was largest in brown rot logs, whereas OTU richness of ectomycorrhizal fungi was largest in soil. 5. Synthesis: The effects of fungal wood decay type on nurse log regeneration of tree seedlings might be partly attributable to water content and fungal communities in the logs. Particularly, rich communities of arbuscular mycorrhizal fungi in brown rot logs could assist in the growth of arbuscular mycorrhizal tree seedlings.

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Author contributions

YF: conceptualization, methodology, writing–original draft and review, supervision, funding acquisition, HK: methodology, writing–review. Both authors contributed to the article and approved the submitted version.

Data availability

The data presented in this study are available on request from the corresponding author.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. **Experimental research and field studies on fungi including the collection of fungal material, are complied with relevant institutional, national, and international guidelines and legislation.**

Abstract

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2. In this study, we examined germination, growth, and survival of six arbuscular mycorrhizal and six ectomycorrhizal tree species on three substrates (brown rot logs, white rot logs, and soil) by seed sowing experiments in a mixed forest dominated by *Pinus densiflora* and*Quercus serrata* . We also analyzed fungal communities in these substrates by rDNA ITS1 sequencing.

3. Some significant substrate effects were found on seedling performance when comparing wood decay types, but these were not clearly consistent across mycorrhizal status of the seedlings. Nevertheless, seedlings of arbuscular mycorrhizal trees tended to show better growth on brown rot logs than on white rot logs, whereas ectomycorrhizal tree seedlings tended to survive better on white rot logs and soil compared to brown rot logs.

4. The fungal community was significantly different across three substrates. Richness of Operational Taxonomic Units (OTUs) of arbuscular mycorrhizal fungi was largest in brown rot logs, whereas OTU richness of ectomycorrhizal fungi was largest in soil.

5. Synthesis: The effects of fungal wood decay type on nurse log regeneration of tree seedlings might be partly attributable to water content and fungal communities in the logs. Particularly, rich communities of arbuscular mycorrhizal fungi in brown rot logs could assist in the growth of arbuscular mycorrhizal tree seedlings.

Keywords

coarse woody debris, microsites for tree regeneration, mycorrhizal type,*Pinus densiflora* , plant-soil feedback, rot type, wood-inhabiting fungi

Introduction

Nurse logs play a vital role in the regeneration process of forest trees. That is important not only in boreal and subalpine coniferous forests but also in temperate and tropical broadleaf forests (Christie and Armesto 2003; Doi et al. 2008; Harmon and Franklin 1989; Papaik and Canham 2006; Sanchez et al. 2009). Decaying logs provide regeneration microsites with better light conditions, reduced litter accumulation, less root competition, and abundant water content. These conditions are particularly advantageous for small-seeded species with limited energy reserves for initial growth, which can still become canopy dominants (Lusk 1995). A better understanding of the mechanisms and factors associated with tree regeneration nurse logs is crucial for predicting future forest dynamics under global climate change.

The properties of the logs affecting seedling regeneration have been the subject of long-standing discussions. For instance, logs must be thick to host many seedlings (Takahashi 1994). Well-decayed logs that are softened, moistened, and covered with abundant moss provide better microsites for seedlings compared to undecayed, hard logs without moss layer (Fukasawa and Ando 2018; Mori et al. 2004; Iijima and Shibuya

2010). Additionally, the tree species of the logs must be taken into account (Orman et al. 2016). Furthermore, the decay type of the logs caused by fungal decomposers has recently gained attention as an important factor influencing seedling regeneration (Fukasawa 2021). Decay type categorizes the physicochemical properties of decaying wood due to decomposer fungi, which exhibit species-specific or strain-specific preferences for different wood components, such as lignin, cellulose, and hemicellulose (Fukasawa 2021). Decay type of basidiomycetes, a phylogenetic group of fungi with strong wood decay abilities, traditionally fall into two categories: brown rot and white rot (Eaton and Hale 1993). In white rot, lignin is selectively or simultaneously decayed along with cellulose and hemicellulose, resulting in fibrous, soft, spongy wood due to the decay of lignin binding the cell walls (Araya 1993). In contrast, brown rot mainly targets cellulose and hemicellulose while leaving lignin with little modification, causing brown rot wood to become brown in colour and with a blocky texture. Brown rot process requires acidic conditions (Espejo & Agosin, 1991), making brown rot wood more acidic than white rot wood (Fukasawa, 2012). Differences in wood decay type can significantly affect seedling communities on the logs and potentially lead to niche separation among dominant tree seedlings in a local context (Fukasawa et al. 2017). However, the factors determining the impact of decay types on tree seedling regeneration are not well-explored.

A review of previous field studies has reported that tree seedlings mainly associated with arbuscular mycorrhizal (AM) fungi tend to regenerate more frequently on brown rot logs than on white rot logs (Fukasawa 2021). In contrast, seedlings mainly associated with ectomycorrhizal (ECM) fungi tend to regenerate more frequently on white rot logs than on brown rot logs (Fukasawa 2021). These mycorrhizal types are formed by distinct fungal groups and are associated with different phylogenetic groups of trees (Smith and Read 2008). Mycorrhizal fungi are abundant in decayed wood in forests, especially in later decay stages (Rajala et al. 2011, 2012, 2015), and are essential for seedling colonization on the logs (Marx and Walters 2006; Fukasawa 2012). Tedersoo et al. (2008) reported that wood decay type influences ectomycorrhizal communities associated with the roots of tree seedlings growing on the logs. Therefore, the difference in mycorrhizal fungi in logs of different decay types might explain the effect of wood decay type on seedling regeneration on the logs. However, communities of arbuscular mycorrhizal fungi have not been compared between white rot and brown rot logs, nor has seedling performance on logs of different decay types been compared among seedlings of different mycorrhizal types.

In the present study, we evaluated the effects of abiotic and biotic properties of three microsites: brown rot logs, white rot logs, and soil, for tree seedling establishment in a secondary mixed forest. We achieved this by sowing seeds of six arbuscular mycorrhizal and six ectomycorrhizal tree species in the field. Fungal communities in the logs were analyzed using metabarcoding of fungal DNA directly extracted from wood sampled from each microsite. We hypothesized that ectomycorrhizal fungal species, which posses organic matter decay abilities, occur more frequently in white rot logs than in brown rot logs due to the high availability of cellulose and hemicellulose in white rot logs. Conversely, arbuscular mycorrhizal fungi, which generally lack organic matter decay abilities, were hypothesized to occur more frequently in brown rot logs. The high concentration of recalcitrant lignin in brown rot logs may reduce colonization by saprotrophic fungi (Lindner et al. 2011), allowing arbuscular mycorrhizal fungi to thrive without competitive exclusion as long as there were host plant roots. Seedling establishment, particularly in its early stages, is determined by three key processes: germination, survival, and growth. We predicted that seedlings of arbuscular mycorrhizal trees would exhibit better growth and survival on brown rot logs than on white rot logs or in soil. In contrast, seedlings of ectomycorrhizal trees would perform better on white rot logs than on brown rot logs or in soil due to the relative dominance of their mycorrhizal symbionts.

Materials and methods

Study site

This study was conducted in a secondary forest dominated by oak (*Quercus serrata*), pine (*Pinus densiflora*), and cedar (*Cryptomeria japonica*) in Mt. Chitose (38°14'N 140deg21'E, altitude 245 m) located in the northern part of the central island of Japan. The site features a gentle northwestern slope with a mean total annual precipitation of 1207 mm, a mean annual temperature of 12.1degC (for the period 1991–2020,

according to the Japan Meteorological Agency), and a maximum snow depth of approximately 1 m at the nearest weather station (Yamagata; 38deg15'N 140deg21'E, altitude 153 m). This area of interest is a shrine forest under the managed of the Forestry Agency of Japan.

Pine wilt disease, attributed to the North American native pinewood nematode *Bursaphelenchus xylophilus* , was first observed in this area in 1982 and led to significant dieback in *P. densiflora* over the past few decades. To prevent the spread of pine wilt disease, the region has undergone deadwood management, including the felling of infected trees and fumigation with pesticides such as methylcarbamodithioic acid ammonium (NCS). Notably, this forest floor lacks dwarf bamboo *Sasa* spp., which typically dominates the understory of many Japanese forests. Previous studies conducted in this site have reported that seedlings of *C. japonica* , *P. densiflora* , *Clethra barbinervis* , and *Ilex crenata* were frequently observed on *P. densiflora* logs (Fukasawa et al. 2017). These studies also revealed that the growth and survival of *C. japonica* seedlings showed negative associations with white rot logs compared with brown rot logs (Fukasawa & Komagata 2017).

Seed-sowing experiment

In an approximately 1 ha tract at the study site, we selected 62*P. densiflora* logs (diameter 19–62 cm, length 62–200 cm) at ten different locations. These logs were well-decayed, falling into decay class IV within a five-class decay system (Fukasawa 2012). They were categorized into one of two decay types—-30 white rot logs and 32 brown rot logs—-based on visual criteria (Araya 1993). To prepare the logs, we removed moss layer and surface vegetations from all the logs (we did not record the species of these), plowed and flattened the tops using a hand axe. Some of the logs had plant roots inside, including remainings of surface vegetation and that grew from soil. We remain with these roots because removing all of these roots were impossible. We set up multiple 5 cm x 5 cm quadrats on the logs by surrounding them with polyvinyl chloride plates (9 mm height with slits, AooYoo, ShenZhen, China) (Fig. 1). Similar quadrats were established on the ground soil at the same ten locations after removing the litter layer.

Mature seeds of six arbuscular mycorrhizal and six ectomycorrhizal tree species were collected from the study site or nearby regions (Table 1). Exceptions were *Abies veitchii* , *Betula ermanii* ,*Chamaecyparis obtusa* , and *Cryptomeria japonica* seeds, which were obtained from the public seed collection of the Forestry and Forest Products Research Institute (Ibaraki, Japan), and *Picea jezoensis* seeds were sourced from a private company (H.I.Tree C's, Saitama, Japan) because it was hard to collect sufficient number of seeds due to a non-masting season in the study area. In November 2019 (for 21 white rot and 23 brown rot logs) or in November 2020 (for additional logs of 9 white rot and 9 brown rot), seeds were sown in the quadrats, with 35–120 seeds per quadrat depending on the species. We prepared nine replicate quadrats for each species on each of the three substrates (brown rot log, white rot log, and soil). In total, 19,170 seeds were sown. To protect the seeds from wind and potential predation by small mammals during the winter, the quadrats were covered with 4 mm mesh nets, securely fastened with metal pegs onto the substrates (Fig. 1).

Starting from the following spring (either April 2020 or 2021), we recorded seed germination and survival for two growing seasons for seeds sown in 2019 and one growing season for seeds sown in 2020, continuing until October 2021. In each quadrat, we recorded the number of live seedlings without individual tagging. These recordings were conducted 13 times in 2020 (from April to November) and 10 times in 2021 (from April to October), including observations every two weeks from April to August and monthly thereafter.

In October 2021, all the seedlings were harvested and transported to the laboratory. Fresh shoot lengths were measured, and the dried weights of shoots and roots were recorded after drying for more than three days in 70 @C. In the case of several seedlings, fresh root subsamples were taken to determine the colonization rate of mycorrhizal fungi.

Physicochemical properties of logs and soil

The canopy openness above each log and soil quadrat was documented by capturing hemispherical images on a cloudy day in June 2020 (for 44 logs and 10 soil subplots) and in June 2021 (for additional 18 logs). This was accomplished using a Canon EOS Kiss X5 camera equipped with a circular fisheye lens (4.5 mm F2.8

EX DC, SIGMA, Kanagawa, Japan). To compute the canopy openness, we employed computer software called CanopOn 2 (available at http://takenaka-akio.org/etc/canopon2/). Additionally, the water content of the logs and soil subplots were measured using a portable soil moisture meter DIK-31F (Daiki, Saitama, Japan) during each seedling survey. The time-series data for water content were averaged for each substrate and subsequently used for the following analyses.

In September 2020 (for 44 logs and 10 soil quadrats) and September 2021 (for additional 18 logs), samples of the substrate were obtained either manually using a rubber glove or a knife due to the softness of the log surfaces. From each substrate (individual logs and soil quadrat), three samples were collected and then combined to create a single sample (totally 72 combined samples), approximately 50 mL each. These samples were transported back to the laboratory and kept in a fridge at around 8 @C for a week, pending chemical analysis.

The samples were pulverized using a blender WB-1 (Osaka chemical, Osaka, Japan) to pass throught a 5 mm mesh. Crushed samples (ca. 60 mL) were subjected to extraction with 200 mL of deionized water in 250 mL polyethylene bottles for 1 h of shaking (100 rpm on a Shaker MK201D (Yamato Scientific, Tokyo, Japan). The pH of the extract was measured using a potable pH meter (LAQUAtwin-pH-11B, HORIBA, Kyoto, Japan). The extract was subsequently filtered using filter paper 5C (ADVANTEC, Tokyo, Japan) and a syringe filter DISMIC25CS (ADVANTEC, Tokyo, Japan). The filtrate was analyzed using an Ion Chromatography system Shim-pack IC (Shimadzu, Kyoto, Japan) with 0.6 mM Na_2CO_3/12mM $NaHCO_3$ as the anion eluent and 2.5 mM oxalic acid as the cation eluent, at a separation column temperature of 40^{o}C. The ion concentrations (Na+, NH4+, K+, Cl-, NO3-, $SO4^{2}$-, Mg^{2}+, Ca^{2}+) were expressed as per 100 g-dried substrate bases. A principal component analysis was conducted to visually represent the variance in nutrient ion composition and project it onto PC vectors (Supplementary Fig. S1).

Fungal communities in logs and soil

In September 2020 (for 44 logs 10 soil quadrats) and September 2021 (for additional 18 logs), substrate samples were collected using a knife. Three samples were taken from each substrate and then combined into a single sample (totally 72 combined samples), approximately 30 mL each. To prevent cross-contamination among samples, the knife was sterilized with 70% ethanol and a burner flame. The collected samples were transported back to the laboratory in a cooler bag with ice and stored at –30@C until DNA extraction.

DNA extraction was carried out from 0.2 g of white rot and brown rot dead wood and 0.3 g of soil freeze-dried samples using the ISOIL for Beads Beating kit (Nippon Gene, Tokyo, Japan) in accordance with manufacture's protocol. Out of a total of 72 samples, DNA extraction was unsuccessful for 7 samples (4 white rot and 3 brown rot samples). For sequencing of the fungal internal transcribed spacer 1 (ITS1) region, we employed the MiSeq sequencing platform with 250 x 2 paired-end reads (Illumina, San Diego, CA, USA). This was done using a two-step PCR protocol with ITS1F_KYO1/ITS2_KYO2 primers (Toju *et al.* , 2012), where the primary amplification included tails for adding indices and Illumina flow cell adapters in the secondary amplification. We used positive and negative controls in the PCR, and positive controls in the MiSeq sequencing. The ITS region is widely recognized as the formal fungal barcode (Schoch *et al.* , 2012; Kauserud 2023). For more details on sample preparation for MiSeq sequencing, please refer to the Supplementary methods.

A total of 4,004,674 reads were obtained after MiSeq sequencing run and chimera check. The data have been deposited in the Sequence Read Archive of the DNA Data Bank of Japan, with accession number*DRA??????????????* . The sequence reads underwent trimming with a minimum quality value of 30, and the 5'- and 3'- primer sequences were subsequently removed from the trimmed reads. Following this, the trimmed reads were denoised using Claident (Tanabe and Toju, 2013) with Assams assembler v0.2.2015.05.10 (Tanabe, 2015). A chimera check was conducted using Claident software with reference to the UNITE database (https://unite.ut.ee, accessed 16th October 2023). The quality-filtered sequences were then classified into molecular OTUs and taxonomically identified using the Claident fungal ITS database (fungi_its_genus), structured after the International Nucleotide Sequence Database (http://www.insdc.org,

accessed 3rd December 2019). This classification was performed at a threshold similarity of 97%, a widely recognized standard for the fungal ITS region (Osono, 2014).

We excluded one white rot and one soil sample with less than 1,000 reads. For each sample, OTUs with less than 0.1% of the total number of reads per sample were removed. Following the filtering process, a total of 3,843,172 reads were retained. Since the OTU numbers reached saturation in this dataset (Supplementary Fig. S2), we did not conduct rarefaction for individual samples to adjust for differences in sequence length. Singleton OTUs were also eliminated from subsequent analyses. As a result, each of the 373 filtered OTUs from 63 samples (25 white rot, 29 brown rot, and 9 soil samples) was cross-referenced with the FUNGuild database and assigned to one of the 11 functional groups: arbuscular mycorrhizal (AM), brown-rot (Bro), ectomycorrhizal (ECM), ericoid mycorrhizal (Erm), fungal parasite (Fup), plant pathogen (Plp), soft-rot (Sof), undefined saprotroph (Sap), white-rot (Whi), and wood decay with unknown decay type (Wod) and unknown functions (Unk) (https://github.com/UMNFuN/FUNGuild, accessed 1st July 2018; Nguyen et al., 2016) (Supplementary Table S1).

Colonization rate of mycorrhizal fungi

The colonization rate (%) of mycorrhizal fungi in the root system of individual seedlings was assessed. For arbuscular mycorrhizal tree species, one seedling was randomly selected from each quadrat on every substrate (n = 9). The roots were initially washed with a 0.005% aerosol OT solution (Wako, Osaka, Japan) in a voltex for 1 min, followed by a 10-min treatment in a hypersonic waterbath. Subsequently, they were rinsed with deionized water twice and cleared by heating in 10% KOH at 100 ^{o}C for over 1 h. After this, cleared roots were rinsed with deionized water, bleached in 0.5% H_2O_2solution for 20 min, followed by another rinse with deionized water, and then fixed in 2% HCl for more than 10 min. The fixed roots were stained with trypan blue and preserved in lactoglycerol (lactic acid 525 mL, glycerin 37.8 mL, deionized water 37.2 mL). Colonization was assessed following the method of McGonigle et al. (1990) under 200x magnification to determine the percentage of root length colonized by arbuscular mycorrhizal fungal structures, including arbuscules, coils, and vesicles.

For ectomycorrhizal trees species, three seedlings were selected from each quadrat on each substrate (n = 27). The colonization rate (%) of ectomycorrhizal fungi was calculated as the percentage of ectomycorrhizal root tips in relation to the total root tips (88.8 tips in average). This assessment was made through direct observation of root systems under a binocular microscope with less than 45x magnification (SZ2–ILST, Olympus, Tokyo, Japan).

Statistical analysis

All statistical analyses were performed using R version 4.0.5 (R core team 2021). To compare the physicochemical properties of substrates, seed germination rate, seedling survival rate, and the measurements of shoot length, dry weight, and mycorrhizal colonization of seedlings among the three substrate categories (white rot log, brown rot log, and soil), we employed the Steel-Dwass test with the *nparcomp* command from the *nparcomp* package. Data from *Alnus hirsuta* were excluded from comparison due to very low germination and survival rates.

For the visualization of fungal taxonomic composition in the substrates, we utilized the *heat_tree* command from the *metacoder*package (Foster *et al.* , 2017). We based our analysis on the occurrence data (presence/absence) of fungal OTUs, as the numbers of sequence reads do not usually reflect the relative abundance of taxa in a sample (Skelton *et al.* , 2019b). To assess the dissimilarities in fungal communities between the samples, we calculated the Raup-Crick index and created non-metric multidimensional scaling (NMDS) ordination plots for all surveyed samples using the *metaMDS* command from the*vegan* package (Oksanen, 2016). The significance of differences in community composition among the substrates was determined by permutational multivariate analysis of variance (PERMANOVA), with 10,000 permutations, using the *adonis* command (Anderson, 2001). Additionally, community variance between samples (calculated using the*betadisper* command) was compared among substrates using analysis of variance (ANOVA) with the *anova* command. The *envfit*command was used to assess the significance of correlations between

environmental variables and fungal community composition. Among the environmental variables listed in Table 2, we selected canopy openness, pH, water content, and nutrient_PC1 for analysis after removing highly correlated variables to reduce multicollinearity.

Fungal OTU richness at each substrate was compared using iNEXT (Chao et al., 2016). Occurrence frequencies of fungal OTUs belonging to specific functional categories, such as arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and plant pathogens, were determined as the percentage of logs on which the OTUs of the focal function were detected relative to the total log numbers. We compared the occurrence frequencies of these functional categories among the substrates using Fisher's exact probability test and Ryan's post hoc comparison (http://aoki2.si.gunma-u.ac.jp/R/src/p_multi_comp.R). These categories were chosen due to their potential effects on seedling growth and mortality (Bayandala et al. 2016; Wulantuya et al. 2020). Indicator species analysis was applied to the OTUs assigned to functions to determine whether their occurrences were indicative of particular substrates, using the *multipatt* command from the*indicspecies* package (Caceres and Jansen 2015).

Generalized linear models (GLMs) and generalized linear mixed models (GLMMs) were employed to analyze the relationships between environmental variables and seedling performance (germination, survival, and growth). In addition to the five factors that showed significant correlations with fungal communities in the NMDS ordination analysis (substrate, canopy openness, pH, water content, nutrient_PC1), the OTU richness of AM or ECM fungi in the fungal community survey was also set as fixed variables. We began by analyzing combined data for AM or ECM trees using GLMM. The OTU richness of AM and ECM fungi were used as factors for models of AM and ECM trees, respectively. For the model explaining germination rate, a matrix of germinated and non-germinated seed numbers [cbind(germinated, non-germinated)] in each quadrat was set as the dependent variable. For the model explaining survival rate, a matrix of survived and dead seedling numbers [cbind(survived, dead)] in each quadrat was set as the dependent variable. Binomial distributions (logit link) were assumed, and unmeasured effects of seedling species and sowing year were set as random factors for germination and survival models. For the models explaining seedling growth, shoot length and dried weight of each seedling were set as dependent variables. Gaussian distribution (identity link) was assumed for the shoot length model, while the dry weight model used a Gamma distribution (log link). The unmeasured effects of substrate identity, seedling species, and sowing year were set as random factors. The best model was selected based on the lowest Akaike Information Criterion (AIC) using backward elimination in the *dredge* function from the *MuMIn* package (Burnham and Anderson 2002). For germination and survival models, corresponded AIC (AICc) was used given the small sample size (Burnham and Anderson 2002).

Subsequently, we analyzed data for individual seedling species by using GLM and GLMM. The same sets of variables used in the models for the combined data of mycorrhizal type were applied to these models. For models explaining germination rate and survival rate, we assumed quasi-Poisson (log link) and quasi-binomial (logit link) distributions, respectively, due to their large data variations (Faraway 2006). The best models were selected based on the lowest AIC or AICc as described above. Pearson's correlation coefficients among the variables were all below 0.7, and the variance inflation factors of the models were less than 5, indicating low levels of multicollinearity.

Pearson's correlation coefficients between colonization rate of mycorrhizal fungi and seedling growth (shoot length, dry weight) were calculated for each tree species, except for *Alnus* seedlings from which we could not obtain enough seedlings.

Results

Physicochemical properties of the substrates

Canopy opennes was irrelevant among the three substrates (Fig. 2). Brown rot logs had higher water content compared to white rot logs and soil. Soil exhibited a higher pH than the logs. Among the nutrient ions, concentrations of Na^+, NH_4^+, and K^+ were significantly higher in white rot logs than in brown rot logs and soil (Fig. 3). In contrast, SO_4^{2-}concentration in soil was higher than in the logs. Cl^-, NO_3^-, and Ca^{2+}

concentrations did not differ across the substrates. PO_4^{3-} concentration was too low to detect quantitatively (< 0.5 mg/L).

Fungal community in the substrates

In total, 373 OTUs were detected, including 192 Ascomycota, 116 Basidiomycota, 13 Glomeromycota, and 9 Mucoromycota, along with 43 of unknown taxonomy (Table S1, Fig. S3). Leotiomycetes, Sordariomycetes, and Eurotiomycetes were the dominant classes in Ascomycota. Agaricomycetes was the most dominant class in Basidiomycota. Among the 373 OTUs, 109 were assigned to one of the ten functional groups. Undefined saprotroph (Sap) contained the largest number of OTUs (47 OTUs), followed by ectomycorrhizal (ECM, 15 OTUs), arbuscular mycorrhizal (AM, 13OTUs), soft rot (Sof, 12 OTUs), white rot (Whi, 7 OTUs), and plant pathogen (Plp, 7 OTUs). The OTU richness of all fungi was nearly saturated against the number of samples (Fig. 4A), with brown rot logs hosting the largest number of fungal OTUs. This was also the case for AM (Fig. 4B), while in the ECM group, soil host the largest number of OTUs (Fig. 4C). Differences in the OTU richness of Plp across the substrates were unclear due to a large overlap in the confidence intervals (Fig. 4D).

NMDS ordination plot and PERMANOVA showed that fungal communities significantly ($P < 0.001$) differed across the substrates (Fig. 5A), although dispersion differed across the substrates ($P = 0.03$). The sampling year had no effect on fungal communities (PERMANOVA, R2 = 0.05, P = 0.07). Among the tested environmental variables, pH, water content, and nutrient_PC1 of the substrates had significant associations with fungal communities (Fig. 5A). The effects of water content and nutrient_PC1 were consistent but the effect of pH was not evident in the dataset focusing on logs (i.e. excluding soil samples) (Fig. 5B). The diameter and length of the logs were not significantly associated with fungal communities.

Forty OTUs were detected as indicative for one of the three substrates (Table 3). The number of indicative OTUs for brown rot logs, white rot logs, and soil were 34, 22, and 76, respectively. All substrates included indicative Sap OTUs. In contrast, all three AM OTUs were found in brown rot samples, and all 12 ECM OTUs were found in soil samples. An indicative Bro OTU (*Leucogyrophana* sp.) and an indicative Whi OTU (*Sistotremastrum* sp.) were found in brown rot samples and white rot samples, respectively.

Seedling demography

Among the total 19,170 seeds sown, 3,562 (18.6%) seeds germinated. Of the 12 tree species tested, *Padus grayana* and *Pinus densiflora* showed germination rates of >50% on all substrates (Fig. 6A). *Ilex crenata* , *Cryptomeria japonica* ,*Abies veitchii* , *Carpinus laxiflora* , and *Betula ermanii* showed germination rates of 20–30%. However,*Chamaecyparis obtusa* , *Toxicodendron trichocarpa* ,*Picea jezoensis* , *Clethra barbinervis* , and *Alnus hirsuta* showed germination rates of <10%. Data of*Alnus hirsuta* were not included in the following analysis due to its very low germination rate. None of the 12 tree species showed a significant difference in germination rate across the three substrates. The seedling species names were represented by their genus names hereafter.

Among the seeds sown in Novermber 2019, *Abies* , *Carpinus* , and *Padus* had already germinated by the first recording on April 16, 2020. The populations decreased gradually during the first gworing season, and remained constant thereafter, even after winter (Fig. S4A).*Cryptomeria* , *Clethra* , *Pinus* , and *Picea* had germination peaks in June, with their population decreasing during summer but remaining constant thereafter, even after winter. Among the seeds sown in Novermber 2020, *Chamaecyparis* , *Ilex* , and*Toxicodendron* showed high survival rates (Fig. S4B, Fig. 6B).*Betula* germinated from April to May and decreased its population to approximately 50% of the original germinants by autumun (Fig. S4B).*Ilex* germinated from June to July and maintained a high survival rate during the first growing season (Fig. S4B).

At the time of seedling harvest in October 2021, 32.7% of the seedlings sown in November 2019 had survived, and 75.8% of the seedlings sown in November 2020 had survived, resulting in a total of 1,422 seedlings harvested. *Padus* seedlings showed a significantly lower survival rate on white rot logs compared to soil (Fig. 6B). *Carpinus*seedlings exhibited a significantly lower survival rate on brown rot logs compared to

soil (Fig. 6B). The dry weight of *Cryptomeria*seedlings was larger on brown rot logs and soil compared to white rot logs (Fig. 7A). The dry weights of *Padus* and *Carpinus*seedlings were larger on soil compared to logs. In contrast, the dry weight of *Toxicodendron* seedlings was larger on the logs compared to soil. The shoot lengths of *Clethra* , *Cryptomeria* ,*Padus* , and *Pinus* seedlings were larger on brown rot logs and soil compared to white rot logs (Fig. 7B). The shoot lengths of*Carpinus* and *Betula* seedlings were larger on soil compared to logs. The colonization rate of arbuscular mycorrhizal fungi was generally high among all the six AM tree species tested and tended to be lower on white rot logs compared to brown rot logs and soil, although the difference between brown rot and white rot logs was not significant, except for *Ilex* (Fig. 7C). The colonization rate of ectomycorrhizal fungi was higher on soil compared to logs in*Abies* and *Picea* seedlings. *Pinus* seedlings showed a significantly higher colonization rate of ECM fungi on brown rot logs and soil compared to white rot logs. *Betula* seedlings exhibited a significantly higher colonization rate of ECM fungi on white rot logs and soil compared with brown rot logs. The colonization rate of ECM fungi in *Carpinus* seedlings was not significantly different across the substrates.

Factors related with seedling performance

GLMM results indicated that substrate was selected in the model explaining germination rate, survival rate, and shoot length of AM tree species and survival rate of ECM tree species (Table 4). Germination rate of AM trees was significantly larger on white rot logs and soil than on brown rot logs (Table 5). Survival rate of AM trees was significanlty larger on soil than on brown rot and white rot logs. Shoot length of AM trees was significantly larger on soil than on white rot logs and was marginally ($P < 0.1$) larger on brown rot logs than on white rot logs. Survival rate of ECM trees was significantly larger on white rot logs and soil compared with brown rot logs. The water content of the substrates was commonly selected as a positive factor for the germination and survival of both AM and ECM trees (Table 4). Canopy openness was selected as a positive factor for dry weight but as a negative factor for the germination of both AM and ECM trees.

GLMs (for germination and survival) and GLMMs (for dry weight and shoot length) for each tree species indicated that the relationships between seedling performance and environmental factors varied among tree species. Substrate had a significant association with the survival rates of *Cryptomeria* , *Padus* , and *Carpinus* , the dry weight of *Cryptomeria* , and the shoot length of *Padus* (Table 6). The dry weight of *Cryptomeria* seedlings was larger on brown rot logs compared to white rot logs (Table 7). The survival rate of*Cryptomeria* seedling was larger on soil compared to brown rot logs. The survival rate and shoot length of *Padus* seedlings were larger on soil compared to brown rot and white rot logs. Among the environmental factors, water content of the logs had the most widespread association with seedling performance, except for *Chamaecyparis* ,*Ilex* , and *Picea* (Table 6). The associations were mostly positive, but in the case of *Carpinus* seedlings, shoot length was negatively associated with the water content of the logs. The association with canopy openness varied among seedling species and performance. The dry weights of *Chamaecyparis* , *Ilex* ,*Abies* , and *Pinus* were positively associated with canopy openness. Similarly, the shoot length of *Chamaecyparis* , germination of *Picea* , and the survival of *Pinus* showed positive associations with canopy openness. However, the germination of*Clethra* and *Toxicodendron* and the survival of *Abies*exhibited negative associations with canopy openness. The pH of the logs had positive associations with the dry weight of *Abies* and*Pinus* , the germination of *Picea* , and the shoot length of*Pinus* , and a negative association with *Ilex* germination. The OTU richness of mycorrhizal fungi had negative associations with the dry weight of *Cryptomeria* and the germination of *Picea* . Nutrient_PC1 did not have any significant associations with seedling performance.

In the seedlings of *Cryptomeria* , *Padus* , *Pinus* and*Betula* , significantly positive correlations were found between their shoot length and mycorrhizal colonization rate (Fig. 8; Table S1). Similarly, significantly positive correlations were found between the dry weight of *Abies* and *Pinus* seedlings and their ectomycorrhizal colonization rate. None of the AM tree seedling showed significant relationships in dry weight with their arbuscular mycorrhizal colonization rate.

Discussion

The present study revealed that the difference in wood decay type affects the germination and growth of

AM tree seedlings and the survival of ECM tree seedlings (Table 5). In the cases of AM trees, shoot length of the seedlings of *Clethra* , *Cryptomeria* , and*Chamaecyparis* tend to be larger on brown rot logs than on white rot logs. Similarly, the dry weight of *Cryptomeria* seedlings was significantly larger on brown rot logs than on white rot logs (Table 7). As the shoot length of *Cryptomeria* and *Padus* seedlings were positively associated with the colonization rate of AM fungi on their roots, which tended to be larger in brown rot logs than white rot logs, a part of the better growth of AM trees on brown rot logs might be attributable to the high colonization rate of AM fungi on seedling roots in brown rot logs in this site. Colonization by AM fungi is essential for nutrient acquisition particularly in substrates with poor nutrient contents such as decaying logs (Fukasawa 2012), and also for protection against pathogens for host plants (Filion et al. 1999). The reason for the rich communities of AM fungi in brown rot logs is not clear. One possible explanation is the effects of pre-experimental vegetation on the logs. In the present study, we used *P. densiflora* logs in decay class IV found in the study site, and such logs usually have some vegetation on them. If the logs were brown rot, the vegetation was dominated by AM trees like *Cryptomeria japonica* (Fukasawa et al. 2017) and *Clethra barbinervis* (Fukasawa 2012). The dominance of AM trees certainly induces the dominance of AM fungi belowground (Sawada et al. 2023), which in turn benefits the successful establishment of AM tree seedlings (Seiwa et al. 2020). Alternatively, recalsitrant lignin-accumulated brown rot logs might not be a good substrate for decomposer fungi (Lindner et al. 2011), leaving room for AM fungi without competitive exclusion. In contrast, white rot logs, rich in cellulose and hemicellulose, might be dominated by decomposer fungi. Actually, the colonization rate of AM fungi was significantly larger on brown rot logs than on white rot logs in *Ilex* seedlings (Fig 7) and this trend was common among the six AM seedlings even though there's no statistical significance. Similarly, previous study reported a higher AM colonization rate on *Cryptomeria* seedlings in brown rot compared to white rot wood in laboratory pot experiments (Fukasawa and Kitabatake 2022).

In addition to the colonization rate, the functions of AM fungi could be promoted in brown rot logs. An important function of AM fungi is absorbing phosphorus from the substrate and providing it to host plants (Smith and Read 2008). During the wood decay process, brown rot fungi employ oxidative reactions of Fe (II) to Fe (III) called the Fenton Reaction to produce hydroxyl radicals, which play a key role in the brown rot type of wood decay (Eaton and Hale 1993). Thus, wood decayed by brownb rot fungi, such as *Rhodonia* (=*Postia*)*placenta* , contains rich iron (Ostrofsky et al. 1997). Since Fe (III) ions react easily to form chemical bonds with phosphorus ions, forming iron phosphate ($FePO_4$), the result is that P is absorbed on the surface of the hydrous Fe (III) oxide fine particles, which are insoluble in water and not directly available to plants (Nanzyo et al. 2004). However, AM fungi are capable of using iron phosphate and making it available to host plants (Bolan and Robson 1987). Although iron ion concentration was not measured in this study, such a unique iron ion condition in brown rot logs might benefit AM trees in their nutrition. Our preliminary investigation into bacterial communities in deadwood found predominant OTUs of iron-reducing bacteria, *Aciditerrimonas* sp., in brown-rot logs but not in white rot logs and soil (data not shown), indicating that iron may play a role in the biotic systems in brown rot logs. This new hypothesis should be further evaluated by field and laboratory experiments.

Another explanation for the better growth and survival of AM tree seedlings on brown rot logs might be their high water content (Fig. 2). It is known that wood decayed by brown-rot fungi significantly increases its water absorption capacity (Karppanen et al. 2008). Water content certainly had positive effects not only on growth and survival but also on germination of AM trees (*Clethra* , *Cryptomeria* ,*Padus* , *Toxicodendron*) and ECM trees (*Abies* ,*Pinus* , *Betula*) (Table 4, 6). Since the seed-sowing experiments were conducted in the field, we cannot separately evaluate the effects of AM fungi and water content on tree seedlings. Nevertheless, in laboratory pot experiments where water conditions were standardized across substrates, we also reported that the AM colonization rate of *Cryptomeria* seedlings was higher in brown rot wood than in white rot wood (Fukasawa and Kitabatake 2022). Thus, the rich AM fungal community in brown rot logs might be crucial for the better seedling performance on bworn rot logs, at least for*Cryptomeria* seedlings. Water content has been rarely compared between brown rot and white rot logs previously, and it is not certain if brown rot logs are always wetter than white rot logs. White rot beech logs contain a high amount

of water when they are well-decayed in the woods (Fukasawa et al. 2009).

In contrast to the better performance of AM tree seedlings on brown rot logs and soil compared to white rot logs, we detected a positive effect of white rot on seedling survival of ECM trees compared with brown rot (Table 5). However, significant difference was not observed in any certain ECM trees (Fig. 6). Furthermore, the colonization rate of ECM fungi was not larger on white rot logs than on brown rot logs, except for *Abies* and *Betula* (Fig. 7), rather larger on brown rot logs than white rot logs in *Pinus* seedlings. Similarly, the OTU richness of ECM fungi was the smallest in white rot logs (Fig. 4). These results indicated that the effect of substrates on ECM seedling performance was less consistent across the seedling species compared with AM trees, and thus it is not clear why the survival of ECM tree seedlings tended to be higher on white rot logs in this site. One possible explanation is the positive effect of pH on ECM tree seedlings (Table 4, 6). Although it was not survival but germination and growth, wood pH was positively associated with the dry weight of *Abies*and *Pinus* seedlings, shoot length of *Pinus* seedlings, and germination of *Picea* seedlings (Table 6). Such a positive effect of pH was not observed in AM tree seedlings, rather it was negative on their germination (Table 4). Although wood pH was not significantly different between brown rot and white rot logs in the present study, it was known that brown rot logs have a lower pH than white rot logs because brown rot fungi produce organic acids such as oxalic acid during their wood decay process (Espejo and Agosin 1991; Fukasawa and Kitabatake 2022). Thus, the positive relationships between wood pH and ECM tree seedlings were in line with our hypothesis that ECM tree seedlings show better regeneration on white rot logs than on brown rot logs. However, the reason why wood pH affects positively on seedling germination of ECM trees but affects negatively on seedling germination of AM trees is not clear. One possible explanation is the indirect effects of pH and wood chemical quality on biotic interactions across plants, mycorrhizal fungi, and associated bacterial communities. Recent studies have reported that rhizosphere bacterial communities are important for the functioning of both AM (Sawada et al. 2023; Xu et al. 2023) and ECM (Wang et al. 2022) fungi on host plants, and such interactions are strongly influenced by litter chemistry and litter-mediated soil properties (Heděnec et al. 2023).

All 12 tree species used in the present study were reported to frequently regenerate as seedlings on decaying logs (Fukasawa 2021). However, none of the tree species showed better performance on the logs (regardless of brown rot or white rot) compared to the soil in the present study. This is probably because some of the advantages on germinating and growing on the logs were reduced in this study. For example, the accumulated thick litter layer on the ground, which prevents the germination and growth of small seedlings, was removed, and bare soil was prepared for seeds. In addition, light condition (canopy openness) was not significantly different among the microsites. Although the water content of soil was significantly lower than that of brown rot logs, such difference might not be critical for seedling performance in the present study, at least, not detrimental to seedling performance on soil. Furthermore, the OTU richness of plant pathogenic fungi was not significantly different among the three microsites (Fig. 4), and negative effects of plant pathogenic fungi on seedling performance on soil (Cheng and Igarashi 1987; O'Hanlon-Manners and Kotanen 2004) were not observed in the present study, probably attributable to the dry soil of the study site. In line with the previous studies, concentrations of nutrients such as potassium and chloride ions were higher in white rot logs (Fukasawa et al. 2017; Ostrofsky et al. 1997; Takahashi et al. 2000) than in brown rot logs and soil. However, remarkable effects of nutrients on seedling performance were not observed. These results suggest that the difference in nutrient condition among brown rot logs, white rot logs, and soil might not be the primary factor determining seedling performance on those substrates.

The present study comprehensively evaluated the abiotic and biotic factors associated with decaying logs of different decay types and soil in relation to tree seedling regeneration. The results indicated that seedlings of AM trees showed better growth on brown rot logs than on white rot logs, which was in line with previous field data and supported our hypothesis. This association might be at least partly attributable to rich communities of AM fungi in brown rot logs. In contrast, ECM tree seedlings tended to survive better on white rot logs and soil compared to brown rot logs, supporting our hypothesis, but the tendency was less consistent across the six AM trees tested, and the mechanism was not clear. Further analysis of other groups of microbes such as bacteria may be valuable for exploring biotic mechanisms of seedling regeneration on

logs of different decay types and soil. Additionally, evaluating seedling performance on these microsites for a larger number of tree species is necessary to reach more general conclusions. Furthermore, laboratory pot experiments with standardized water conditions and substrate sterilization are needed to evaluate the importance of abiotic factors for seedling performance.

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Figure captions

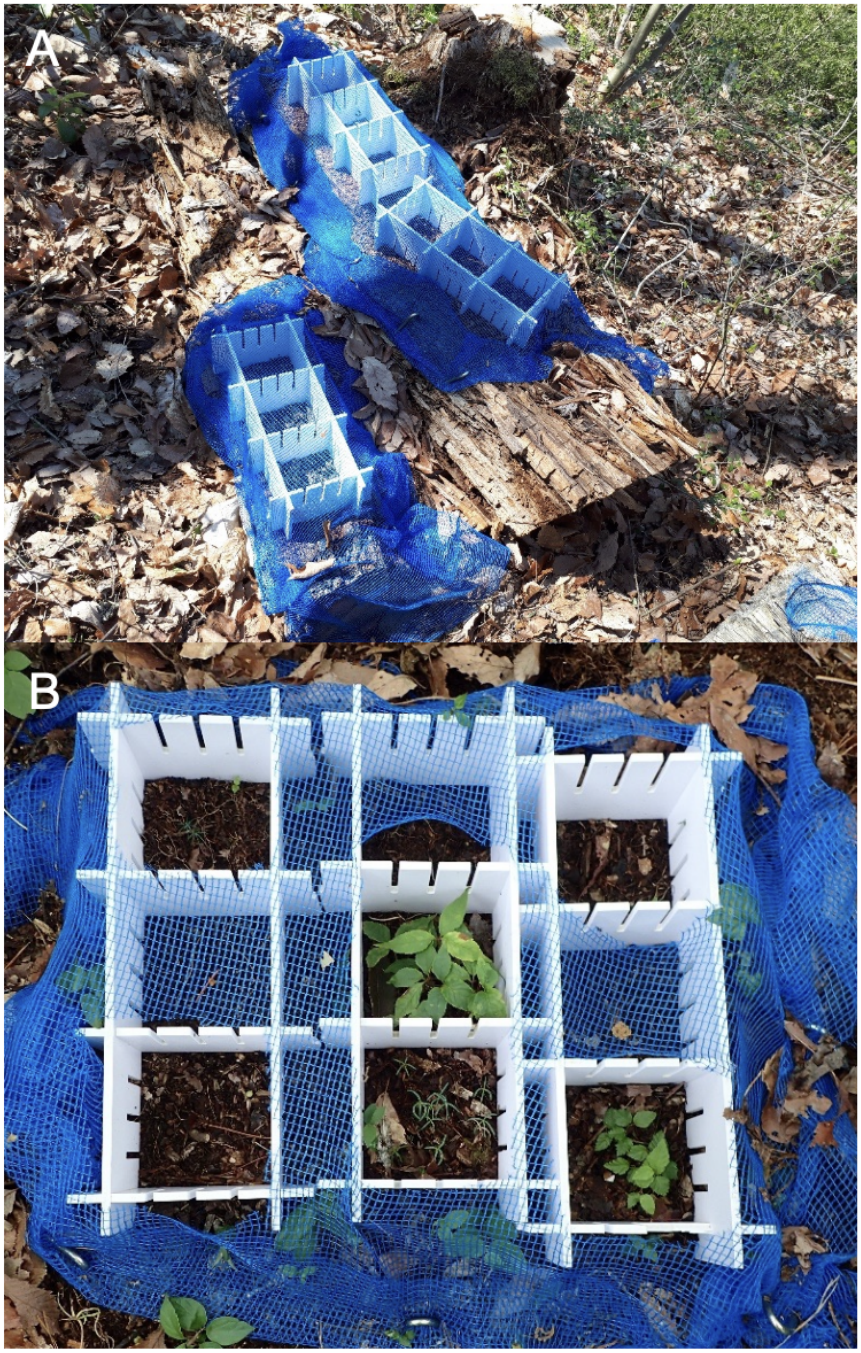

Fig. 1 Arrangements of 5 cm x 5 cm quadrats on logs (A) and soil (B). The mesh cover on each quadrat was removed after germination.

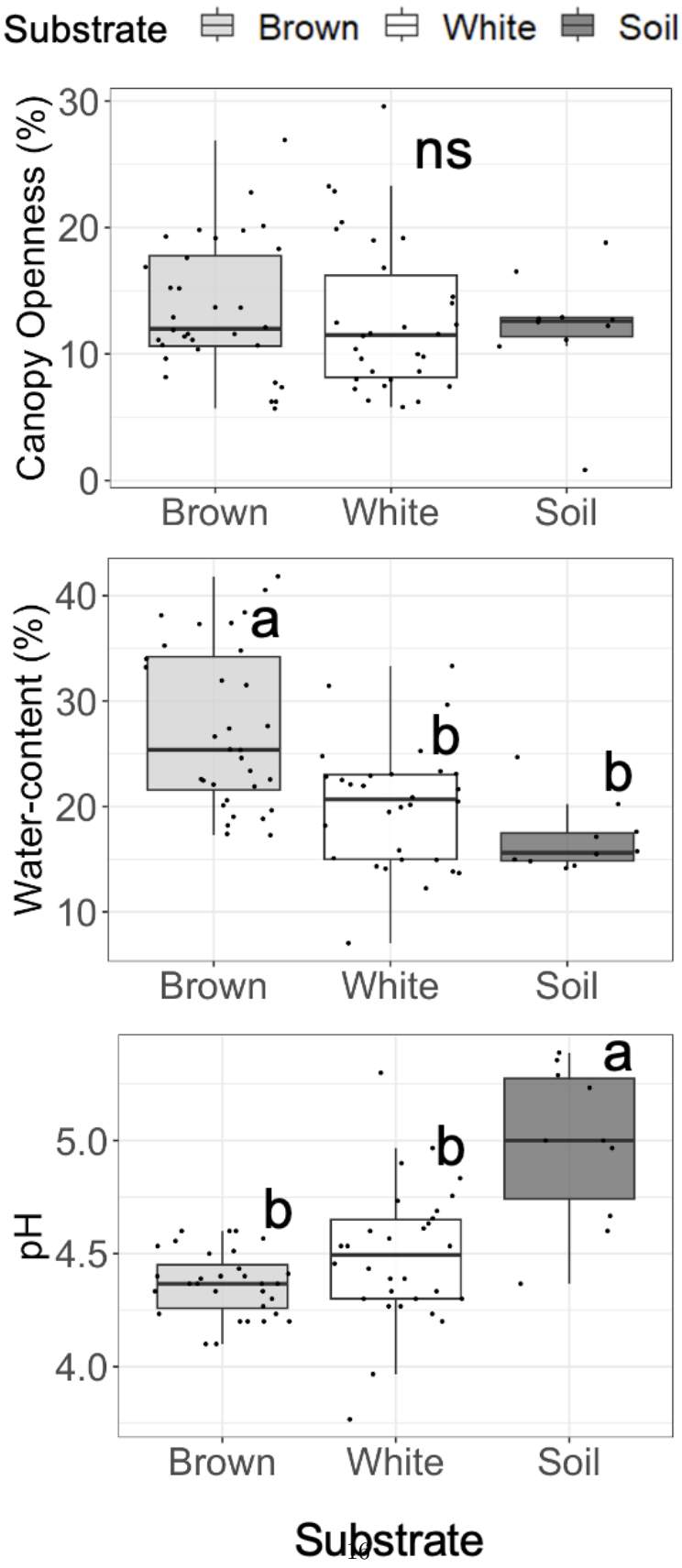

Fig. 2 Physicochemical properties of the substrates: brown rot logs (n = 32); white rot logs (n = 30); soil (n = 10). Different letters indicate a significant difference between the substrates (Steel-Dwass test, $P < 0.05$). Ns indicate no significant differences across the three substrates (Steel-Dwass test, $P > 0.05$).

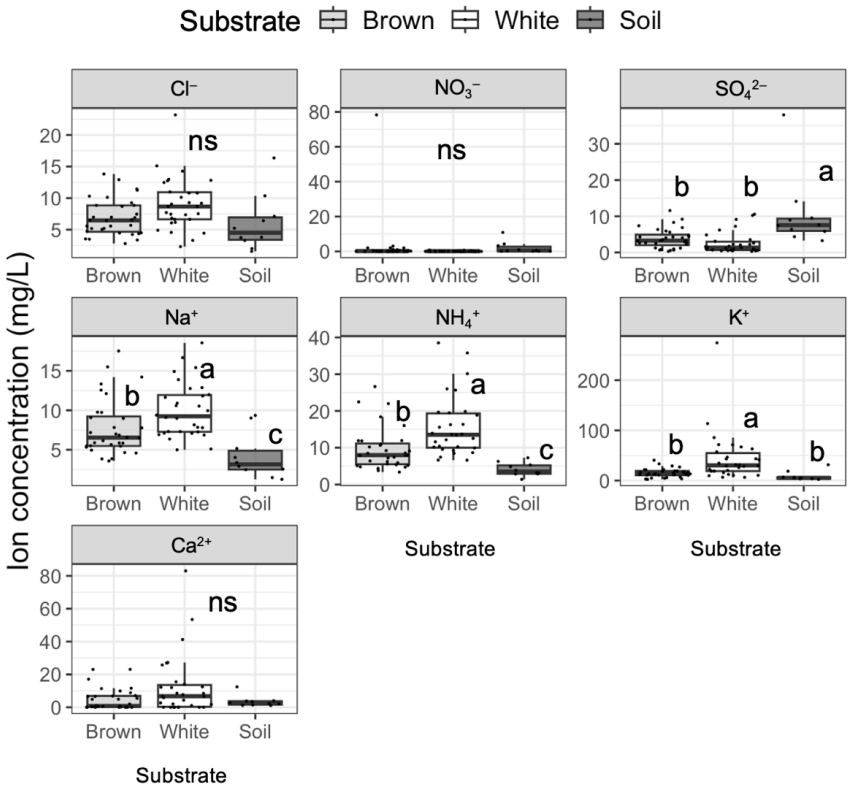

Fig. 3 Nutrient ion concentrations of the substrates: brown rot logs (n = 32); white rot logs (n = 30); soil (n = 10). Different letters indicate a significant difference between the substrates (Steel-Dwass test, $P < 0.05$). Ns indicate no significant differences across the three substrates (Steel-Dwass test, $P > 0.05$).

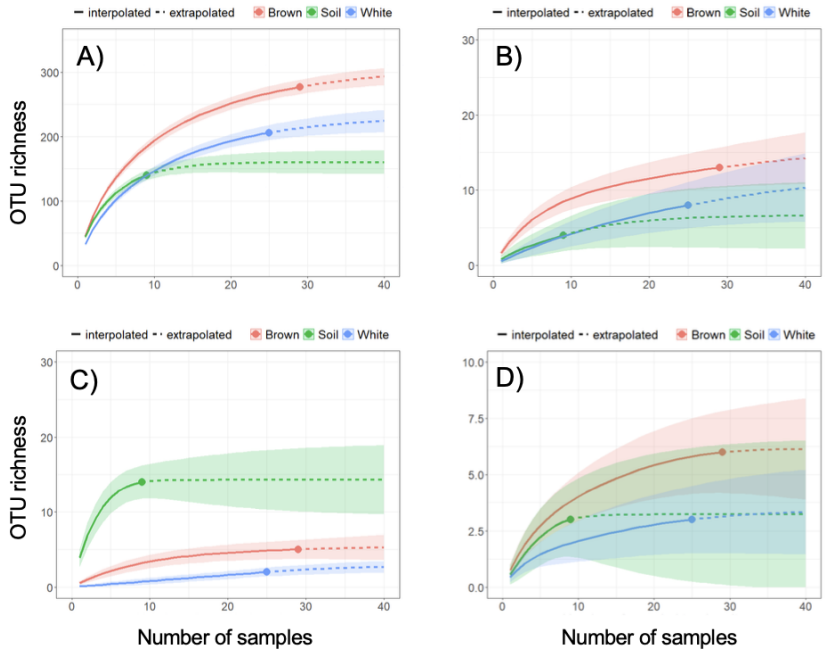

Fig. 4 Fungal OTU richness accumulation curves plotted against the number of samples for each substrate. (A) all fungi, (B) Arbuscular mycorrhizal fungi, (C) Ectomycorrhizal fungi, (D) Plant pathogenic fungi.

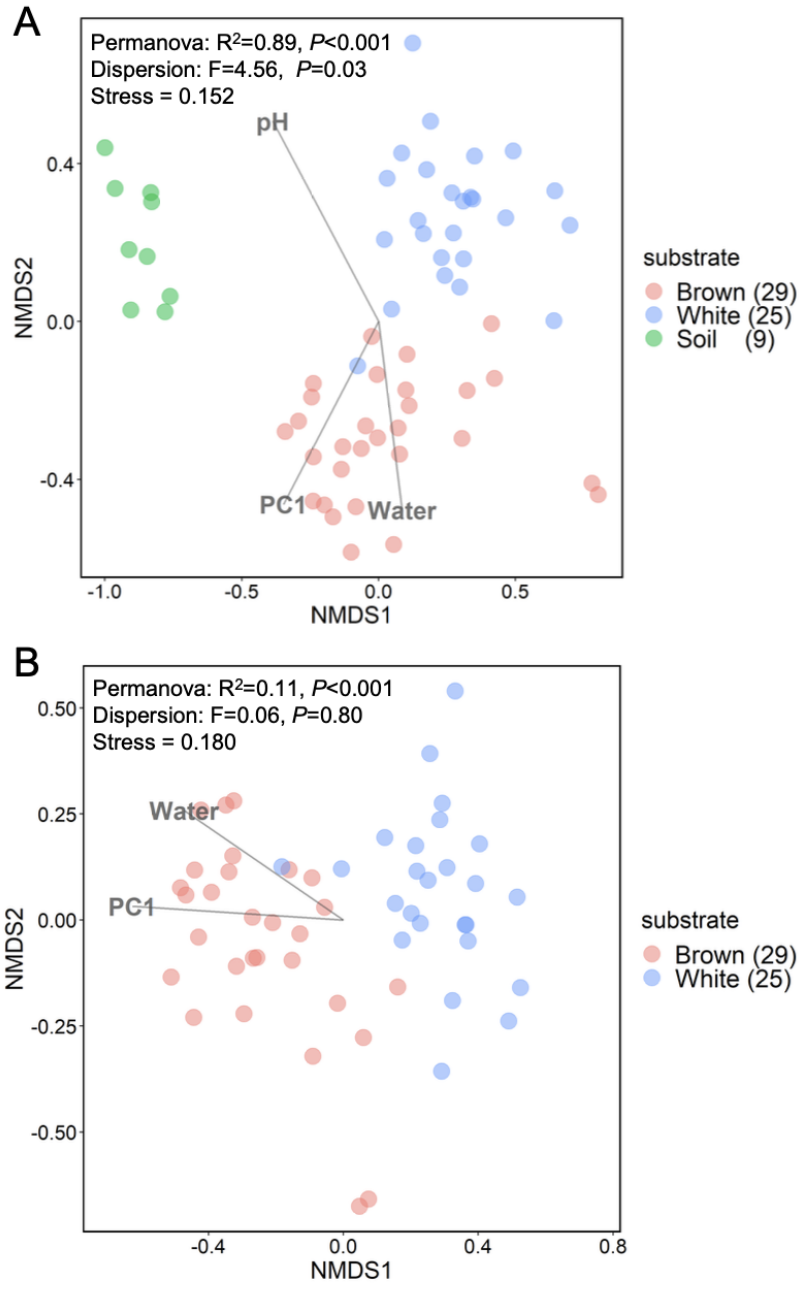

Fig. 5 NMDS ordination plot illustrating the dissimilarity of fungal communities in brown rot logs, white rot logs, and soil samples. (A) Comparison among all three substrates. (B) Comparison between brown rot and white rot logs. Environmental variables with significant relationships to fungal community structure are displayed as vectors: water, water content; PC1, nutrient_PC1.

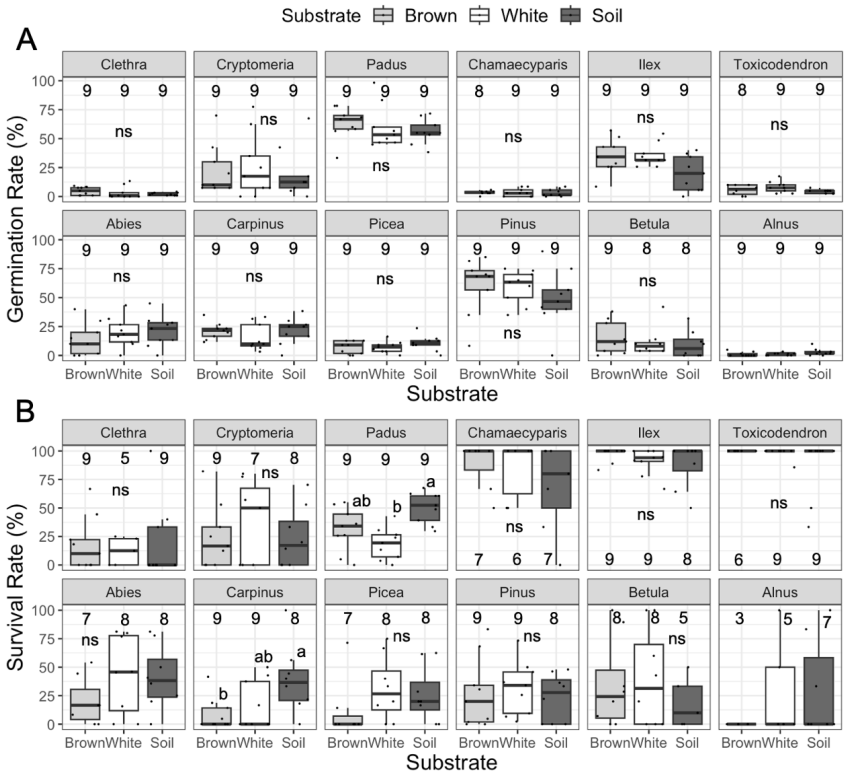

Fig. 6 Germination rate (A) and survival rate (B) of 12 tree species on brown rot logs, white rot logs, and soil. Different lowercase letters on the boxes indicate a significant difference across the substrates (Steel-Dwass test, $P < 0.05$). Ns indicate no significant differences across the three substrates (Steel-Dwass test, $P > 0.05$). Numbers indicate the number of replicates, which was originally 9 but was reduced in some cases due to accidental disturbances by animals. Upper row: arbuscular mycorrhizal trees. Bottom row: ectomycorrhizal trees.

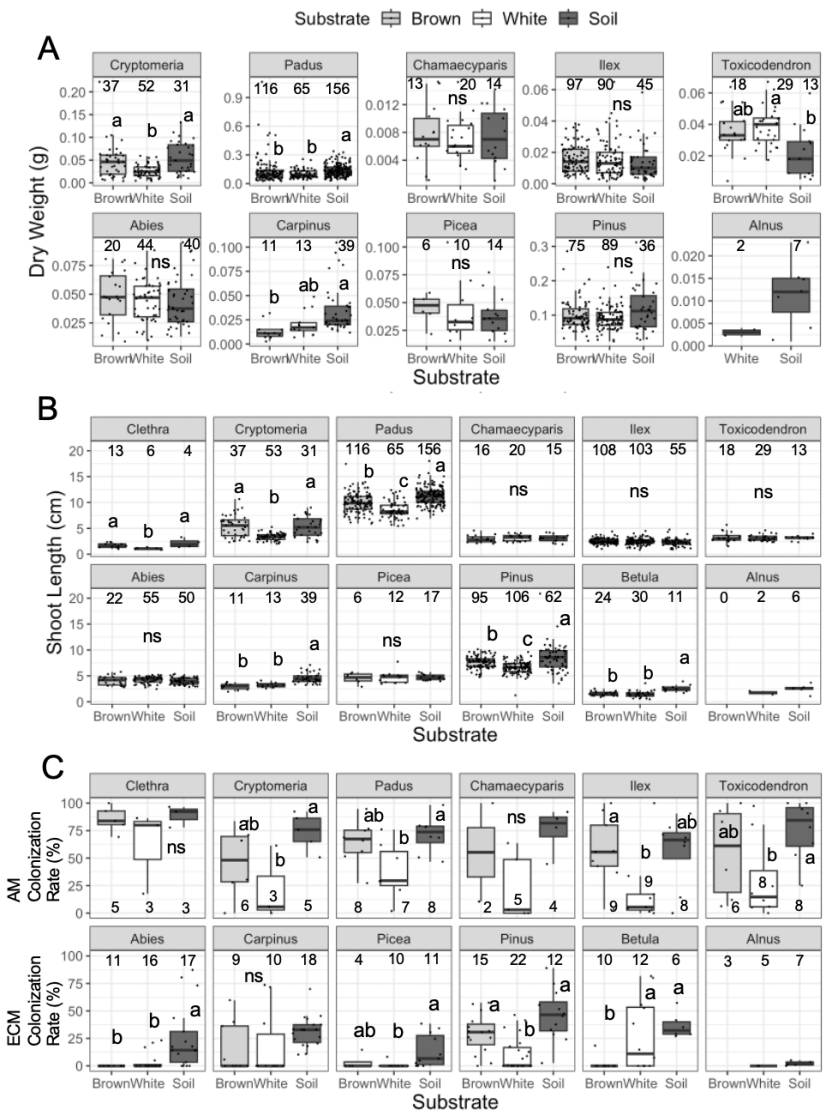

Fig. 7 Dry weight of the entire seedling (A), shoot length (B), and colonization rate of mycorrhizal fungi (C) of the tested tree species on brown rot logs, white rot logs, and soil. Different lowercase letters on the boxes indicate a significant difference across the substrates (Steel-Dwass test, $P < 0.05$). Ns indicate no significant differences across the three substrates (Steel-Dwass test, $P > 0.05$). Numbers indicate the number of replicate seedlings used for the measurements. Upper row: arbuscular mycorrhizal trees. Bottom row: ectomycorrhizal trees. Dry weight was not measured for *Clethra* and *Betula* seedlings because they were too lightweight.

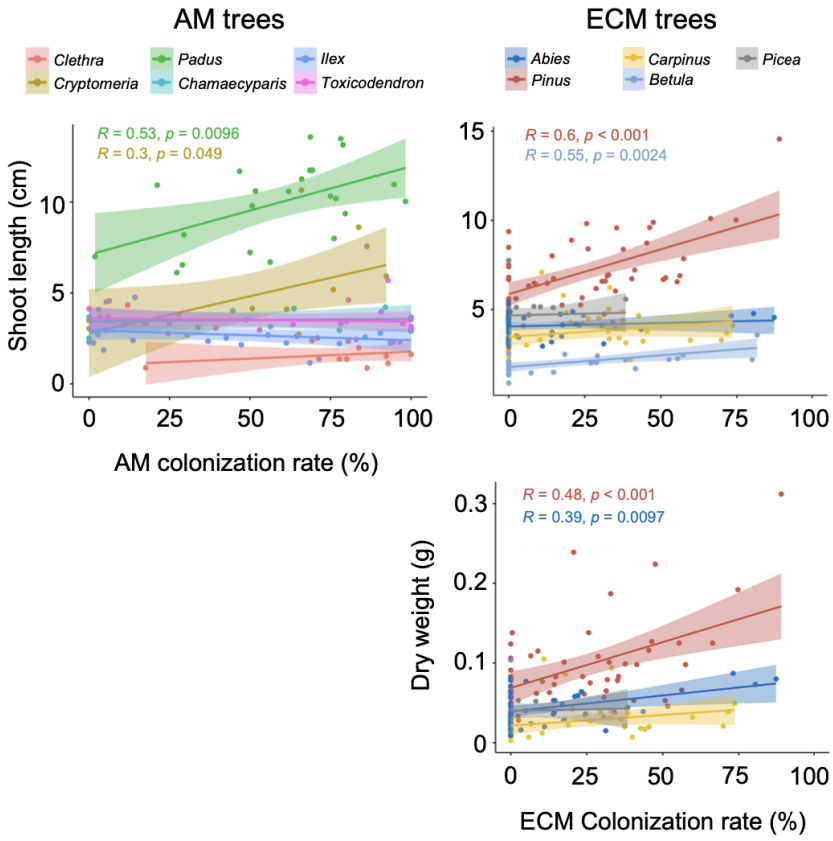

Fig. 8 Relationships between seedling performance (shoot length, dry weight) and the colonization rate of mycorrhizal fungi. Left: seedlings of arbuscular mycorrhizal trees. Right: seedlings of ectomycorrhizal trees. If the relationship was statistically significant ($p< 0.05$), the Pearson's correlation coefficient (R) and$p$ -value were displayed in the figure. Relationships between the dry weight of AM trees and the colonization of AM fungi were not presented because there were no significant relationships.

Table 1 List of seed species used, their mycorrhizal types, and the number seeds sown in each quadrat.

Species	Mycorrhizal type	Sowing	Number per quadrat
Chamaecyparis obtusa (Siebold et Zucc.) Endl.	AM	Nov. 2020	70
Clethra barbinervis Siebold et Zucc.	AM	Nov. 2019	120
Cryptomeria japonica (L.f.) D.Don	AM	Nov. 2019	40
Ilex crenata Thunb. var. *crenata*	AM	Nov. 2020	35
Padus grayana (Maxim.) C.K.Schneid.	AM	Nov. 2019	60
Toxicodendron trichocarpum (Miq.) Kuntze	AM	Nov. 2020	40
Abies veitchii Lindl.	ECM	Nov. 2019	60
Alnus hirsuta Turcz. var. *sibirica* (Spach) C.K.Schneid.	ECM	Nov. 2020	60
Betula ermanii Cham.	ECM	Nov. 2020	50
Carpinus laxiflora (Siebold et Zucc.) Blume	ECM	Nov. 2019	60
Picea jezoensis (Siebold et Zucc.) Carrière var. *jesoensis*	ECM	Nov. 2019	55
Pinus densiflora Siebold et Zucc.	ECM	Nov. 2019	60

Table 2 List of environmental variables recorded in this study for explaining seed germination, seedling survival, and growth.

Variable	Description	Data type	Min, **mean**, max
Substrate	White- and brown-rot logs and soil	Category	–
Openness	Canopy openness above the substrate, %	Continuous	0.9, **13.1**, 29.6
Diameter	Diameter of the logs, cm	Continuous	18.8, **31.2**, 62
Length	Length of the logs, cm	Continuous	62.0, **92.7**, 200
pH	Substrate pH	Continuous	3.8, **4.5**, 5.4
Water	Substrate water content, %	Continuous	7.0, **22.9**, 41.8
Nutrient_PC1	Principal component 1 in PCA of substrate nutrient ions	Continuous	–243.8, **0.0**, 25.8
ECMF richness	OTU richness of ectomycorrhizal fungi in the substrate	Continuous	0, **0.8**, 5
AMF richness	OTU richness of arbuscular mycorrhizal fungi in the substrate	Continuous	0, **1.1**, 10
ECMF infection	Infection ratio of ectomycorrhizal fungi in seedling root, %	Continuous	0, **18.4**, 89.0
AMF infection	Infection ratio of arbuscular mycorrhizal fungi in seedling root, %	Continuous	0, **54.2**, 100

Table 3 Fungal OTUs detected as indicative of one of the three substrates.

OTU_ID	Substrate	Function	Taxa	Stat	*P*
OTU_1171	Brown	AM	Glomeraceae sp.	0.358	0.0284
OTU_2226	Brown	AM	Glomeraceae sp.	0.358	0.0263
OTU_1653	Brown	AM	*Glomus* sp.	0.349	0.0418
OTU_3243	Brown	Bro	*Leucogyrophana* sp.	0.349	0.0436
OTU_925	Brown	Sap	*Mortierella* sp.	0.696	0.0001
OTU_1080	Brown	Sap	*Mortierella* sp.	0.569	0.0006
OTU_799	Brown	Sap	*Hyphoderma* sp.	0.48	0.0021
OTU_2641	Brown	Sap	*Mollisia* sp.	0.412	0.0142
OTU_1259	Brown	Sap	*Mollisia* sp.	0.358	0.0253
OTU_2899	Brown	Sof	*Scytalidium* sp.	0.385	0.0138
OTU_1595	White	Sap	*Sugiyamaella* sp.	0.737	0.0001
OTU_1827	White	Sap	*Hyaloscypha* sp.	0.489	0.0013
OTU_3258	White	Sap	*Stypella vermiformis*	0.361	0.0354
OTU_1473	White	Whi	*Sistotremastrum* sp.	0.775	0.0001
OTU_1135	White	Wod	*Botryobasidium* sp.	0.546	0.0017
OTU_3033	Soil	ECM	*Russula* sp.	0.674	0.0001
OTU_1999	Soil	ECM	*Tomentella* sp.	0.59	0.0004
OTU_41	Soil	ECM	*Elaphomyces* sp.	0.5	0.0013
OTU_51	Soil	ECM	*Tomentella* sp.	0.5	0.0021
OTU_1717	Soil	ECM	*Russula* sp.	0.5	0.002
OTU_316	Soil	ECM	*Russula* sp.	0.413	0.0066
OTU_246	Soil	ECM	*Thelephora* sp.	0.4	0.0199
OTU_303	Soil	ECM	*Tomentella* sp.	0.4	0.0174
OTU_1056	Soil	ECM	*Sarcodon* sp.	0.4	0.0178
OTU_2514	Soil	ECM	*Rhizoscyphus* sp.	0.4	0.0196
OTU_2861	Soil	ECM	*Thelephora* sp.	0.4	0.0172
OTU_3027	Soil	ECM	*Russula* sp.	0.345	0.0288
OTU_2845	Soil	Erm	*Oidiodendron* sp.	0.5	0.0013
OTU_1785	Soil	Plp	*Colletotrichum* sp.	0.4	0.0169
OTU_2323	Soil	Sap	*Umbelopsis* sp.	0.918	0.0001
OTU_2063	Soil	Sap	*Mortierella* sp.	0.886	0.0001
OTU_3112	Soil	Sap	*Geminibasidium* sp.	0.886	0.0001

OTU_529	Soil	Sap	*Sagenomella* sp.	0.837	0.0001
OTU_2733	Soil	Sap	*Mortierella* sp.	0.713	0.0002
OTU_2019	Soil	Sap	*Saitozyma podzolica*	0.686	0.0001
OTU_3025	Soil	Sap	*Cladophialophora* sp.	0.674	0.0002
OTU_1451	Soil	Sap	*Penicillium* sp.	0.4	0.0171
OTU_189	Soil	Sap	*Cladophialophora* sp.	0.345	0.0284
OTU_139	Soil	Sof	*Trichoderma* sp.	0.583	0.0003
OTU_2831	Soil	Sof	*Trichoderma* sp.	0.4	0.0176

Table 4 GLMM results illustrating the relationships between germination rate, survival rate, dry weight, and shoot length of seedlings from arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species and environmental factors.

Mycorrhizal type	Seedling performance	n	Variables substrate	PC1	pH	Water	Openness	OTU richness +
AM trees	Germination	146	SIG	–0.0039***	–0.3781**	0.0699***	–0.0386***	0.0346
	Survival	130	SIG	–0.0029		0.0818***		
	Dry weight	689					–0.0014	
	Shoot length	738	SIG					
ECM trees	Germination	146			0.3492***	0.0371***	–0.0328***	
	Survival	123	SIG			0.0747***	0.0573***	
	Dry weight	378					0.0262	
	Shoot length	529			0.6706			

*, $P < 0.05$; **, $P < 0.01$; ***,$P < 0.001$.

+ OTU richness of mycorrhizal fungi: AM fungal OTU richness for AM trees, and ECM fungal OTU richness for ECM trees.

PC1, nutrient_PC1.

Table 5 Multiple comparisons among the estimates for arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree seedling performance concerning the substrates where the relationships with substrates were selected as a factor in the GLMM in Table 4.

Mycorrhizal type	Seedling performance	Substrate combination	Estimate*	SE	Z	*P*
AM	Germination	White–Brown	0.3044	0.1129	2.697	0.019
		Soil–Brown	0.5136	0.1276	4.024	<0.001
		Soil–White	0.2091	0.1184	1.766	0.18
	Survival rate	White-Brown	–0.1022	0.2009	–0.509	0.867
		Soil–Brown	1.3022	0.1907	6.828	<0.001
		Soil–White	1.4044	0.1897	7.405	<0.001
	Shoot length	White-Brown	–0.6088	0.2875	–2.118	0.085
		Soil–Brown	0.6001	0.3521	1.705	0.201
		Soil–White	1.2089	0.3641	3.32	0.003
ECM	Survival rate	White-Brown	0.9066	0.1436	6.315	<0.001
		Soil–Brown	1.1842	0.1774	6.676	<0.001
		Soil–White	0.2776	0.1454	1.909	0.134

* When estimate for substrate combination White–Brown was positive, white-rot logs have positive effect on the seedlings compared to brown-rot logs.

Table 6 GLMM results showing the relationships between seedling performance of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species and environmental factors.

Seedling	Performance	n	Substrate	PC1	pH	Water	Openness	OTU richness^{+}
Clethra (AM)	Germination	26				0.0949***	-0.1201**	0.0930
	Survival	22				0.0683***		
	Shoot length	22						
Cryptomeria (AM)	Germination	26				0.0800***		
	Survival	23	SIG			0.1879***		
	Shoot length	112						
	Dry weight	111	SIG					-0.2582**
Padus (AM)	Germination	25				0.0183***	-0.0127	
	Survival	25	SIG			0.0761***		
	Shoot length	305	SIG			0.0121*		
	Dry weight	305						
Chamaecyparis (AM)	Germination	25						
	Survival	19						
	Shoot length	50					0.0218*	
	Dry weight	47					0.0530*	
Ilex (AM)	Germination	19			-1.0400**			
	Survival	18		0.0842				0.5427
	Shoot length	190						
	Dry weight	167					0.0567*	
Toxicodendron (AM)	Germination	25					-0.0867*	
	Survival	23						
	Shoot length	59						
	Dry weight	59				0.0428*		
Abies (ECM)	Germination	27						
	Survival	23					-0.2098*	
	Shoot length	127				0.0124		
	Dry weight	104			0.5180*	0.0823**	0.0590**	
Carpinus (ECM)	Germination	26						
	Survival	25	SIG					
	Shoot length	55				-0.0245**		
	Dry weight	55				-0.0343		
Picea (ECM)	Germination	26			1.6652**		0.0719*	-0.1724*
	Survival	22						
	Shoot length	34						
	Dry weight	28						
Pinus (ECM)	Germination	25				0.0273*		
	Survival	24					0.1063*	
	Shoot length	250			0.2990*	0.0122		
	Dry weight	187			0.5260**		0.0264*	
Betula (ECM)	Germination	23				0.0063**		
	Survival	19						
	Shoot length	61						

*, $P < 0.05$; **, $P < 0.01$; ***,$P < 0.001$.

+ OTU richness of mycorrhizal fungi: AM fungal OTU richness for AM trees, and ECM fungal OTU richness for ECM trees.

Alnus data were not analyzed because its sample size was very small.

Dry weight of *Clethra* and *Betula* seedlings were too light to measure.

PC1, nutrient_PC1.

Table 7 Multiple comparisons of the estimates for tree seedling performance concerning the substrates where the relationships with substrates were selected as a factor in the models in Table 6.

Seedling	Performance	Substrate combination	Estimate	SE	Z	P
Cryptomeria (AM)	Dry weight	White - Brown	–0.74	0.31	–2.41	0.04
		Soil - Brown	0.15	0.4	0.39	0.92
		Soil - White	0.9	0.4	2.24	0.06
Cryptomeria (AM)	Survival	White - Brown	0.89	0.52	1.7	0.2
		Soil - Brown	1.67	0.68	2.47	0.04
		Soil - White	0.79	0.6	1.3	0.39
Carpinus (ECM)	Survival	White - Brown	0.53	0.8	0.67	0.78
		Soil - Brown	1.51	0.7	2.14	0.08
		Soil - White	0.97	0.68	1.43	0.33
Padus (AM)	Surival	White - Brown	–0.26	0.33	–0.78	0.71
		Soil - Brown	1.6	0.36	4.5	< 0.001
		Soil - White	1.86	0.32	5.88	< 0.001
Padus (AM)	Shoot length	White - Brown	–0.04	0.1	–0.41	0.91
		Soil - Brown	0.25	0.1	2.4	0.04
		Soil - White	0.29	0.08	3.62	0

* When estimate for substrate combination White–Brown was positive, white-rot logs have positive effect on the seedlings compared to brown-rot logs.