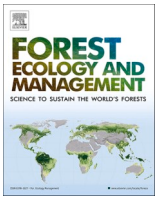
Forest Ecology and Management 572 (2024) 122197

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Long-term nitrogen addition in a boreal forest affects wood-inhabiting fungal communities and influences wood decomposition

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ARTICLE INFO

Keywords:
Fertilization
Fungal DNA
Soil properties
Wood decay

ABSTRACT

Addition of nitrogen (N) to forest soil may alter wood decay rates and fungal community structure and richness. In a northern Sweden *Pinus sylvestris* L. forest, two levels of ammonium nitrate were applied annually from 1971 to 2008. In 2007 we initiated an investigation into wood decay (assessed through mass loss) and fungal responses using stakes of trembling aspen (*Populus tremuloides* Michx.) and loblolly pine (*Pinus taeda* L.) wood installed at different soil depths in plots where zero (N0), low (34 kg; N1), or high (68 kg; N2) levels of N were applied. Stakes were located either horizontally on the surface of the organic horizon, at the interface between the mineral and organic horizons, or vertically in the mineral soil. For litter and mineral soil, fertilizer treatment was not significant for any soil chemical or physical property. Overall, pine and aspen wood stake mass loss was less than 35 % three years after deployment. Notably, the N1 treatment had the most pronounced effect on wood decay, and significantly accelerated aspen decomposition at the organic horizon surface in the second and third years. Analysis of fungal DNA extracted from the wood stakes revealed fluctuations in fungal richness and community composition depending upon stake location and duration since deployment. Fungal richness was notably higher in surface aspen stakes under N0 and N1 treatments and in surface pine stakes under N0 in the second year, though richness generally decreased with time as stake decay increased. Fungal community composition also varied by stake location and time since deployment. These results indicate that prolonged N addition can affect fungal richness, which may in turn affect wood decomposition rates. Further research is needed to clarify the nature and persistence of long-term soil N-addition effects on organic matter decomposition and soil microbial communities.

1. Introduction

Fungi, adapted to tolerate the low soil pH levels in boreal forests, have a dominant role in decomposing organic matter, including wood and other detrital residues (M. Högberg et al., 2007; Lindahl et al., 2007). The rate of decay is linked to nutrient availability (Renvall, 1995) on these chronically N-deficient soils (Tamm, 1991), especially decay occurring on the soil surface (Gosz, 1981; Hunt et al., 1998). Thus, fungal decay can be impacted by nitrogen (N) additions to boreal forest soil (Tamm, 1991). Nitrogen additions can have a negative (Jones et al., 2012; Lilleskov et al., 2001), neutral (Morrison et al., 2016), or positive effect on soil fungal richness or composition, depending on the soil horizon (Gadgil and Gadgil, 1978). Even though long-term N additions may alter wood decay in the mineral soil, differences in chemical, physical, and biological properties, combined with the inherent variability of forest floor and mineral soil, make the N effect on decay less clear (Homann et al., 2001). Furthermore, the duration and magnitude of N addition can alter soil properties (Chappell et al., 1999), thereby leading to more complex and persistent impacts on wood decay rates.

Increased N availability in forests can shift fungal biodiversity,

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https://doi.org/10.1016/j.foreco.2024.122197
Received 29 April 2024; Received in revised form 17 July 2024; Accepted 18 July 2024
Available online 17 September 2024
0378-1127/Published by Elsevier B.V.

acidify soils, and alter soil carbon (C) dynamics and wood decay processes (Robinson, 2002). Studies have indicated that elevated N additions, either through deposition or fertilization, can lead to reduced C allocated to ectomycorrhizal fungi (e.g., Kjøller et al., 2012; Lilleskov et al., 2001). Further, nitrophilic fungal taxa (e.g., *Laccaria, Paxillus*) can thrive under increased N conditions, while nitrophobic fungal taxa (e.g., *Tricholoma, Piloderma*) are adversely affected (Lilleskov et al., 2001). Moreover, N addition can influence fungal decomposition of wood by altering extracellular enzyme activity and production as well as soil pH, which modulates the reactivity of these degradative enzymes (Bååth et al., 1995). Addition of N also changes microbial community composition and nutrient acquisition strategies, potentially leading to reduced decomposition rates in conditions of high N (Freedman et al., 2016; He et al., 2021).

In addition to the influence of N addition on fungal diversity and structure, wood decomposition in boreal forests is governed by several other factors, including temperature, moisture, soil contact, and wood chemistry (Hart et al., 2024; Shorohova and Kapitsa, 2014; Zhou et al., 2007). However, lignin and cellulose contents of wood, combined with forest management regime, can be overriding factors governing wood decay rates (e.g., Finér et al., 2016; Wang et al., 2019). Moreover, wood location (i.e., on the soil surface or within the mineral soil) can affect decay rates due to differing microclimatic conditions that influence microbial colonization and activity (Remsburg and Turner, 2006). Competition between fungal species can impede colonization within and along wood surfaces, slowing decay rates (Boddy and Hiscox, 2016; Lustenhouwer et al., 2020).

Therefore, our study objective was to better understand the impacts of long-term N additions on wood decay rates and fungal community structure at different depths in the litter and mineral soil at the previously established long-term (1971—2006) fertilization site near Norrliden, Sweden (Tamm et al., 1999; M. Högberg et al., 2010; P. Högberg et al., 2024). We monitored the decomposition of trembling aspen (*Populus tremuloides* Michx.; hereafter aspen) and loblolly pine *(Pinus taeda* L.; hereafter pine) wood stakes for three years (2008—2010) after deployment (2007) to determine mass loss and fungal community structure. These two species have different wood properties (e.g., N content, lignin type, lignin to cellulose ratio) that favor different wood-decomposing fungal communities (Blanchette 1984). Our hypotheses were twofold: long-term N additions (1) would alter soil properties and, therefore, wood stake mass loss for all soil—wood stake location (i.e., on the surface of the organic horizons, at the surface organic horizon-mineral soil interface, or in the mineral soil), and (2) would promote different fungal richness and community structure dependent on stake location and species.

2. Materials and methods

2.1. Study area

Our study was overlain on the long-term *Pinus sylvestris* L. fertilization experiment in northern Sweden near Norrliden (64°21'N, 19°46' E; Tamm et al., 1999; M. Högberg et al., 2010; P. Högberg et al., 2024). The 1971—2008 experiment included 30- x 30-m plots receiving annual N fertilization rates of 0 (N0), 32 (N1), or 68 (N2) kg N/ha respectively, as ammonium nitrate,except in 1991. For additional details on fertilization see P. Högberg et al., 2006; Tamm et al., 1999, P. Högberg et al. (2011), and P. Högberg et al. (2024). The N0 plots received only background N deposition of approximately 3 kg/year (P. Högberg et al., 2011). We randomly selected three plots of each N rate to serve as replicates, avoiding plots that also received phosphorus or potassium fertilization. The soil is classified as a Haplic Podzol (FAO, 1988) or Typic Haplocryod (US Soil Taxonomy; P. Högberg et al., 2006) developed on a flat plateau of glacial till (P. Högberg et al., 2006). Average air temperature is 1 °C and mean annual precipitation is 570 mm (M. Högberg et al., 2014).

2.2. Wood stakes

Wood stake preparation and insertion is fully detailed in Jurgensen et al. (2006), (2020). Briefly, surface (15 cm long) and mineral (20 cm long) stakes of aspen and pine were cut from 2.5 cm × 2.5 cm kiln-dried, knot-free, sapwood stakes of 40 or 50 cm lengths; the middle 10 cm was kept as a control (time = 0) to determine initial wood stake properties (Jurgensen et al., 2020). Prior to insertion, the top of each mineral soil stake was coated with a wood sealer to reduce moisture loss after insertion.

In June 2007, we placed 25 aspen and 25 pine stakes horizontally on the surface of the litter layer (hereafter surface stakes), horizontally at the interface between the mineral and organic horizons (hereafter interface stakes), and vertically into the mineral soil (hereafter mineral soil stakes) in each N treatment plot (3 N rates x 2 species x 3 stake locations x 25 stakes x 3 replicates = 1350 total stakes). Mineral soil stakes were inserted into square holes made ~30 cm apart by a soil coring tool to minimize soil compaction at the wood—soil interface. Each stake was inserted so that the top was even with the mineral soil surface and then covered with the surface organic horizons.

In June 2008, 2009, and 2010, five stakes of both wood species (aspen or pine) were randomly sampled from the three soil locations in each N treatment plot (3 N rates x 2 species x 3 soil locations x 5 stakes x 3 replicates = 270 total stakes per year). Immediately upon extraction and after removing any adhered soil, stakes were weighed to determine moisture content. After promptly sampling for fungal DNA sequencing (see next section), stakes were partially dried, and shipped to the College of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI, United States of America (USA) for further laboratory processing.

In the laboratory, stakes were dried for 48 h at 105 °C and then weighed. Decomposition (mass loss) of stakes was measured by subtracting the dry weight of individual surface, interface, and mineral soil stakes from the dry weight of its corresponding unexposed laboratory control. Mass loss averages for each replicate (n=5) were used as treatment observations in statistical analyses.

2.3. Fungal ITS sequencing from wood samples

Within 24 hours of removing stakes from the soil, we collected wood samples from four randomly selected stakes of each N addition rate, wood species, stake location, and replicate (4 stakes x 3 N addition rates x 2 wood species x 3 stake locations x 3 replicates = 216 stakes/sample year) for fungal DNA analysis. On one side of each stake, we cleaned an area of ~2 cm^2 of adhering soil at both stake ends with a sterile razor blade before drilling.

A DNA-sterile drill bit was used to extract wood shavings that were then placed into 1.2 ml strip tubes in a 96-well format and covered with filter-sterilized cell lysis solution (Lindner and Banik, 2009). Wood shavings were collected by drilling a hole almost through the stake, ~2 cm from each end of each stake (i.e. two drill holes per wood stake). Tubes were sealed and frozen before shipment to the United States Department of Agriculture (USDA), Forest Service, Center for Forest Mycology Research (Madison, WI, USA) for fungal community analysis. All fungal samples were frozen to −80 °C until DNA was extracted. Fungal DNA was extracted from each wood sample following the methods of Lindner and Banik (2009) modified for use with 200 µL strip tubes as per Lorch et al. (2013). Briefly, contents of the 1.2 ml tubes were incubated for 1—2 hours at 65 °C and centrifuged 5 min at 3700 rcf at room temperature. Supernatant (100 µL) was transferred to 200 µL strip tubes. DNA was extracted using a glass milk extraction protocol and then amplified using the fungal specific primer pair ITS1F/ITS4. Resulting amplicons were cloned and eight random clones were chosen from each drill sample for reamplification and sequencing (16 clones per stake). Sequences were identified to fungal taxon using BLAST searches of the GenBank database with similarities of 97 % or more used to denote

species identification and 90—97 % for genus identification. Furthermore, the clone sequences were compared among themselves to identify conspecificity within the dataset.

2.4. Soil sampling and analyses

We randomly selected one sampling point, designated with a 30-cm diameter metal ring, adjacent to the wood stakes in each N treatment plot (3 N rates x 3 replications = 9 total samples). Within the ring we measured the depth of the surface litter (inclusive of the S [mixed fresh litter, mosses, lichens], F [decomposing fragments], and H [highly decomposed] horizons) and placed all the organic material that was less than 0.5 cm in diameter into a paper bag. Because of the rocky nature of the mineral soil, it was impossible to excavate soil of similar volumes to determine soil bulk density. Therefore, in each plot we dug one small soil pit greater than 10 cm in diameter and removed all soil material to a depth of 10 cm. In close proximity, we then excavated a second hole of similar diameter to a depth of 20 cm, and soil from the 10—20 cm depth retained. We used the expanding polyurethane foam method (Page-Dumroese et al., 1999) to obtain hole volume at the 0—10 and 10—20 cm mineral soil depths. A total of 27 soil samples (3 N rates x 3 depths [surface organic matter, 0—10, 10—20] x 3 replicates) and the foam cores were shipped to the USDA Forest Service, Rocky Mountain Research Station (Moscow, ID, USA) to determine soil physical and chemical properties and to measure foam core volumes for bulk density calculations.

Soil samples were dried 24 h at 105 °C, weighed, sieved through a 2-mm mesh screen to remove rocks, and re-weighed to calculate soil fine fraction bulk density (Page-Dumroese et al., 1999). The sieved soil was analyzed for C and N (LECO CN analyzer, St. Joseph, MI, USA) and organic matter by weight loss after 8 h ignition at 375 °C. Soil C, N, and organic matter were corrected for fine-fraction bulk density and expressed on a per hectare basis. Soil characterization data at the time of our study is shown in Table 1. Rock content for individual soil pits ranged from 11—69 % of the total soil weight.

2.5. Statistical analyses

2.5.1. Wood stakes

Fungal richness and composition data were analyzed using R (R Core Development Team, 2019) and Primer (Anderson et al., 2008). Fungal presence/absence data were recorded in the form of an operational taxonomic unit (OTU) table. Because data were not normally distributed, we tested a logit transformation. The transformation failed to significantly alter the outputs or data distribution therefore we analyzed non-transformed data. Our independent variables were N rate, sample date, stake species, and stake location and our dependent variables were stake mass loss and fungal richness. We analyzed main effects using nonparametric tests (Mangiafico, 2021) and Scheirer-Ray-Hare ('scheirerRayHare' functions, 'rcompanion' package) to analyze full models for any significant interactions. If none were detected, we employed Kruskal–Wallis ('kruskal.test') to test individual main effects against dependent variables. Dunn's test ('dunnTest' function) was used for pairwise differences. For statistical significance we assumed α=0.05. The explanatory power of wood stake species in the ANOVA (F-value) was large, which may have confounded the separation of the N-rate affect from other model variables. Thus, we analyzed the impact of N additions on wood stake mass loss and fungi separately and found stake species was significant (mass loss, p <0.001; richness, p <0.01). Therefore, we calculated percent sum of squares to quantify the effect each factor had on the response variables. We used the 'Rmisc' package to calculate means and standard errors (Hope, 2013).

For fungal communities, we analyzed Jaccard distance matrixes of fungal presence/absence data via a permutational MANOVA (perMANOVA), after removal of samples that detected no fungal OTUs (5 samples in total). Pairwise comparisons between N rates were analyzed via perMANOVA and we tested for homogeneity of dispersions from the centroids via betadisper tests (Anderson et al., 2008). Results were visualized using Metric MDS ordination that employed 999 bootstrap averages of the centroid; this illustrated where 95 % of the centroid averages lie within multivariate space. We used an analysis of variance (ANOVA) to assess the contribution of specific fungal taxa (i.e., taxa exhibiting the greatest average presence across treatments) to differences between N rates, stake species, stake location, and soil depth.

2.5.2. Soil samples

We analyzed soil physical and chemical properties using an ANOVA (SAS ver 9.2) with $\alpha = 0.05$ for significance. The factors in the ANOVA model were treatment (N level) and soil horizon (forest floor, mineral soil 0—10 and 10—20 cm).

3. Results

3.1. Soil analysis

We detected no significant ($\alpha = 0.05$) differences among the N rate treatments on pH, bulk density, organic matter, total C, or total N in the forest floor and mineral soil samples collected in 2010. Forest floor total N in N1 and N2 was trending to be greater than N0 ($p = 0.118$). Further, organic matter content in the 0—10 cm mineral soil depth in the N1

Table 1
Average forest floor, and mineral soil properties (±SE) in the control (N0) and each nitrogen rate (N1 and N2) from the Norrliden, Sweden long-term N-addition study site. Samples were collected in 2010.

Treatment	pH	Bulk density		Organic matter	Total carbon	Total nitrogen	Rock-fragments
		Total (Mg/m^3)	Fine	(Mg/ha)			(percent)
Forest floor							
N0	3.8 (0.1)	.	.	347 (46)	163 (18)	3 (1)	.
N1	3.9 (0.1)	.	.	396 (69)	208 (36)	7 (2)	.
N2	3.7 (0.1)	.	.	340 (71)	189 (27)	7 (1)	.
***p*-value**	0.118			0.802	0.560	0.118	
0—10 cm							
N0	4.0 (0.2)	1.1 (0.1)	0.9 (0.2)	97 (24)	15 (4)	0.6 (0.2)	24
N1	4.1 (0.3)	1.1 (0.1)	0.9 (0.1)	211 (17)	22 (4)	0.8 (0.1)	22
N2	3.8 (0.1)	1.3 (0.1)	0.9 (0.1)	101 (41)	15 (2)	0.7 (0.1)	44
***p*-value**	0.611	0.063	0.954	0.055	0.257	0.372	
10—20 cm							
N0	4.7 (0.1)	1.1 (0.1)	0.8 (0.1)	64 (23)	9 (2)	0.5 (0.1)	34
N1	4.0 (0.3)	1.2 (0.1)	0.8 (0.2)	56 (41)	8 (3)	0.3 (0.1)	33
N2	4.4 (0.1)	1.3 (0.1)	0.9 (0.2)	61 (17)	9 (1)	0.4 (0.1)	37
***p*-value**	0.192	0.584	0.831	0.629	0.791	0.490	

treatment was trending to be higher than the N0 and N2 treatments ($p = 0.055$). As expected, total C in the mineral soil decreased with increasing soil depth.

3.2. Wood stake mass loss

Sample year and stake species were significant for mass loss (Fig. 1; $p \leq 0.001$). Mass loss increased with sample year, and overall, aspen stakes exhibited greater mass loss than pine stakes. Because we found that sample year, N rate, stake species, and stake location interacted to affect mass loss ($p \leq 0.05$), we examined the response of each stake species for a given location ($p \leq 0.001$ for aspen and pine). Based on the percent sums of squares, stake species accounted for 7.3 % of the variation in mass loss. Within stake species, stake location accounted for more of the variation in mass loss (4.2 %, aspen; 6.1 %, pine) than did N rate (2.2 %, aspen; 2.1 %, pine).

3.2.1. Aspen stakes

Overall, stake location was significant for aspen stake mass loss (Fig. S1, $\alpha = 0.05$); mass loss was greater at the interface than at the soil surface or in the mineral soil. Sample year was also significant for mass loss (Table S1). Specifically, surface aspen stakes in the N1 treatment had greater mass loss in sample years 2009 and 2010 than did stakes in N0 and N2 (Fig. 1), which had similar mass loss. No significant pairwise treatment differences were detected (Table S3).

3.2.2. Pine stakes

Nitrogen rate was not significant for pine stake mass loss at any stake location although the mineral soil stakes generally trended toward more mass loss than surface and interface stakes (Fig. S2; Table S1).

3.3. Fungal richness

Of the 432 wood stakes sampled, 427 yielded amplification products for cloning, which resulted in 517 identified fungal OTUs. Fungal richness for both stake species decreased with sample year, and overall, pine samples had greater richness than aspen stakes (Fig. 2). Overall, stake species accounted for < 1 % of the observed variation in fungal richness based on the percent sums of squares. In addition, wood quality (lignin, cellulose contents) affected fungal richness variability among N treatments (N0, N1, N2) in 2008. Within stake species, stake location accounted for < 1 % of the variation in fungal richness for aspen and pine. Nitrogen rate accounted for 2.1 % and 2.7 % of the variation observed for aspen and pine, respectively. We found an inverse relationship of richness and mass loss (Figs. 1, 2, and S5) that strengthened with time. Generally, fungal richness declined faster in the N1

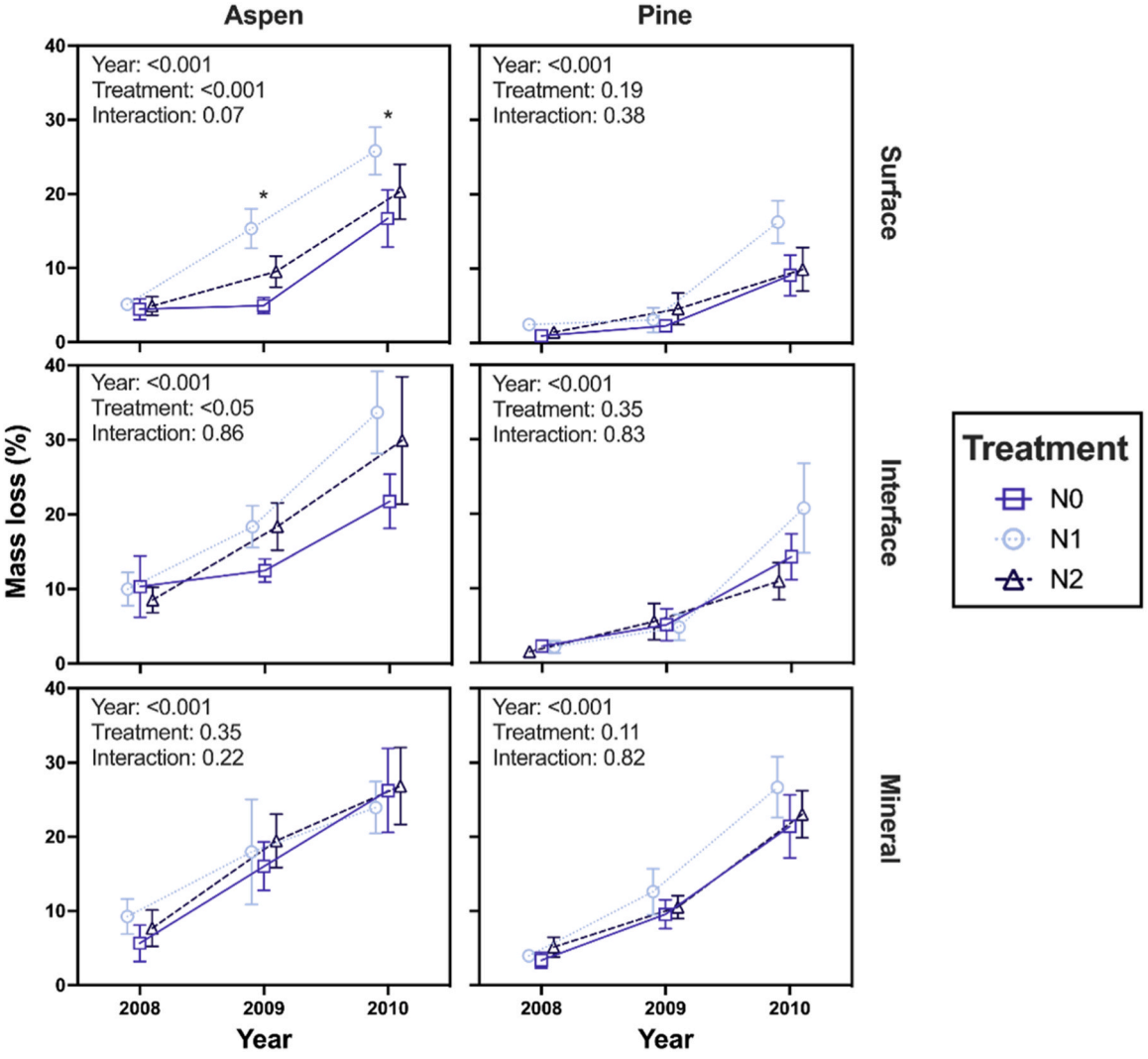

Fig. 1. Means and standard error of percent mass loss of aspen and pine stakes over time for each stake location. Asterisks denote a statistical difference ($\alpha = 0.05$) among N rates for that year. N rate was only significantly different for stakes placed on the surface. Kruskal-Wallis and Scheirer-Ray-Hare test p-values are listed in each plot.

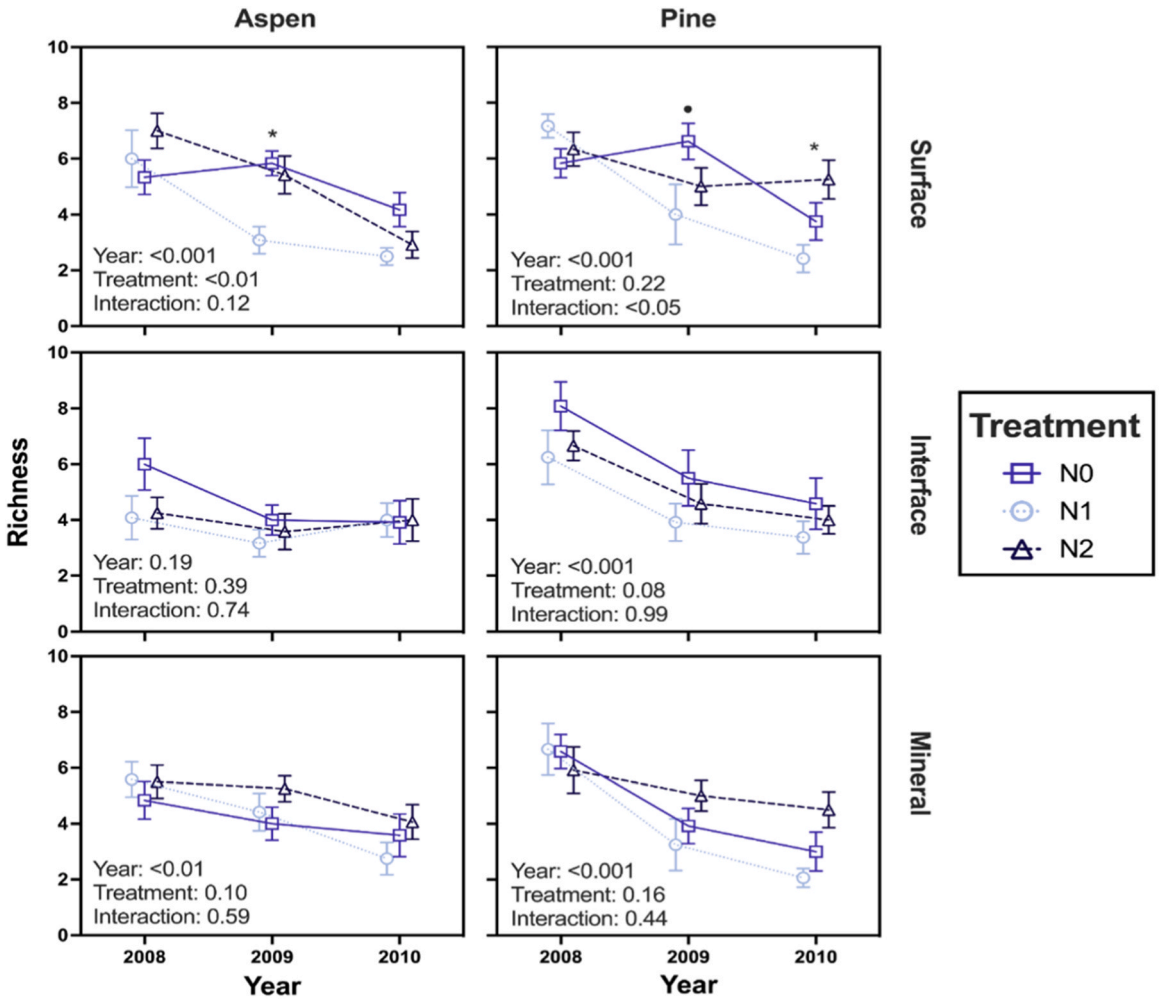

Fig. 2. Means and standard error of fungal richness for aspen and pine stakes over time for each stake location. Asterisks denote a statistical difference ($\alpha = 0.05$) among N rates for that year, and points denote marginal statistical difference ($\alpha = 0.10$). N rate was only significantly different for stakes placed on the surface. Kruskal-Wallis and Scheirer-Ray-Hare test p-values are listed in each plot.

treatment, and this treatment tended to have lower fungal richness overall. Lower fungal richness corresponds to greater mass loss across wood stake species and location.

3.3.1. Aspen stakes

Fungal richness for aspen stakes was relatively similar across stake locations and N rates (Fig. S3). Stake location was not significant ($p = 0.12$). N rate was significant for surface stakes in 2009 (Table S4; Fig. 2); N1 had less richness than either N0 or N2.

3.3.2. Pine stakes

Nitrogen rate was significant for pine stake fungal richness; surface stakes in N0 in 2009 had marginally greater fungal richness than either N-addition plot, but in 2010 N2 had statistically greater richness than N1 ($\alpha = 0.05$; Fig. 2). Stake location was also significant, with mineral soil stakes having less richness than surface or interface stakes (Fig. S4).

3.4. Fungal community composition

Overall, all main effects were significant (all $p \leq 0.001$) for fungal community composition, as were the interactions of sample year × N rate × stake location ($p \leq 0.05$), sample year × stake location ($p \leq 0.001$), sample year × stake species ($p < 0.001$), sample year × N rate ($p \leq 0.001$), stake location × N rate ($p < 0.001$), stake location × stake species ($p \leq 0.001$), and N rate × stake species ($p \leq 0.001$). Because of these multiple significant interactions, we examined fungal community composition for each stake species across all sampling years in each stake location (Fig. 3) to evaluate community changes over time and in response to N rate.

3.4.1. Aspen stakes

For aspen surface stakes, N rate (pseudo-F_{2107}=2.24; $p \leq 0.01$) and sampling year (pseudo-F_{2107}=2.22; $p \leq 0.01$) were significant for fungal community composition, but their interaction was not (pseudo-F_{4107}=1.01; $p = 0.41$). Except for the first sample date (2008), composition in N0 tended to be distinct from either N1 or N2 (Fig. 3). While N1 and N2 had similar compositions within a given sampling year, these compositions tended to shift with progressive sampling years (Fig. 3) and likely reflects advancing wood decay. Except for *Mollisia cinerea* and *Pseudoplectania nigrella*, the N0 treatment had the lowest frequency of fungal taxa (Table 2). In 2010, surface aspen in the N1 and N2 treatments did not have different fungal communities (Fig. 3).

Nitrogen rate (pseudo-F_{2205}=4.20; $p \leq 0.01$) and sampling year (pseudo-F_{2205}=4.01; $p \leq 0.01$) were significant for community composition on interface stakes, as was the N rate × sampling year interaction (pseudo-F_{4205}=2.05; $p \leq 0.01$). The interaction is likely due to sampling year being significant for all N rates in 2008 and 2009. Community composition of the interface stakes was generally different from the surface stakes (Fig. 3). In addition, the N0 treatment had a different community composition than the N1 and N2 treatments. Similar to

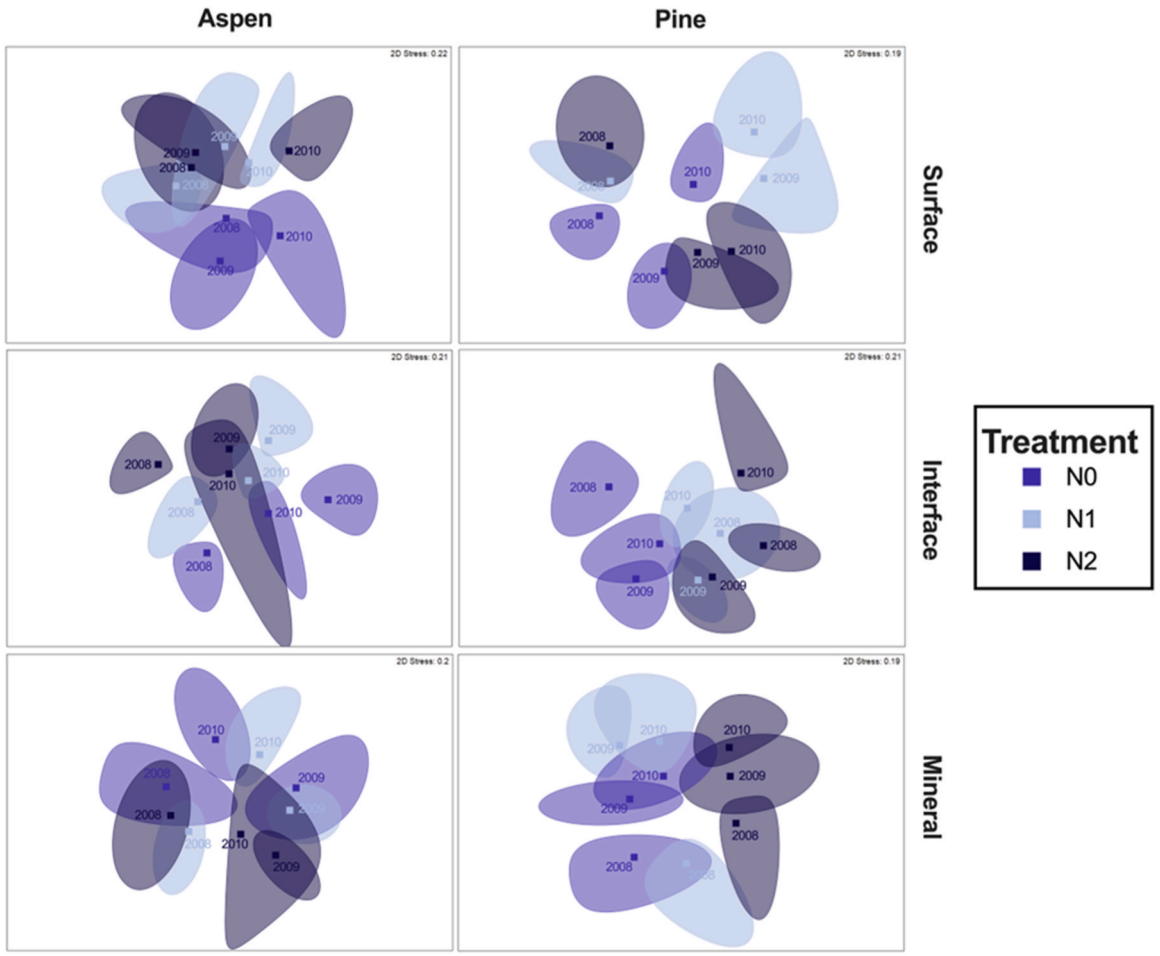

Fig. 3. Non-metric multidimensional scaling (NMDS) plots using Jaccard distances to assess nitrogen treatment effects for each sampling year associated with aspen and pine stakes placed at three soil depths (i.e., surface, interface, and mineral). We employed bootstrap averages to determine where 95 % of the centroid averages lie withing multivariate space. The mean centroid is indicated by the treatment symbol with 95 % confidence ellipses.

surface stakes, *M. cinerea* was observed more often in N0 as compared to either N1 or N2 ($p = 0.06$; Table 2).

Nitrogen rate for aspen mineral soil stakes (pseudo-F_{2194}=2.73; $p \leq 0.01$), sampling year (pseudo-F_{2194}=3.79; $p \leq 0.01$), and their interaction (pseudo-F_{4194}=1.30; $p < 0.05$) were significant. The significant interaction is likely due to no N rate fungal community differences observed in 2008, while in 2009 and 2010 the N2 community was different than that in N0 and N1 (Fig. 3). Only two fungal species identified on the mineral aspen stakes (*Phanerochaete velutina* and a *Cenococcum* sp.) showed significant treatment differences (Table 2).

3.4.2. Pine stakes

For pine surface stakes, N rate (pseudo-F_{2190}=4.26; $p \leq 0.01$), sampling year (pseudo-F_{2190}=3.89; $p \leq 0.01$), and their interaction (pseudo-F_{4190}=1.71; $p \leq 0.01$) were significant for fungal community composition. While N rate was significant every sampling year, the interaction is likely due to divergence in fungal communities from 2008 to 2010, especially when comparing N0 and N2 to N1. When N rate was significant for the presence of fungal taxa on pine surface stakes, the N1 and N2 treatments generally had greater numbers identified (Table 2). *Phanerochaete sanguinea* and *P. velutina* were identified more often in N1 ($p \leq 0.001$ for both species; Table 2) while *Pholiota scamba* and *Pezoloma ericae* were identified more often in N2 ($p \leq 0.001$ and $p \leq 0.01$, respectively; Table S5). Notably, the saprophytic fungus *P. scamba* was not identified on N1 surface stakes.

Significant differences in nitrogen rate for pine interface stakes (pseudo-F_{2189}=4.18; $p \leq 0.01$) and sampling year (pseudo-F_{2189}=3.07; $p \leq 0.01$) were detected, but the interaction was not significant (pseudo-$F_{4,97}$=1.16; $p \leq 0.10$). *Phanerochaete velutina* was identified more often in N0 and N1 but was absent in the N2 treatment (Table 2). In addition, a species of *Coniochaeta* and *Phialocephala fortinii* fungi were observed most often in N0 as compared to either N1 or were absent in N2 ($p \leq 0.001$ for both rates; Table 2). We also identified *Acrodontium antarcticum* and a *Sporothrix* sp. on pine interface stakes, generally associated with N1 and N2 ($p \leq 0.001$; Table 2). Notably, *P. velutina* and the *Coniochaeta* sp. were not observed in N2, and the *Sporothrix* sp. was absent in N0 during our sampling periods.

Nitrogen rate (pseudo-F_{2105}=2.44; $p \leq 0.01$), sampling year (pseudo-F_{2105}=2.04; $p \leq 0.01$) were significant for community composition in mineral soil stakes (Fig. 3), but the interaction was not (pseudo-F_{4105}=1.01; p=0.50). We identified *Pholiota mixta* and *A. antarcticum* more often from N2 as compared to N0 and N1 ($p \leq 0.001$ and $p \leq 0.01$, respectively; Table 2). A *Sporothrix* sp. was present most often in N2, intermediate in N1, and not observed in N0 ($p \leq 0.01$; Table 2).

4. Discussion

4.1. Soil properties

Contrary to our first hypothesis, we detected no significant differences in soil properties during our sampling in 2010. This may be due to the small sample size (n=9) and the inherent variability of forest soils, particularly the organic horizons (Muukonen et al., 2009). Previous work, for example, noted that the combined litter layers at Norrliden

Table 2
Mean fungal taxa frequency in drill shavings for all three sampling years combined in each stake species at each stake location in the control (N0) and each nitrogen rate (N1 and N2) from the Norrliden, Sweden long-term fertilizer study site.

Stake species and location	Fungal taxa	Fertilizer treatment			*p*-value
		N0	N1	N2	
Aspen					
Surface	*Ascocoryne cylichnium*	0.04b	0.06ab	0.18a	≤0.05
	Lachnellula fuscosanguinea	0.00b	0.11b	0.18a	≤0.01
	Mollisia cinerea	0.30a	0.00b	0.07b	0.001
	Phanerochaete velutina	0.03b	0.20a	0.06b	≤0.01
	Pseudoplectania nigrella	0.14a	0.12a	0.00b	≤0.01
	Sistotrema brinkmannii	0.09	0.12	0.15	ns
Interface	*Acrodontium antarcticum*	0.21b	0.38ab	0.47a	≤0.01
	Mollisia cinerea	0.17a	0.05b	0.09ab	0.06
	Phanerochaete velutina	0.06b	0.27a	0.10b	≤0.01
	Phialocephala sp.	0.19a	0.05b	0.00b	≤0.001
	Phialocephala sp.	0.17a	0.03b	0.03b	≤0.001
	Sporothrix sp.	0.01b	0.06b	0.23a	≤0.001
Mineral	*Acrodontium antarcticum*	0.18	0.24	0.32	ns
	Cenococcum sp.	0.29a	0.14ab	0.07b	≤0.01
	Coniochaeta sp.	0.06	0.14	0.14	ns
	Phanerochaete velutina	0.05b	0.24a	0.06b	≤0.001
	Phialocephala sp.	0.11	0.13	0.08	ns
	Phialocephala sp.	0.10	0.05	0.11	ns
Pine					
Surface	*Ascocoryne cylichnium*	0.13	0.04	0.06	ns
	Hamamotoa lignophila	0.15	0.14	0.06	ns
	Helotiaceae sp.	0.11	0.03	0.10	ns
	Helotiaceae sp.	0.03	0.07	0.08	ns
	Infundichalara minuta	0.1	0.14	0.13	ns
	Pezoloma ericae	0.11ab	0.01b	0.18a	≤0.001
	Phanerochaete sanguinea	0.02b	0.24a	0.04b	≤0.001
	Phanerochaete velutina	0.12ab	0.24a	0.01b	≤0.001
	Pholiota scamba	0.02b	0.00b	0.15a	≤0.001
Interface	*Acrodontium antarcticum*	0.11b	0.38a	0.51a	≤0.001
	Coniochaeta sp.	0.24a	0.01b	0.00b	≤0.001
	Hyaloscypha Boudier sp.	0.03	0.11	0.12	ns
	Phanerochaete velutina	0.21a	0.22a	0.00b	≤0.001
	Phialocephala fortinii	0.20a	0.07b	0.06b	≤0.05
	Sporothrix sp.	0.00b	0.13a	0.19a	≤0.001
Mineral	*Acrodontium antarcticum*	0.11b	0.10b	0.29a	≤0.01
	Cenococcum sp.	0.14	0.13	0.08	ns
	Phanerochaete velutina	0.16	0.19	0.08	ns
	Phialocephala fortinii	0.07	0.08	0.09	ns
	Pholiota mixta	0.11b	0.07b	0.29a	≤0.001
	Sporothrix sp.	0.00b	0.07ab	0.13a	≤0.01

For each fungal taxa within each stake species and location, N treatment values with the same letter are not significantly different. "ns" indicates no significant differences among treatments. Some fungal genera are repeated as identification to species was inconclusive.

generally had less than 1 Mg/ha of N with the N0 treatment having significantly less N than N1 and N2 (P. Högberg et al., 2011). In 2010 the litter layers had 3–7 Mg/ha of N, but N treatment was not significant. In addition, in 2010 our litter analyses for C:N for N0 (54) is greater than the C:N reported (~38) in P. Högberg et al. (2024). However, our data for N1 (C:N 29) and N2 (C:N 27) are, similar to a previous report (P. Högberg et al., 2024).

The high N-loads associated with the N2 plots did result in losses of base cations but did not result in negative tree-growth responses (P. Högberg et al., 2006). These authors noted that pH of the surface organic horizon was higher in the N2 plots as compared to the N0 and N1 plots, although we only found that the surface organic horizons had a lower pH as compared to the mineral soil. Chronic N additions can lower soil pH and disrupt decomposition by altering fungal community composition (Morrison et al., 2016) which may be a reason that stakes on the soil surface or at the interface had lower mass loss than in the mineral soil. Further, fungi that decompose higher quality substrates favor aspen stakes (Keiser et al., 2011; Strickland et al., 2009a, b).

4.2. Stake mass loss and fungal communities

After three years, the lowest rate of applied N (i.e., N1) had, in general, the greatest effect on wood stakes, similar to results observed with other common substrates (e.g., litter) found in boreal forest ecosystems (Hogervorst et al., 2003; Allison et al., 2009). Many non-symbiotic microbes are C-limited (Smith and Paul, 1990; Ekblad and Nordgren, 2002) and, in our study, the wood stakes may have supplied an additional C-substrate for the microbial community. However, microbial activity can also be constrained by N-availability (Zhang and Zak, 1998). For example, prior studies show that in pure cultures, low levels of N addition enhanced decomposition by basidiomycetes (Fog, 1988), while high N levels can suppress organic matter decomposition (e.g., Findlay, 1934; Zadraizil and Brunnert 1980) and decrease overall microbial biomass (Bååth et al., 1981). At the Norrliden study site, we observed that the rate of N addition had minimal effect on wood stake mass loss or fungal richness, but strongly affected community composition. Furthermore, mass loss was strongly regulated by stake location on or in the soil.

In general, interface and mineral soil stakes decayed faster than surface stakes, likely because the litter layer, which averaged 5 cm in depth across the plots, helped retain stake moisture and moderate temperature fluxes, conditions conducive to decomposition (A'Bear et al., 2013). Forest floor moisture content can be seasonally higher (Tan et al., 2005), but the mineral soil generally retains moisture for a longer period, and which can lead to an increase in wood decay in the mineral soil (Finér et al., 2016). At Norrliden we did not collect soil moisture data, however at other geographic locations, wood stake moisture content and mass loss were positively correlated (Finér et al., 2016; Page-Dumroese et al., 2021). In addition, stake fungal communities varied with from the surface to the mineral soil, leading to different decomposer communities. It is unknown whether the more stable climate regimes at the interface or in the mineral soil, when combined with potential differences in the decomposer community, would lead to greater or lesser decomposition rates. The linkages among mass loss, wood species, and fungal decomposers communities should, however, be considered because these factors will likely also drive root decay (Jurgensen et al., 2020).

A common relationship between wood quality and decomposition rate is evident across multiple tree species (Ulyshen et al., 2020; Finér et al., 2016; Page-Dumroese et al., 2019). Notably, most studies find that aspen stakes decompose more rapidly than pine stakes (e.g., Page-Dumroese et al., 2021), a phenomenon often attributed to the relatively low lignin content of aspen (Hu et al., 2018; Wang et al., 2018). Moreover, the addition of N has frequently been observed to positively affect decomposition rates in forest soils (Chen et al., 2016; Freedman et al., 2016; Manning et al., 2008; van der Wal et al., 2007). Our findings build

upon these studies, providing further insight into the intricate ways that changes in nutrient availability can influence decomposition rates in boreal forest soils. While the significant effects of N1 additions were observed only for aspen on the soil surface, it is noteworthy that both aspen and pine stakes generally exhibited a higher overall decay rate in the N1 treatments across all locations. This trend is consistent with the findings of P. Högberg et al. (2006), who noted that low rates of N addition contributed to increases in stand volume more effectively than higher N amendment rates. Nitrogen applications might also supply N for the microbial community, thus promoting energy allocation towards cellulase production and the breakdown of accessible cellulose and hemicellulose in wood (Carreiro et al., 2000; van der Wal et al., 2007). Soil N applications can inhibit oxidative enzyme production by microbes and reduce organic matter decomposition rates by altering the competitive balance between enzymatic lignin mineralization and non-enzymatic lignin oxidation (FAO 1988; Fukasawa et al. 2009; Lindner et al. 2011; Marais et al. 2020; Muukkonen et al. 2009; R Core Development Team 2019; Vishniac 1996). Moreover, the absence of greater wood stake mass loss in the high N treatment could be attributed to a fungal utilization limit of N, possibly reaching a threshold (Zhong et al., 2015). Additionally, factors such as the initial amount of N added, soil pH, and the soil and wood stake C:N ratios could also play roles in shaping the fungal community structure (M. Högberg et al., 2014), and consequently, affect wood stake mass loss.

Wood stake decay studies in the eastern USA suggests that abiotic properties (i.e., soil temperature and moisture) are the primary drivers of decay rate (Adams et al., 2021; Ponder et al., 2017). In the drier, western USA, no clear region-wide drivers of wood stake decay were evident along a climatic gradient from north to south but local site factors (i.e., soil texture and microclimate) were better related to decay rate (Page-Dumroese et al., 2021), similar to the findings of Bradford et al. (2014). However, in some ecosystems, biotic factors (i.e., fungal richness and community composition) can overwhelm the effects of abiotic properties and, therefore, control decay rates (Maynard et al., 2018; Hu et al., 2021).

4.3. Fungal communities and richness

On this same experimental site, an earlier study initiated after 14 years of N additions, found that soil fungal communities from the organic horizon were related to soil C:N and pH (M. Högberg et al., 2014). We may be observing similar effects of N additions (Keiser et al., 2011; Manning et al., 2008; Strickland et al., 2009b) in 2008–2010. Other possible indirect effects would be long-term N applications leading to an increase in plant concentrations of N or an alteration in soil pH that alters fungal or bacterial community structure to result in altered decomposition rates during the later stages of decomposition (Berg and Matzner 1997; M. Högberg et al., 2014).

In our study, we observed an inverse relationship with fungal richness and mass loss: a decrease in fungal richness corresponded with an increase in mass loss (Figure S5). This may be due to competitive interactions among species, with greater richness leading to more competitive interactions and thus less resource allocation to wood decomposition. We found that, in general, fungal richness was lowest within the mineral soil where we observed the greatest mass loss. Fresher wood contains more labile C components (e.g., cellulose) than older wood, the later containing more chemically complex components (e.g., lignin) (Wang et al., 2019). Thus, this decrease in richness with time is perhaps due to rapid loss of easily degraded cellulose by a generalized groups of fungi that leave behind lignin that is only utilized by a few fungal taxa (Rajala et al., 2012) that specialize in production of ligninolytic enzymes and dominate at the later stages of decompostion (Schimel, 1995; Boddy, 2001; Rajala et al., 2012).

Fungal community composition was more responsive to N rates than mass loss and fungal richness. Some fungal species tended to be associated with the same N rate across soil depth. For example, *P. velutina* tended to be present more often across all stake locations for aspen and pine stakes in N1 plots. This cord-forming fungus may not be able to capitalize on increased soil N associated with the N2 treatment to colonize the wood stakes (Boddy, 1993; Fukasawa and Kaga, 2020). Another example is *A. antarcticum* that was only present in the interface and mineral soil layers and tended to be present most often in the N2 treatment, suggesting that greater access to N is needed for it to colonize the wood stakes. Together these findings indicate that N amendments define the composition of the decomposer community, but these communities are associated with similar amounts of wood stake mass loss, suggesting some level of functional redundancy within these communities. Wood decay in the boreal forest is likely affected by biotic and abiotic factors, namely wood composition (quality) and location within the soil profile. In addition, in some environments solar radiation can be a factor in altering decay processes by selecting for microbes that tolerate high temperatures, low moisture, and/or UV radiation (Caldwell et al., 2007; Gallo et al. 2009). Incoming solar radiation can also degrade lignin (Day et al., 2007) and result in increased wood mass loss. At Norrliden, photodegradation was not likely a factor driving wood stake mass loss as plots were installed under similar amounts of canopy coverage.

5. Conclusion

In a boreal forest in northern Sweden, N addition rate, wood stake species, and stake location within the soil profile all shaped wood stake decomposition and the associated composition of fungal communities. Disentangling the complex relationships among these factors will, however, require studies aimed further toward identifying the mechanisms leading to our current observations. In addition, future work should attempt to confirm and explain our observation that N additions significantly affected wood stake decomposition on the surface of the forest floor. Importantly, because we found that stake location on or in the soil was the most important factor controlling decomposition, more investigations should focus on sub-surface decomposition with continuous measurements of microclimate conditions (i.e., soil temperature and moisture for each stake placement location). Finally, effects of N fertilization on decomposition of wood across a broad range of lignin-to-N ratios would further improve our understanding of how exogenous N influences wood decomposition, fungal community structure, and potentially unveil the optimum amount of N for wood decomposition and fungal community composition. Our study emphasizes the importance of incorporating multiple factors in a field-experiment to fully understand the decomposition process, but it also highlights that, although factors interact to affect a response, one factor may be the main driver of that response. Here we found that stake location and stake species were the main drivers of wood stake mass loss, while N rate was the main driver of fungal community composition.

CRediT authorship contribution statement

Michelle A. Jusino: Writing – review & editing, Data curation. **Mark T. Banik:** Writing – review & editing, Resources. **Katelyn Alexander:** Writing – original draft, Visualization, Formal analysis. **Michael S. Strickland:** Writing – review & editing, Formal analysis. **Martin F. Jurgensen:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Joanne M. Tirocke:** Writing – review & editing, Resources. **R. Kasten Dumroese:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Deborah S. Page-Dumroese:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Daniel L. Lindner:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Derek N. Pierson:** Writing – review & editing.

Disclaimer

The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or US government determination or policy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

We gratefully acknowledge the scientists and supporting staff involved in the installation and maintenance of this study site. We also thank the lab technicians who analyzed the wood stakes. We acknowledge the USDA Forest Service Northern and Rocky Mountain Research Stations for their support of this project.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2024.122197.

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