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Nitrogen addition increases mass loss of gymnosperm but not of angiosperm deadwood without changing microbial communities

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ABSTRACT

Enhanced nitrogen (N) deposition due to combustion of fossil fuels and agricultural fertilization is a global phenomenon which has severely altered carbon (C) and N cycling in temperate forest ecosystems in the northern hemisphere. Although deadwood holds a substantial amount of C in forest ecosystems and thus plays a crucial role in nutrient cycling, the effect of increased N deposition on microbial processes and communities, wood chemical traits and deadwood mass loss remains unclear. Here, we simulated high N deposition rates by adding reactive N in form of ammonium-nitrate (40 kg N ha^{-1} yr^{-1}) to deadwood of 13 temperate tree species over nine years in a field experiment in Germany. Non-treated deadwood from the same logs served as control with background N deposition. Our results show that chronically elevated N levels alters deadwood mass loss alongside respiration, enzymatic activities and wood chemistry depending on tree clade and species. In gymnosperm deadwood, elevated N increased mass loss by +38 %, respiration by +37 % and increased laccase activity 12-fold alongside increases of white-rot fungal abundance +89 % ($p = 0.03$). Furthermore, we observed marginally significant ($p = 0.06$) shifts of bacterial communities in gymnosperm deadwood. In angiosperm deadwood, we did not detect consistent effects on mass loss, physico-chemical properties, extracellular enzymatic activity or changes in microbial communities except for changes in abundance of 10 fungal OTUs in seven tree species and 28 bacterial OTUs in 10 tree species. We conclude that N deposition alters decomposition processes exclusively in N limited gymnosperm deadwood in the long term by enhancing fungal activity as expressed by increases in respiration rate and extracellular enzyme activity with minor shifts in decomposing microbial communities. By contrast, deadwood of angiosperm tree species had higher N concentrations and mass loss as well as community composition did not respond to N addition.

1. Introduction

Increased atmospheric nitrogen (N) deposition stemming from anthropogenic activities such as fossil fuel combustion and fertilization alters global N cycling and has drastic effects on ecosystems worldwide (Galloway et al., 2004; Galloway et al., 2008). Especially in systems that

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are naturally N limited, such as boreal and temperate forests, consequences of chronically elevated N levels threaten ecosystem functioning by biodiversity loss (Isbell et al., 2013), a reduction of microbial community activity (Edwards et al., 2011; Janssens et al., 2010) and soil acidification (Sutton et al., 2011) with the gravity of effects depending on individual ecosystem sensitivity (Bobbink et al., 2010). Furthermore, anthropogenic N depositions result in an overall alteration of N cycling through changes in leaching, mineralization and nitrification (Pardo et al., 2011; Zak et al., 2011). Higher N deposition has been shown to increase carbon sequestration in forest soils by slowing plant litter decomposition (Lovett et al., 2013; Zak et al., 2011; Zak et al., 2017). In Europe and North America, levels of anthropogenic N deposition have been declining since 1990 (Schmitz et al., 2019) with studies estimating current additional N through atmospheric deposition between 15 (Schwarz et al., 2014) to 25 kg ha^{-1} $year^{-1}$ for these regions (de Vries et al., 2014). Schwede et al. (2018) project 10–20 kg ha^{-1} $year^{-1}$ of atmospheric N deposition in Central Europe, with hotspots in the Netherlands, Northern Germany and Italy exceeding 25 kg N ha^{-1} $year^{-1}$. Du et al. (2014) report a mean of 3.5 kg N ha^{-1} $year^{-1}$ for the US with highest deposition rates measured in the Midwest at 5.4 kg N ha^{-1} $year^{-1}$ due to intense agriculture and livestock breeding.

The response of forest soil systems to elevated N levels has been studied extensively with varying and frequently contradicting results (Allison et al., 2007; Carreiro et al., 2000; Edwards et al., 2011; Morrison et al., 2018; Treseder, 2008). For instance, Carreiro et al. (2000) reported enhanced decomposition in litter with low lignin content and a decrease in high-lignin substrates. Bioavailable N has been shown to affect decomposition processes via promoting cellulolytic enzyme activity and gene expression and inhibiting these processes for ligninolytic enzymes (Edwards et al., 2011; Knorr et al., 2005; Waldrop et al., 2004; Waldrop and Zak, 2006; Wang et al., 2010), with impact on C storage in forest ecosystems through increasing mass loss of leaf litter at intermediate stages and decreasing mass loss at late stages of decomposition. Two mechanisms are proposed to be responsible for this effect: (i) a decoupling of degradation of polysaccharides and polyaromatics, increasing the activity of cellulose degrading enzymes and repressing the production and activity of lignin modifying enzymes such as manganese peroxidase and laccase (Edwards et al., 2011; Frey et al., 2014; Morrison et al., 2018), and (ii) shifts in microbial community composition (Entwistle et al., 2018; Lilleskov et al., 2019; Moore et al., 2021), to higher abundances of saprotroph fungal species (Morrison et al., 2018).

While we have a basic understanding of how elevated N levels shape decomposition processes in leaf litter of temperate and boreal forests, we are lacking knowledge on the impact of N on other large carbon pools in forests. Carbon sequestered in deadwood amounts to about 8 % of total global carbon stocks in forests (Pan et al., 2011). Deadwood has an ecological function in forest ecosystems by offering a habitat to bacteria, fungi, insects and other micro fauna (Merganicova et al., 2012) and acting as a key component in water, nutrient and carbon storage and cycling (Rajala et al., 2012; Stokland et al., 2012). Similar to their role in soil systems, fungi - especially white-rot Basidiomycota - enable deadwood decomposition through the production of extracellular ligninolytic and cellulolytic enzymes to break down woody biomass (Stokland et al., 2012). While their contribution to the breakdown of woody biomass might appear relatively small compared to fungi, bacteria play an active role in deadwood decay as well, for example via nitrogen fixation (Groß et al., 2022) and thereby alleviation of nutrient constraints (Rinne et al., 2017). Bacterial communities in deadwood have been shown to follow successional patterns (Hoppe et al., 2015) and to be controlled by wood physico-chemical parameters such as C:N ratio, pH and water content as well as fungal community composition (Moll et al., 2018).

Understanding deadwood decomposition is crucial for modeling forest C cycles (Seibold et al., 2021), however, the altering influence of chronically elevated N deposition has so far not gained much attention and studies on the effect of N deposition on deadwood and its decomposition process in the field have remained sparse (Bebber et al., 2011; Qiao et al., 2016; Purahong et al., 2018). To our best knowledge, there are only two publications investigating the direct effects of artificially enhanced N deposition on deadwood decomposition. Bebber et al. (2011) exposed European beech (*Fagus sylvatica*) wood blocks inoculated with two cord-forming fungal species to enhanced N levels of 2.8 kg ha^{-1} $year^{-1}$ and found higher mass loss for those blocks exposed to higher N availability levels. This was attributed to the alleviated nutrient constraints for both fungal taxa, enabling both fungal species to higher wood decomposing activity by a higher amount of available N for metabolism and growth. In contrast, Qiao et al. (2016) reported a negative effect of N addition on decomposition of wood substrates in a laboratory experiment due to the microbial community switching from breaking down lignocellulose to less complex substrates. Purahong et al. (2018) investigated the effect of two years of enhanced N deposition on deadwood of thirteen temperate tree species in a field experiment, the same experiment this study is based on. The authors found no significant changes in either enzymatic activity or fungal community composition.

Strong differences in wood physico-chemical properties such as lignin content and pH values between tree species and clades (angiosperms and gymnosperms) lead to heterogeneous decomposition patterns (Weedon et al., 2009). However, overall C:N ratios of wood from recently died trees are high (Filipiak, 2018; Hoppe et al., 2016) and although N accumulates during decomposition due to breakdown of woody biomass and the activity of N-fixing bacteria (Rinne et al., 2017), deadwood C:N ratios remain high compared to other substrates in forest ecosystems (e.g. 377 in *Fagus sylvatica*, 630 in *Picea abies*, Purahong et al., 2018). Decomposer communities in deadwood are therefore subjected to higher N limitations than their leaf litter counterparts (Tláskal et al., 2017). Consequently, mere extrapolation of such N effects on deadwood (based on results from forest soil studies) might lead to unrealistic results. Considering the effects of elevated N deposition on litter decomposition, we assume that any effects of N deposition on deadwood decay and its microbial communities would carry implications for overall forest C cycling, either through higher C sequestration due to slower decomposition or through higher amounts of CO_2 released by more rapid depletion of wood components.

To examine the impact of elevated N deposition on deadwood decomposition in a field setting, we conducted an experiment in a temperate forest in Central Germany. As mentioned above, first results after two years of artificially enhanced N deposition were published by Purahong et al. (2018) with no visible effect on mass loss, fungal communities or enzymatic activities. Here, we have aimed at characterizing the long-term effect of elevated N levels on deadwood decomposition by analyzing deadwood parameters after nine years of exposure to elevated N. Since N is one of the most important macronutrients (Bani et al., 2018) and deadwood a highly N limited substrate (Stokland et al., 2012), we hypothesized that artificially enhanced N deposition would lead to higher extracellular enzymatic activity of wood-decaying fungi accompanied by increased respiration rates and subsequently increased mass loss of logs (hypothesis 1). Building on this, we also expected to see changes in wood chemical properties such as lignin content and pH after long-term exposition to enhanced N deposition. Although Purahong et al. (2018) did not observe significant shifts in fungal community composition, we expected to see a long-term effect of elevated N deposition on microbial communities in terms of composition and ecology of the present fungal taxa (hypothesis 2).

2. Material and methods

2.1. Study site and design

The N experiment is a sub experiment of the BELongDead project (Kahl et al., 2015), which is a part of the DFG funded research platform Biodiversity-Exploratories (Fischer et al., 2010). The experiment was

situated on five different forest plots at the Hainich National Park in the state of Thuringia, Germany. Located in Central Germany (51.097324 N 10.458212E) between 285 and 550 m above sea level, the average annual temperature at the Hainich National Park ranged from 6.5 to 8 °C with an annual precipitation of 500 to 800 mm (Fischer et al., 2010). Distances between plots varied between 0.4 and 28.7 km until 2017, when the entire experiment was moved to a natural beech forest stand for logistic reasons. Due to its status as a highly restricted conservation area, most forest stands at the Hainich National Park do not underlie forestry management. European (*Fagus sylvatica*) beech acts as the main tree species (83 % of forest cover), 17 % of forest area are covered by coniferous woods dominated by *Picea abies* (Fischer et al., 2010).

The experiment was made up by 26 log halves per plot belonging to 13 temperate tree species in two tree clades (nine angiosperm species: *Acer* spp. *Betula pendula* L., *Carpinus betulus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Populus* spp., *Prunus avium* (L.) L., *Quercus* spp., *Tilia* spp.; four gymnosperm species: *Larix decidua* Mill., *Picea abies* (L.) H. Karst, *Pinus sylvestris* L., *Pseudotsuga menziesii* (Mirb.)) and two treatments (nitrogen addition and control without addition) with five replicates, adding up to 130 logs in total. All logs were cut during the winter months of 2008/2009 and originate from the same region in Thuringia. Until the N experiment was ready to be launched in 2011, all logs were stored (with soil contact) at the experimental site.

At the start of the experiment in 2011, the logs were in early stages of the decomposition process. For the experiment, every log was sawed up longitudinally (40 cm length, 30 cm diameter) and placed in a separate plastic box (Euro packaging box 60 × 40 × 32 cm, Auer GmbH, Amerang, Germany) to inhibit soil contact and therefore contamination through additional N or alteration of the fungal community. Initially these boxes were necessary for ^{15}N labeling and respiration measurements (Purahong et al., 2018). Small holes on the box edges enabled drainage to prevent precipitation from collecting inside and tile pieces underneath the logs ensured natural air flow within each box. Increased nitrogen deposition was simulated by spraying treatment logs with 5 ml of ammonium nitrate solution (40 g NH_4NO_3 l^{-1}) in varying intervals three times a year until 2020, resulting in an extra annual deposition of 40 kg N ha^{-1} $year^{-1}$. The control group logs of the experiment were sprayed with 5 ml of distilled water. N leaching was projected to amount to 2 to 3 g N $year^{-1}$ m^{-2} for the log area. Background N deposition is not included in this calculation and amounts to ca. 15 kg N ha^{-1} $year^{-1}$ in the study region (Schwarz et al., 2014). Additional information on the experimental setup can be found in Purahong et al. (2018).

2.2. Sample collection

The experiment was first launched in September 2011; the last sampling campaign took place in 2020. Since most of the deadwood logs were in late stages of decomposition by then, the experiment was removed afterwards.

Deadwood samples were collected during eight campaigns since 2011. Wood shavings were taken by drilling ca. 30 cm into the log with a wood auger (10 mm diameter, 450 mm length) and collected in sterile sample bags. To avoid cross-contamination, the auger was flamed and wiped down with ethanol in between drillings. Additionally, 10–15 cm sections of each log were cut for respiration rate measurements. Samples for DNA extraction were transported to the lab at 0 °C and stored at −20 °C until further processing. For incubation and respiration rate measurements, samples were transported and stored under cool conditions (~5 °C).

2.3. Mass loss

To calculate mass loss over the experimental run time, field moist weight of logs was measured at eight occasions using a laboratory scale. To determine the dry mass or water content of logs, fresh wood shavings were weighed directly after drilling, dry weight was measured after drying at 60 °C for a minimum of two days. Loss of woody mass by drillings of previous sampling campaigns was accounted for via calculation of mean volumes and masses of drilling holes and subtracting those values from dry weights of logs. Mass loss (Ml) was calculated as follows:

$$Mass\ loss\ [\%] = \frac{(dry\ weight_{2011}[kg] - dry\ weight_{2020}[kg])}{dry\ weight_{2011}\ [kg]} \times 100.$$

2.4. Respiration rate

Respiration of deadwood was measured under controlled conditions using a subsample as described in Groß et al., 2022. Samples were weighed into 350 ml glass jars and moistened with distilled water to achieve about 50 % of the maximum water holding capacity. Jars were closed with a screw cap with an integrated septum and rinsed for 30 min with synthetic air (20 % O_2 and 80 % N_2, Riessner Gase GmbH, Lichtenfels, Germany), followed by incubation in the dark at 20 °C for 48 h. Subsequently, gas samples of 100 µl were taken with a syringe 20 and 48 h after flushing with synthetic air and immediately measured with a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA, USA). The chromatograph was calibrated using certified gas standards (10,000 and 20,000 ppm CO_2, Riessner Gase GmbH, Lichtenfels, Germany). The respiration rate (µg C g^{-1} h^{-1}) was calculated using the following equation:

$$respiration\ rate = \frac{\Delta CO_2}{\Delta t} \times \frac{V_{gas}}{DW} \times \frac{P_{air}}{R \times T_{air}} \times M$$

where ΔCO_2 Δt^{-1} (ppm h^{-1}) is the change in CO_2 concentration during incubation, V_{gas} (m^3) is the gas volume of the incubation flasks, DW (g) is the dry weight of the deadwood sample, P_{air} (Pa) is the air pressure, R (8.314 J K^{-1} mol^{-1}) is the gas constant, T_{air} (K) is the air temperature, and M (g mol^{-1}) is the molar mass of C. After the incubation, wood samples were dried at 103 °C until mass constancy.

2.5. Enzymatic activities

We measured the activities of five extracellular enzymes, three of which are associated with oxidative lignin modification and two with hydrolytic polysaccharide degradation (i.e. of cellulose and hemicelluloses) in the aqueous extracts of the drilling samples with three technical replicates per wood sample. Laccase (EC 1.10.3.2), manganese peroxidase (EC 1.11.1.13) and manganese-independent peroxidases [EC 1.11.1.7/14/16, Kellner et al., 2014] were measured spectrophotometrically with ABTS [2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid)] as a substrate following the protocols of Arnstadt et al. (2016a) and Leonhardt et al. (2019). Manganese-independent peroxidase activities encompass an unspecific summarizing peroxidase measure, i.e. lignin peroxidase (EC 1.11.1.14), versatile peroxidase (EC 1.11.1.16), phenol-oxidizing peroxidases (EC 1.11.1.7) and dye-decolorizing peroxidase (EC 1.11.1.19). For the activities of endocellulase (EC 3.2.1.4) and endoxylanase (EC 3.2.1.8), AZO-cellulose and AZO-xylan were used as substrates following the protocol of Megazymes (Bray, Ireland) adapted from Větrovský et al. (2011). All measurements were carried out on a TECAN Infinite 200 (Tecan Group, Männedorf, Switzerland) using single-use microtiter 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany).

2.6. Wood chemistry

For the extraction, samples were milled (IKA11, IKA Werke GmbH Co.KG, Staufen, Germany) with the addition of 2 parts dry ice to avoid heating. 3 g of wood samples were used for aqueous extraction using 30 ml distilled H_2O and were shaken for 2 h at 180 rpm and 6 °C (Certomat MOII, Sartorius AG, Göttingen, Germany). The extracts were filtered (Sterilflip, 10 µm, Merck Millipore, USA) and centrifuged for 10 min at

15,000 rpm and 10 °C. The pH was measured in these aqueous extracts with a pH meter.

C and N concentrations in milled deadwood samples were analyzed using a Vario Max CN element analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) at the Department of Soil Ecology, University Bayreuth (Germany) and the Institute for Bioanalysis, University of Applied Sciences and Art Coburg. To determine dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), milled deadwood samples were weighted into 100 ml PE bottles, using 1.5 g for angiosperm and 2 g for gymnosperm tree species. 15 ml 0.05 M K_2SO_4 (Chemsolute, Th. Geyer GmbH & Co.KG, Renningen, Germany) were added and all bottles were shaken overhead for 60 min at 20 °C. The extract was then filtered at 5 °C using cellulose acetate filters (0.45 µm, Sartorius Stedim Biotech GmbH, Göttingen, Germany). Samples were diluted with distilled water at a 1:1 ratio, and sent to the Analytical Chemistry Lab, University Bayreuth (Germany) for the analysis of DOC and DON using a multi N/C 2100 TOC analyzer (Analytik Jena GmbH, Jena, Germany).

Klason lignin content and acid-soluble lignin in milled deadwood samples (planetary ball mill Pulverisette 7, Retsch, Idar-Oberstein, Germany, for 5 min at 5650 rpm) were measured as described in Arnstadt et al. (2016a) following aqueous extraction and drying at 60 °C. Briefly, Klason lignin content was determined gravimetrically after sequential hydrolysis using 72 % sulfonic acid at 30 °C for 1 h and 2.4 % at 120 °C for 1 h. Acid soluble lignin was measured via UV absorbance of the hydrolysate at 205 nm ($\epsilon = 100$ g cm $^{-1}$).

2.7. DNA extraction, PCR and amplicon sequencing

Wood samples were milled to a fine powder for DNA extraction using an analytical mill (Mixer mill MM400, Retsch, Idar-Oberstein, Germany) with the addition of dry ice. DNA was isolated using 0.2 g milled wood samples and the Quick-DNA Fecal/Soil Microbe Kit (Zymo Research, Irvine, USA) following the manufacturers protocol. For amplification of the fungal ITS2 region, we used of the primer pair fITS7 (forward) and ITS4 (reverse) (Ihrmark et al., 2012) as described in Leonhardt et al., 2019. The bacterial 16S region was amplified using the primers 515F (forward) and 806R (reverse) (Caporaso et al., 2012) as described in Moll et al., 2018. Briefly, target PCRs were performed in triplicates in 25 µl reactions using 12.5 µl DreamTaq MasterMix (ThermoFisher Scientific, Waltham, USA), 1 µl of each primer (10 pmol) and ca. 20 ng template DNA. Cycling conditions for ITS PCR included denaturation for 5 min at 95 °C, 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min 15 s and a final elongation at 72 °C for 10 min. For 16S, the cycling protocol included the following steps: denaturation for 3 min at 94 °C, 32 cycles: 94 °C for 45 s, 50 °C for 1 min, 72 °C for 1 min 30 s and final elongation 72 °C for 10 min. After pooling of the technical replicates PCR products were purified (PCR Purification Kit, Analytik Jena GmbH & Co. KG, Jena, Germany) and quantified via fluorescence spectrophotometry (Cary Eclipse, Agilent Technologies, Waldbronn, Germany). In preparation for sequencing, an Index PCR was performed using the Nextera XT Library Preparation Kit (Illumina, san Diego, USA) and the protocol described in Moll et al. (2021). Final libraries were sequenced on an Illumina MiSeq platform at the Department of Soil Ecology of the Helmholtz Centre for Environmental Research (UFZ), Halle (Saale). All raw sequences were submitted in the SRA of NCBI under accession number PRJNA379275.

2.8. Bioinformatics

Raw read FASTQ files were processed using the linux-based snakemake implementation dadasnake (Weißbecker et al., 2020, version 0.10.3) of the DADA2 algorithm (Callahan et al., 2016; version 1.14). Reads were searched for both primer sites and primer sequences using cutadapt (Martin, 2011, version 4.1). Denoising, error estimation, chimera removal and merging were all performed using DADA2. For bacterial communities, trunc length was set to 170 base pairs (bp) for forward and 130 bp for reverse reads with a minimum trunc quality (truncQ) of 13 and a maximum expected error (maxEE) of 0.2. Merging was performed using a minimum overlap of 12 bp with no mismatches allowed. For fungal communities, these properties were set to a minimum trunc quality of 15 bp and a maxEE value of 3. ITS reads were merged using a 20 bp minimum overlap with two mismatches allowed. According to current best practices (Brandt et al., 2021), amplicon sequence variants (ASVs) as constructed by DADA2 were then clustered into operational taxonomic units (OTUs) using a 97 % similarity cutoff with VSEARCH (Rognes et al., 2016). Taxonomic classification was performed using mothur (Schloss et al., 2009) and its Naive Bayesian classifier combined with UNITE (Abarenkov et al., 2022; version 9.0) and SILVA (Quast et al., 2013; Yilmaz et al., 2014; release 138) as reference databases for fungal ITS and bacterial 16S sequences respectively. In cases with uncertain results (e.g. species that are unlikely to appear in Germany), sequences were searched manually against the NCBI non-redundant nucleotide sequence database using blastn (excluding environmental sample, metagenomes or unidentified organisms). In a final filtering, 16S sequences with less than a minimum length of 245 bp and >275 bp length were omitted to filter out mitochondrial and plastid sequences. For functional annotation of fungal taxa, we used the FungalTraits database (Põlme et al., 2020).

2.9. Statistical analysis

Data processing, visualization and statistical tests were conducted in R (R Core Team 2021, Version 4.2.3) using RStudio (Posit Software 2023, Version 2023.03.0 + 386). Normality and homoscedasticity of all variables were checked via Shapiro-Wilk- and Fligner-Killeen's tests. Since no variables met the requirements for parametric testing, we tested for differences in wood physico-chemical parameters, mass loss and respiration rate at the end of the experiment via Kruskal-Wallis-Tests as a non-parametric variance analysis at $\alpha < 0.05$. Correlations were checked using Kendall's *tau* rank correlation ($\alpha = 0.05$).

To model the impact of elevated N availability on mass loss over time, we fitted a linear mixed effects model (LMM) using restricted maximum likelihood (REML) and the *lme4* R package (Bates et al., 2015) based on the commonly used model for deadwood decomposition first proposed by Olson (1963):

$$y_t = y_0 \times e^{-kt}$$

where t is the timeframe and k is the decay constant, which we calculated according to the formula mentioned in the same publication (Olson, 1963; Harmon et al., 1986; Arnstadt et al., 2016b). Edelmann et al. (2023) suggest linear and sigmoid models as better options for modeling deadwood mass loss. We decided to use Olson's formula due to the unusual log size and the few environmental parameters available for the entire experiment run time as well as the artificial set up, which prevented soil contact. The model was fitted to the entire available mass loss data (t_0/2011 – t_7/2020). We chose LMMs to account for the violation of the assumption of independence by repeated measurements of each log. Fixed effects included treatment (two-level factor with C = control and N = treatment) and time (days). Log ID and tree species were set as a nested random effect (1|tree species/Log ID) to allow intercepts to vary between tree species and to account for repeated measurements of individual logs. The distribution of residuals was checked graphically using QQ- and scatterplots (residuals vs. predicted values, residuals vs. fitted values). Conditional and marginal R^2 were calculated using the *r.squaredGLMM* function (MuMIn package, Barton, 2022) and the significance of model terms was tested using a X^2 test (function *drop1* with test = *"Chisq"* from the same R package). A summary table for the model was extracted using the *tab_model* function provided in the R package sjPlot (Lüdecke, 2022). We decided against splitting the data set into angiosperm and gymnosperm species for modeling due to the relatively

low sample size in gymnosperms.

Before further microbial community analysis, OTUs assigned to mitochondria, chloroplasts, eukaryotes and Archaea were filtered from the bacterial dataset. Some samples had to be removed (KB107_C and KB107_N) due to unclear labeling. For further analysis, both OTU tables were transformed to relative abundances using the function *decostand* from the R package vegan (Okasanen et al., 2019) (except for calculation of α diversity).

To gain insight into community structures under long-term elevated N deposition, we conducted a Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance on both tree species and clade level for fungal and bacterial communities using the functions *vegdist* and *wcmdscale* (vegan package). Scores were extracted using *scores* from the same package for visualization using ggplot2 (Wickham, 2016). Additionally, we tested whether the treatment had a significant impact on community composition using a Permutational Analysis of Variance (PerMANOVA) at 999 permutations using the function *adonis2* (vegan package, Okasanen et al., 2019).

We performed Differential Abundance Analysis (DAA) using the R package DESeq2 (Love et al., 2014) based on Wald's test after applying a prevalence threshold of 10 % and centered log-ratio transformation (CLR) at $\alpha = 0.05$ using control (C) as reference level.

To test for differences in alpha diversity measures, we calculated Shannon's indices for both bacterial and fungal communities based on OTU tables rarefied to an even sequencing depth of 10,000 reads using the function *rarefy_even_depth* from the package phyloseq (McMurdie and Holmes, 2013) and tested using Kruskal-Wallis tests on clade and tree species levels (Fig. 1). Furthermore, we tested for changes in relative abundances of fungi that are associated to different rot types (i.e. white rot, brown rot) and growth forms on the genus level (i.e. filamentous mycelium, yeast) using Wilcoxon rank sum test ($\alpha = 0.05$) on the relative abundances OTU table and information provided by the FungalTraits database (Põlme et al., 2020).

For data visualization and general data preparation, we used the tidyverse family of R packages (Wickham, 2016).

3. Results

3.1. Mass loss and respiration rate

Chronically enhanced N deposition affected deadwood mass loss, but the effect varied depending on tree species. Mass loss (abbreviated as Ml in this section) increased in gymnosperms (i.e. *Pseudotsuga*, *Pinus, Picea, Larix*) by about +38 % under elevated N deposition (Ml_C: 38.15 ± 18.64

Table 1
Mean mass loss (± SD) of untreated (control) and treated (N addition) deadwood of 13 temperate tree species after nine years, and mean differences between control and treatment. *P*-values ($\alpha = 0.05$; italic script: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$).

Tree species	Control mass loss [%]	N addition mass loss [%]	Difference [%]	p-Value
Angiosperms				
Acer	64.58 ± 19.6	63.85 ± 16.02	−1.13	1
Betula	65.58 ± 13.09	78.76 ± 10.57	+20.09	0.12
Carpinus	79.23 ± 9.91	76.52 ± 16.85	−3.42	0.75
Fagus	79.04 ± 10.43	60.17 ± 17.21	−23.88	*0.07*
Fraxinus	42.94 ± 7.07	38.70 ± 7.78	−9.88	*0.08*
Populus	59.94 ± 17.58	57.42 ± 21.63	−4.2	0.6
Prunus	64.47 ± 37.88	62.22 ± 26.06	−3.48	0.81
Quercus	38.05 ± 7.42	36.97 ± 8.26	−2.83	0.47
Tilia	65.08 ± 19.38	62.42 ± 18.52	−4.09	0.92
Total	62.33 ± 20.67	58.88 ± 20.61	−5.53	0.44
Gymnosperms				
Larix	40.91 ± 13.83	44.99 ± 10.58	+9.99	0.75
Picea	53.80 ± 15.35	64.33 ± 12.48	+19.58	0.35
Pinus	20.85 ± 15.35	37.79 ± 10.66	+81.25	*0.08*
Pseudotsuga	37.06 ± 17.24	63.25 ± 21.77	+70.65	*0.08*
Total	38.15 ± 18.64	52.59 ± 17.86	+37.84	**0.04***
All	54.65 ± 22.90	56.91 ± 19.87	+4.14	0.67

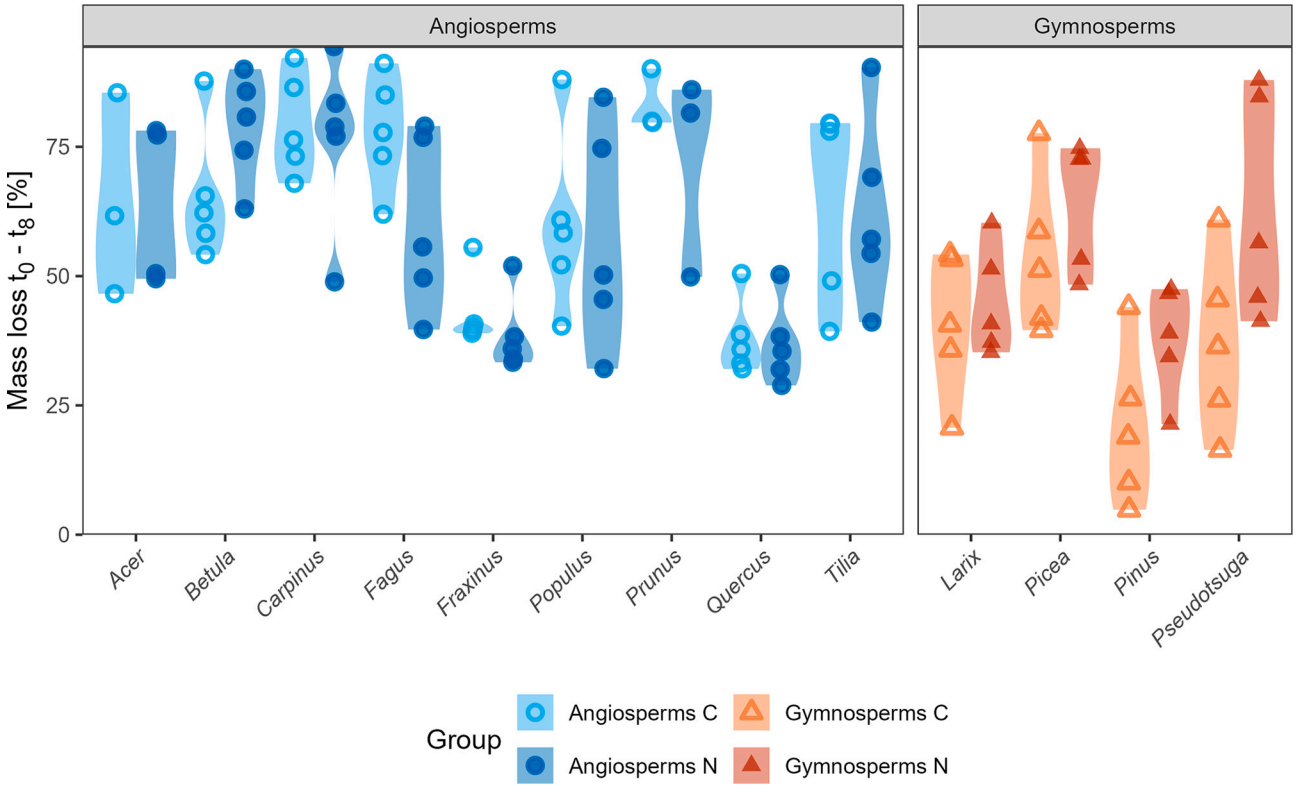

Fig. 1. Mass loss in thirteen temperate tree species after nine years of exposure to artificially elevated N deposition (+40 kg ha^{-1} $year^{-1}$).

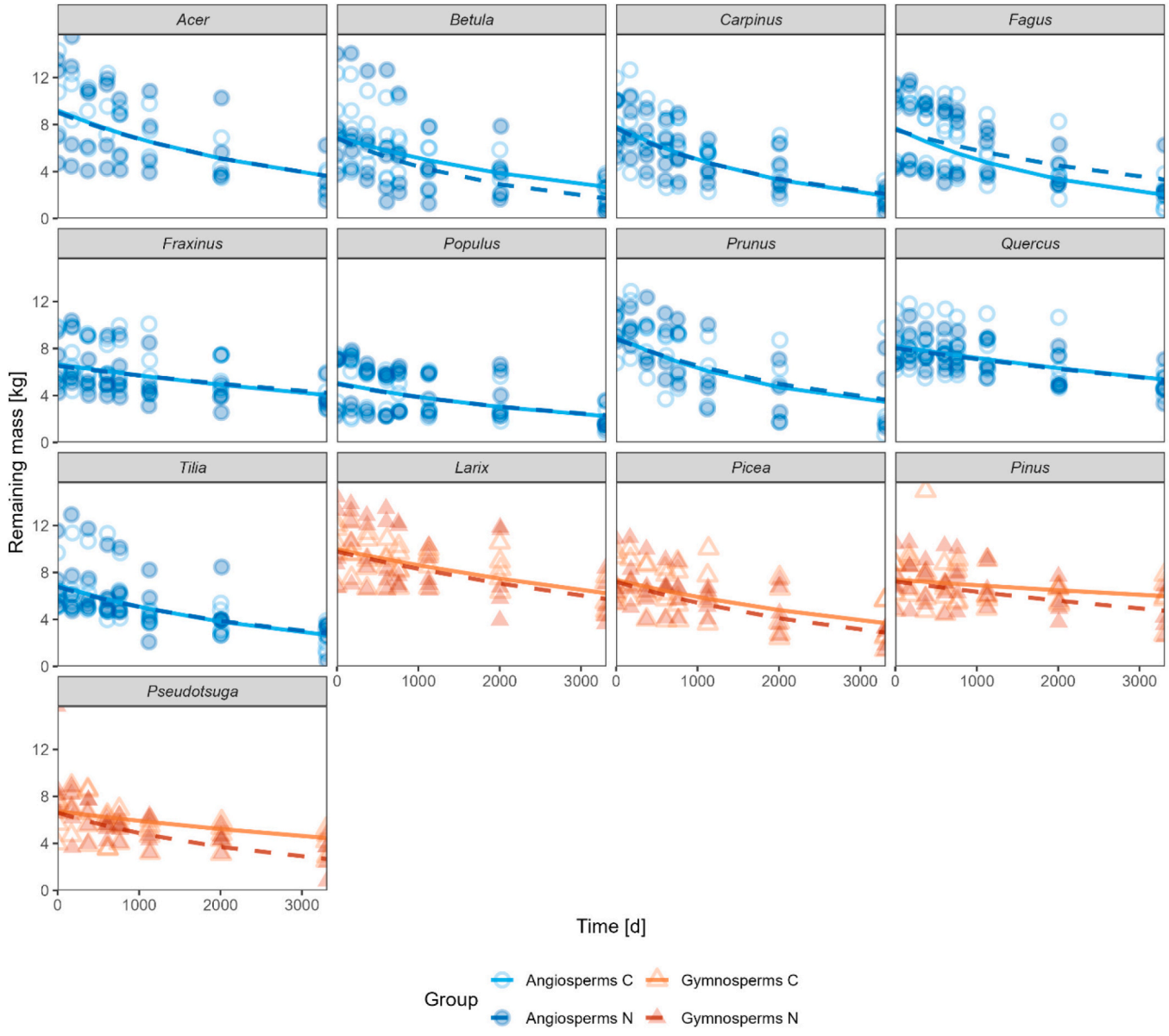

Fig. 2. Mass loss in deadwood of 13 temperate tree species without treatment (C) or with N addition of 40 kg ha^{-1} $year^{-1}$ (N) over a nine-year period, using an exponential model (Olson, 1963).

%; Ml_N: 52.59 ± 17.86 %, Table 1, Fig. 2, $p_{Gymno} = 0.04$; absolute mass loss difference after nine years: 14.4 %). Differences in mass loss were marginally significant in *Pseudotsuga* (Ml_C: 37.06 ± 17.24 %, Ml_N: 63.25 ± 21.77 %, $p_{Pseudotsuga} = 0.08$) and *Pinus* (Ml_N: 20.85 ± 15.35, Ml_C: 37.79 ± 10.66, $p_{Pinus} = 0.08$), in the case of which N led to an increase in mass loss of +71 % and + 81 %, respectively (Table 1).

In angiosperms (i.e. *Acer*, *Betula*, *Carpinus*, *Fagus*, *Fraxinus*, *Populus*, *Prunus*, *Quercus*, *Tilia*), elevated N deposition led to an overall decrease in mass loss of −5.5 %, however, this difference was not statistically significant ($p_{Angio} = 0.439$). In *Fagus* logs, we detected a marginally significant decrease of −24 % ($p_{Fagus} = 0.07$) as well as in mass loss (−10 %) in *Fraxinus* logs (Ml_C: 42.94 ± 7.07, Ml_N: 38.70 ± 7.78, $p_{Fraxinus} = 0.08$).

Our model with considerable explanatory power (marginal $R^2 = 0.51$, conditional $R^2 = 0.922$) did not show a significant impact of the fixed factor treatment ($\beta = -0.02$, confidence interval = −0.14–0.10, $p = 0.78$) on mass loss over the experiment run time and all logs (Table 2, Fig. 2). Half-life did not differ between control and treatment when compared across all logs (Kruskal-Wallis test, $p = 0.76$) and we did not observe significant differences in angiosperms ($p = 0.11$). In gymnosperms, artificial N deposition led to a significant decrease in log half-life ($p = 0.04$, Fig. S4).

Kruskal-Wallis tests revealed marginally significant ties of treatment to an increased respiration rate in gymnosperm logs ($p_{Gymno} = 0.08$, Table 3, Fig. 3). Respiration correlated positively with overall mass loss in both angiosperms and gymnosperms, however, this relationship was only significant in angiosperms ($p < 0.01$ for treatment and control) (Fig. S1). Overall, respiration rates increased by +37 % in gymnosperms under elevated N availability, highest with +65.5 % increase in *Pinus* and lowest with +15.6 % increase in *Pseudotsuga* (Table 3). Respiration rates in angiosperms were highly variable and did not show a clear response to the N treatment (Table 3, Fig. 3).

Table 2
Linear mixed effects model summary; k: decay rate; treatment: two level factor, baseline = control; τ_{00}: estimates of random effects variation; ICC: interclass correlation coefficient ($\alpha = 0.05$; italic script: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Predictors	log(mass loss)		
	Estimates	Confidence interval	p
(Intercept)	2.07	1.92–2.15	<0.001 ***
k * days	0.00	0.00–0.00	<0.001 ***
Treatment [N]	−0.02	−0.14–0.10	0.782
Random effects			
σ^2	0.02		
$\tau_{00\ Log\ ID:tree\ species}$	0.11		
$\tau_{00\ tree\ species}$	0.02		
ICC	0.84		
Observations	1016		
Formula (R Syntax)	ln(Mass loss) ~ (k * Days) + C/N + (1|tree species/Log ID)		

3.2. Enzymatic activities and wood chemistry

Extracellular enzyme activities measured were highly variable as illustrated by the high standard deviations (Table S1). Of five enzymatic activities we measured, only laccase activities were significantly higher under artificial N deposition in gymnosperms with an increase of up to +1219 %, whereas they did not differ in angiosperms (Fig. 4, $p_{Gymno} = 0.02$, $p_{Angio} = 0.98$). In gymnosperms, this seems to be mainly driven by *Pseudotsuga* samples, where the differences between control and N-treatment samples were substantial ($laccase_C$: 2.13 ± 4.77 mU g^{-1}; $laccase_N$: 41.40 ± 59.68 mU g^{-1}, +1844 %, Fig. S2). We also observed increases in laccase activity in the other gymnosperm species (Table S1). Regarding the other four enzymes we measured, we did not observe any significant changes in activity levels (Table S1).

Artificial N deposition significantly lowered C:N ratios in both gymnosperm and angiosperm logs (gymnosperms: $C:N_C$: 595 ± 294, $C:N_N$: 373 ± 210, $p_{Gymno} = 0.02$; angiosperms: $C:N_C$: 231 ± 124, $C:N_N$: 166 ± 106, $p_{Angio} = 0.04$, Fig. S3). On average, C:N ratios decreased by −37 % in gymnosperms and − 28 % in angiosperms. N concentrations increased by +78 % in gymnosperms (N $concentr._C$: 0.109 ± 0.07 %, N $concentr._N$: 0.194 ± 0.143 %) and + 29 % in angiosperms (N $concentr._C$: 0.309 ± 0.24 %, N $concentr._N$: 0.398 ± 0.24 %).

C content was significantly higher ($p_{Angio} = 0.03$) in angiosperm treatment logs (C_C: 46.3 ± 0.85 %, C_N: 46.83 ± 1.05 %), however, this difference is quite small (+1.2 %), while for gymnosperms C content did not differ. We did not observe differences for DOC, but similar to N content, gymnosperms contained +99 % more DON (DON_C: 0.06 ± 0.15 mg g^{-1}, DON_N: 0.12 ± 0.09 mg g^{-1}, $p_{Gymno} = 0.002$) and angiosperms +79 % more DON (DON_C: 0.16 ± 0.18 mg g^{-1}, DON_N: 0.29 ± 0.25 mg g^{-1}, $p_{Angio} = 0.001$) under elevated N deposition. Water content, pH and Klason and acid-soluble lignin content did not differ between treatments, neither on tree species nor on tree clade level (Table S1, Fig. S3).

3.3. Community composition

3.3.1. Fungi

A total of ca. 17 million sequences resulted in 1522 ASVs after quality trimming, filtering and chimera removal, which were then clustered into 879 OTUs. Most abundant OTUs regardless of treatment groups include OTU_0065 (*Mycena galericulata*, gymnosperms: 3.6 %, angiosperms: 6.6 %), OTU_0233 (*Hyphodontia pallidula*, gymnosperms: 2.1 %) and OTU_0237 (*Phlebia livida*, angiosperms: 4.7 %) (Table S3).

Overall, we did not detect significant changes in fungal communities on the tree clade level (Table 4). A PerMANOVA revealed weak treatment effects on fungal community composition in *Acer* (Table 4, p =

Table 3
Mean respiration rates (± standard deviation) of 13 temperate tree species after nine years of elevated N deposition, difference between control (C) and treatment (N) and p-values ($\alpha = 0.05$; italic script: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$).

Tree species	Control mean respiration [μg C g^{-1} h^{-1}]	N addition mean respiration [μg C g^{-1} h^{-1}] ± SD	Difference [%]	p-value
Angiosperms				
Acer	27.47 ± 15.4	19.91 ± 6.84	−27.49	0.48
Betula	14.73 ± 7.21	22.56 ± 7.79	+53.17	0.12
Carpinus	24.12 ± 9.86	24.19 ± 6.15	+0.30	0.62
Fagus	18.57 ± 7.78	15.63 ± 4.95	−15.81	0.58
Fraxinus	7.28 ± 1.84	9.62 ± 5.76	+32.18	0.92
Populus	18.90 ± 10.25	15.63 ± 4.45	−17.32	0.81
Prunus	23.78 ± 5.23	11.85 ± 4.53	−50.18	**0.02***
Quercus	5.71 ± 4.38	6.21 ± 4.72	+8.70	0.92
Tilia	16.66 ± 9.85	22.28 ± 9.59	+33.76	0.18
Total	16.66 ± 10.04	16.24 ± 8.35	−1.28	0.81
Gymnosperms				
Larix	5.35 ± 1.78	6.69 ± 3.28	+30.59	0.47
Picea	8.08 ± 1.53	11.29 ± 3.12	+39.70	0.17
Pinus	6.56 ± 1.61	10.86 ± 4.80	+65.63	0.18
Pseudotsuga	7.71 ± 3.42	8.91 ± 4.78	+15.58	0.75
Total	6.92 ± 2.32	9.51 ± 4.13	+37.37	*0.08*
All	14.22	14.2	−0.16	0.31

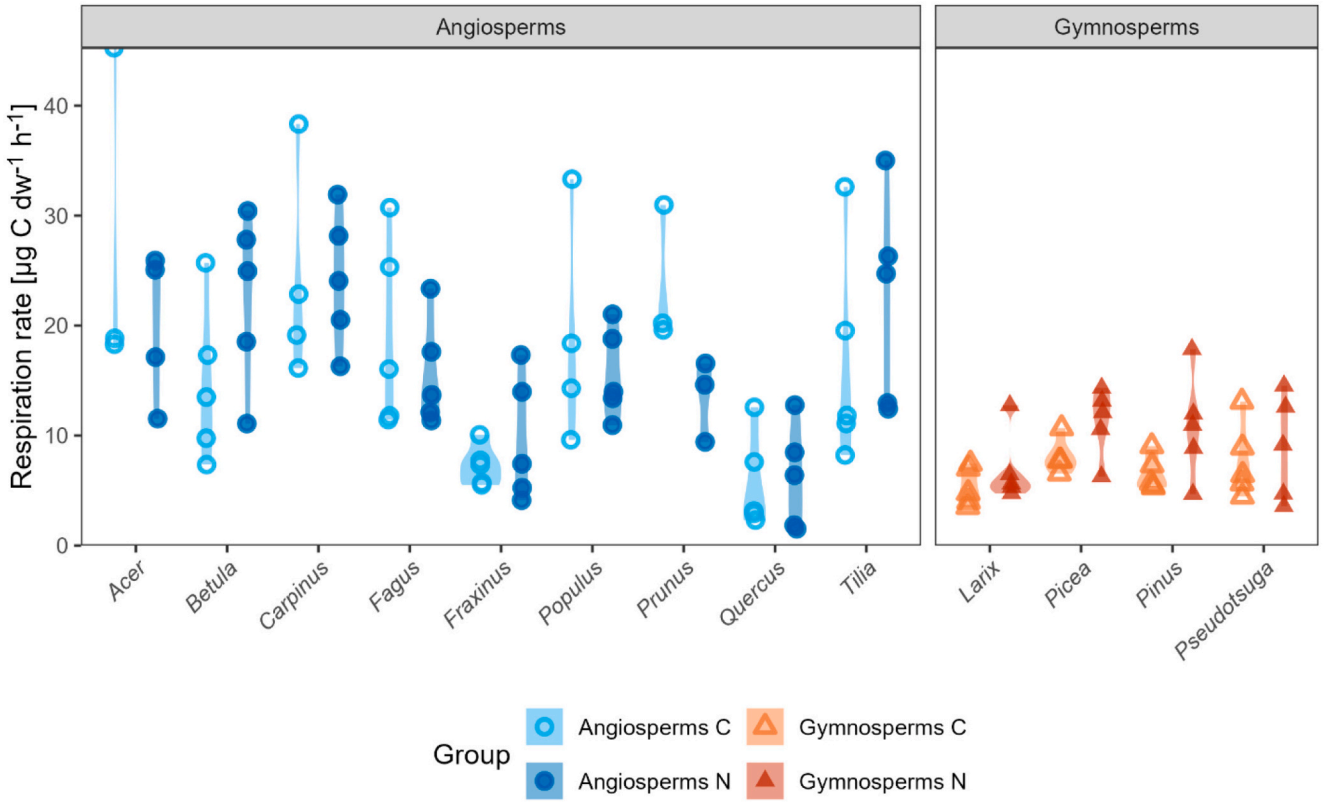

Fig. 3. Respiration rate in thirteen temperate tree species after nine years of exposure to +40 kg ha^{-1} $year^{-1}$ elevated N deposition.

0.03, $R^2 = 0.25$). PCoA of fungal communities separated into samples from angiosperm and gymnosperm tree species shows no clear separation between treatment and control groups (Fig. 5).

We did not observe any changes in species richness or Shannon's index due to enhanced N deposition on the tree species or clade level. Using a Wilcoxon rank sum test for assessing differences in relative abundances of taxa associated to different rot types, we detected an +89 % increase in abundance of white-rot fungi in coniferous tree species from 6.1 % in control logs to 11.5 % in treatment logs ($p = 0.03$, Fig. 6). This effect did not translate to the tree species level. These changes can mainly be attributed to increases in abundance of *Hyphodontia pallidula* (30.5 %) and *Resinicium bicolor* (8.4 %) in logs exposed to enhanced N deposition in *Picea*, which are both not present in control logs and an increase in abundance of *Hypochnicium* sp. from 2.7 % to 9.6 % under elevated N deposition in *Pinus*.

Using DAA, we observed changes in centered log-ratio abundances of 10 OTUs across seven tree species (Table S3), but without any consistent trend and no re-occurring OTUs. The abundance of two fungal OTUs decreased significantly under elevated N deposition (OTU_0050 *Helicogloea insularis* in *Fraxinus* samples, log2fold change −23.3, and OTU_0711 *Dermateaceae* in *Larix* samples, log2fold change −11). Eight fungal OTUs increased in abundance (most notably OTU_0567 *Leptodontidium* ssp. in *Larix*, log2fold change +24.6, and OTU_0109 *Mycena haematopus* in *Tilia*, log2fold change +24.3). Using FungalTraits, we classified seven OTUs as predominantly wood or litter saprotrophs, except for OTU_0175 (*Athelia bombacina*), which is characterized as a lichen parasite and/or fungal decomposer. In samples of *Carpinus*, *Pinus*, *Populus*, *Prunus*, *Pseudotsuga* and *Quercus*, we did not observe any significant changes in abundances of single OTUs.

3.3.2. Bacteria

17,864 ASVs were constructed from quality filtered and trimmed reads, which were clustered into 6655 OTUs. After a second filtering to discard OTUs that were assigned to either chloroplast or mitochondria, 6514 OTUs remained for further analysis. Most abundant OTUs across the dataset include OTU_00685 (*Roseiarcus*, gymnosperms: 4.4 %, angiosperms: 2.3 %, putative methanotrophic), OTU_00681 (*Bradyrhizobium*, gymnosperms: 4.4 %, angiosperms: 5.2 %, associated with nitrogen fixation) and OTU_00683 (*Burkholderia*, gymnosperms: 4.3 %, angiosperms: 4.4 %, associated with nitrogen fixation). We also detected notable abundances of methanotrophic, i.e. methylotrophic bacteria, notably OTU_00701 (*Methylovirgula*, gymnosperms: 3.2 %, angiosperms: 2.8 %, Vorob'ev et al., 2009) and OTU_00684 (*Methylocystis*, angiosperms: 2 %).

Using a PerMANOVA, we found a marginally significant treatment effect on bacterial community composition in gymnosperm deadwood only, although treatment did explain a low amount of variation (Table 4, $p = 0.06$, $R^2 = 0.03$). Corresponding to our findings for fungal communities, PCoA of bacterial communities did not show a clear separation between treatment and control groups as well (Fig. 5).

Alpha diversity did not change on either tree species or clade level in either Shannon's index or observed species richness except for *Populus* samples (Shannon's index, $p = 0.03$, Table S4).

Applying DAA to this dataset, we identified a total of 28 OTUs whose abundance changed significantly between control and treatment communities in 10 tree species (Table 5), however, only two of those (OTU_00763, Genus *Actinospica* and OTU_00901, Genus *Legionella*) reoccurred in two different tree species. In total, abundances of 16 OTUs significantly increased under elevated N deposition while it decreased for twelve OTUs. We did not record any significant changes in bacterial abundances in *Fraxinus*, *Larix* and *Picea* samples.

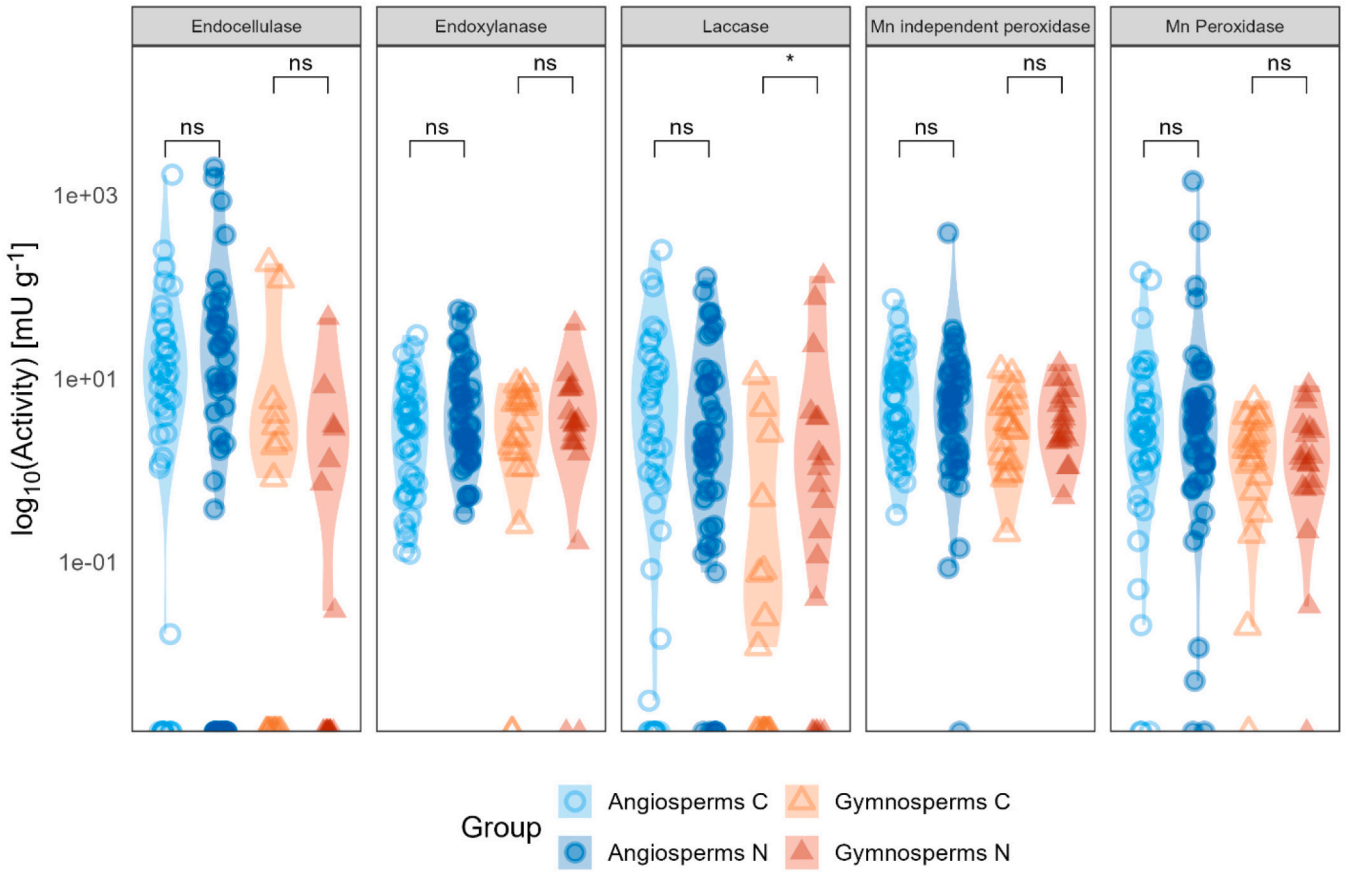

Fig. 4. Enzymatic activities in thirteen temperate tree species after nine years of +40 kg ha^{-1} year^{-1} elevated N deposition; parantheses: Differences in activity tested using Kruskal-Wallis tests, α = 0.05; *: p < 0.05, **: p < 0.01, ns: not significant).

Table 4
PerMANOVA results for fungal and bacterial communities in relation to treatment; Df = Degrees of freedom, SSQ = Sum of squares (α = 0.05; italic script: p < 0.10, *: *p* < 0.05, **: *p* < 0.01).

Tree species	Df	Fungi				Bacteria			
		SSQ	R^2	F	Pseudo F	SSQ	R^2	F	Pseudo F
Acer	1	0.64	0.25	1.65	**0.03** *	0.35	0.22	1.4	0.09
Betula	1	0.46	0.13	0.21	0.23	0.17	0.11	1.01	0.42
Carpinus	1	0.35	0.13	1.16	0.29	0.15	0.08	0.74	0.72
Fagus	1	0.35	0.1	0.87	0.68	0.18	0.09	0.79	0.78
Fraxinus	1	0.27	0.1	0.91	0.59	0.16	0.09	0.77	0.85
Populus	1	0.27	0.08	0.65	0.94	0.15	0.076	0.58	0.91
Prunus	1	0.36	0.16	0.76	0.5	0.14	0.13	0.57	0.7
Quercus	1	0.3	0.1	0.85	0.5	0.26	0.14	1.26	0.15
Tilia	1	0.2	0.03	0.49	0.91	0.22	0.11	0.1	0.42
Angiosperms	1	0.34	0.01	0.77	0.83	0.32	0.02	1.28	0.16
Larix	1	0.24	0.08	0.7	0.85	0.18	0.01	0.89	0.59
Picea	1	0.47	0.15	0.35	0.18	0.33	0.25	2.6	**0.02** *
Pinus	1	0.49	0.14	1.31	0.13	0.17	0.14	1.32	0.14
Pseudotsuga	1	0.17	0.66	0.57	0.1	0.12	0.11	0.95	0.64
Gymnosperms	1	0.5	0.03	1.21	0.22	0.23	0.04	1.42	*0.06*

4. Discussion

To examine the impact of elevated N levels on deadwood decomposition, we investigated samples from deadwood logs of 13 temperate tree species exposed to artificial nitrogen levels of +40 kg ha^{-1} year^{-1} over a nine-year period in a temperate forest field experiment. Our model did not show a significant impact of elevated N levels over time across all species, which can be attributed to high variation between tree species and clades (angiosperms and gymnosperms) and comparatively low sample sizes. Nevertheless, we detected effects of chronically elevated N deposition at the time of the last sampling campaign. In gymnosperm deadwood, we observed enhancing effects of N on respiration rate and laccase activity and thus accelerated decay (i.e. mass loss) at the last sampling in 2020 accompanied by increases in

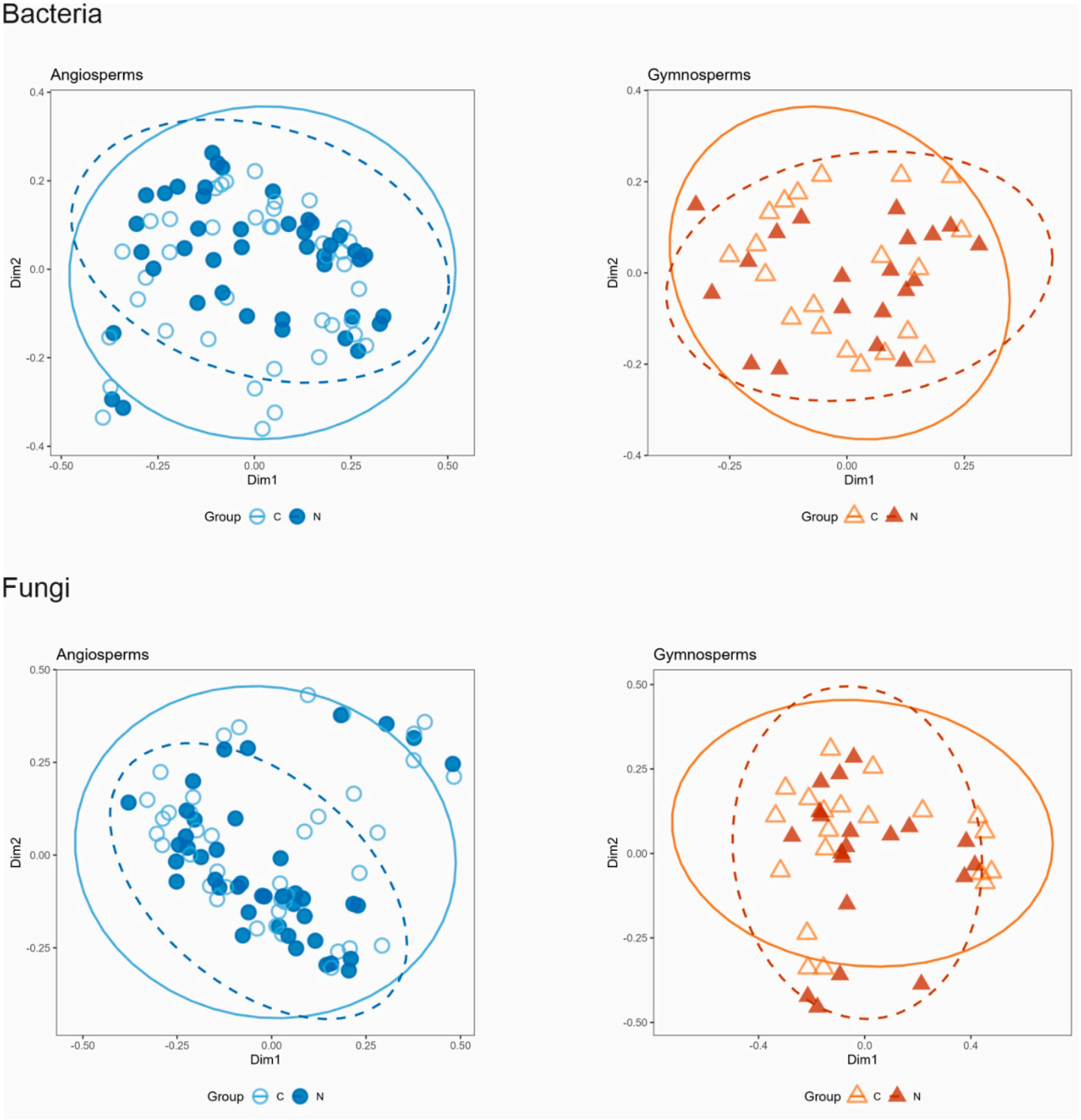

Fig. 5. Principal coordinates analysis (PCoA) displaying bacterial and fungal community structure after nine years of chronically elevated N deposition (solid lines: control, dashed lines: treatment).

abundance of white-rot fungi, but without significant shifts in community composition of neither bacteria (only marginal) nor fungi. Deadwood log half-life of all gymnosperm tree species was significantly reduced under enhanced N deposition. In deadwood of angiosperm tree species, the response was more heterogeneous, however none of our results proved to be statistically significant. While our results show tendencies for individual tree species responses to N addition, more biological replicates might be needed to be able to explore these effects further.

4.1. Alterations of mass loss in relation to fungal and bacterial community composition

We can consider our first hypothesis H1 that additional N would increase mass loss, enzymatic activities and microbial respiration confirmed based on the present results for gymnosperm deadwood only. We observed different effects depending on tree clade and tree species consistent with our expectations that tree species effects would persist under elevated N deposition. Our results indicate that N deposition has a

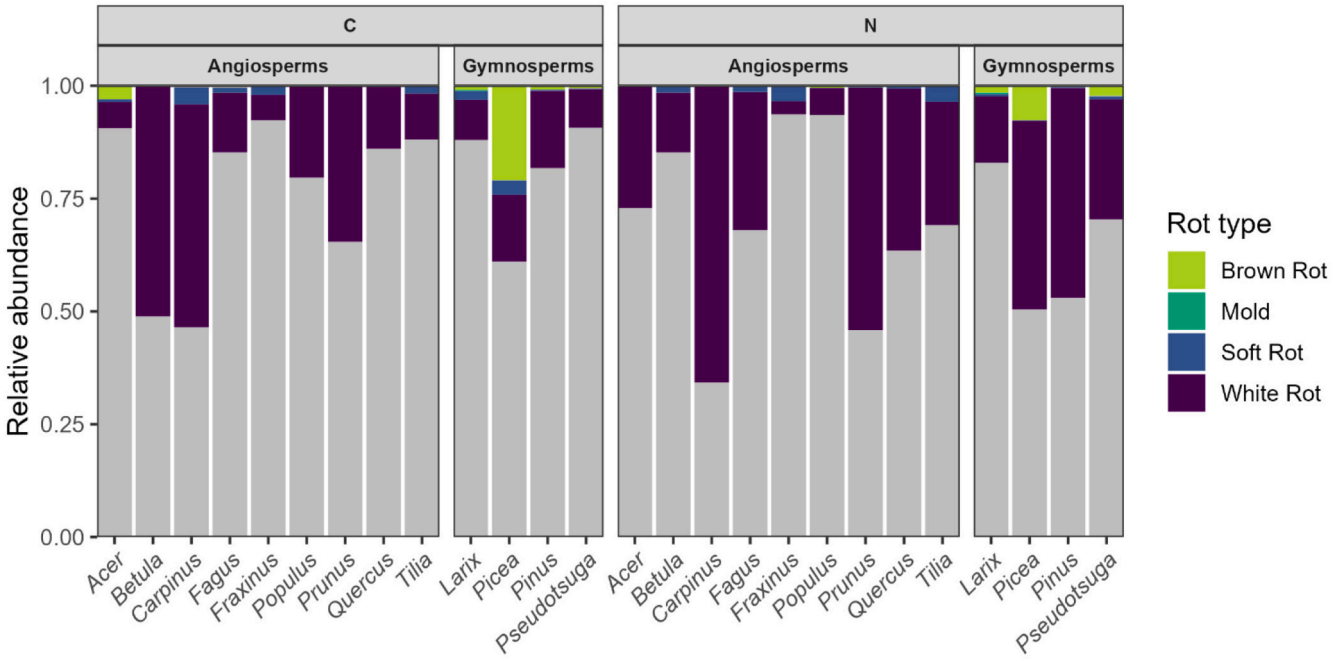

Fig. 6. Relative abundances of different rot types (assigned using FungalTraits) in fungal communities of 13 temperate tree species (grey color: No information available).

moderate long-term effect on gymnosperm deadwood in terms of physiology (increased microbial, i.e. fungal extracellular enzyme activity and respiration rate).

Regarding our H2, we did observe marginal changes in the bacterial community in gymnosperm deadwood only, partially confirming the hypothesis. While we did not find any significant shifts in fungal communities on either the tree species or tree clade level, we observed a significant increase (+89 %) in the abundance of white-rot fungi in gymnosperm deadwood, which can mostly be attributed to three corticoid fungal taxa who are more abundant under long-term elevated N deposition. Considering the significant differences in both DON and total N in the treated samples and the resulting lower C:N ratios, we concluded that the addition of ammonium nitrate resulted in higher N availability in the logs and thus altered the substrate quality and properties for the microbial community.

In gymnosperms, we found a long-term effect of elevated N availability resulting in +38 % higher overall mass loss accompanied by marginally significant higher respiration rates of +37 % and 12-fold higher laccase activities. The major contributors of the latter and other extracellular enzyme activities are fungi, particularly white-rot Basidiomycota (Lundell et al., 2014). We did record changes in abundances of white-rot fungi, which can be traced down to mostly three species (*Resinicium bicolor*, *Hypochnicium* sp., *Hyphodontia pallidula*) in gymnosperm deadwood, however, these did not translate to detectable effects on the total fungal community composition as illustrated by the results of our PerMANOVA and PCoA. Furthermore, those fungal species did not yield significant results in our DAA, making their shifts in abundance based on molecular techniques only difficult to interpret. Nevertheless, those increases could partially explain the higher mass loss we observed in gymnosperm deadwood, as all three species are known to be common white-rotters in central Europe (Krieglsteiner, 2000).

Our DAA revealed two fungal OTUs whose abundance significantly increased according to differential abundance analysis (*Athelia bombacina* and *Leptodontidium* spp.) in *Larix* and *Picea* samples, but those are unlikely to cause significant differences in mass loss alone. Changes in fungal and bacterial community composition have frequently been observed under artificially elevated N deposition (e.g. Lilleskov et al., 2019). Since we did not record substantial shifts of the fungal communities ($p = 0.22$) and only marginally of the bacterial communities ($p = 0.06$) on either tree species or tree clade level, we attribute the higher activities of laccase in our experiment (at least to some extent) to the improved N availability for the decomposers, i.e. for the fungal community in the wood substrate and increases in abundances of single species profiting from alleviated nutrient constraints.

In angiosperms, the effect of enhanced N availability was less pronounced, leading to increased mass loss i.e. in *Betula*, while mass loss was negatively affected or unaffected in deadwood of other tree species (*Fagus*, *Quercus*). This is reflected in the heterogeneous responses in respiration rates and enzymatic activities as well. On the tree species level, we saw higher but non-significant activities of endoxylanase and endocellulase (i.e. in *Betula*), which does match to effects of enhanced N deposition commonly observed in leaf litter and soil resulting in the promotion of cellulolytic enzymatic activities (Waldrop and Zak, 2006). We observed changes in abundances of only seven of altogether 879 fungal OTUs in angiosperm deadwood with for example a strong decrease in abundance of *Helicogloea insularis* in *Fraxinus* samples, suggesting that there might be individual reactions of fungal species towards elevated N deposition. However, we do not think that those single species could be responsible for large differences in mass loss and microbial activity in the analyzed logs.

Generally speaking, gymnosperm deadwood has higher C:N ratios than angiosperm deadwood (Bantle et al., 2014) and contains less nutrients (Arnstadt et al., 2016b). Our results are in line with H1, as fungal communities in gymnosperm deadwood are subjected to higher N limitation than their counterparts in angiosperm deadwood. However, we did not observe a decrease in lignin content with increasing laccase activity, which seems contradictory given the substantial mass loss in

Table 5
Results of differential abundance analysis, taxonomic information includes the last taxonomic rank assigned to an OTU, α = 0.05, adj. p = adjusted p-value.

Domain	Tree species	OTU	Base mean	Log2Fold Change	Adj. p	Taxonomic information
Fungi	*Acer*	OTU_0439	58.78	23.41	<0.01	*Annulatascales* spp.
		OTU_0801	46.93	9.47	<0.01	*Menispora manitobaensis*
	Betula	OTU_0154	1478.78	14.10	<0.01	*Trechispora stevensonii*
	Fagus	OTU_0410	27.47	22.91	<0.01	*Nectriaceae*
	Fraxinus	OTU_0050	53.13	−23.29	<0.01	*Helicogloea insularis*
	Larix	OTU_0576	166.82	24.64	<0.01	*Leptodontidium* spp.
		OTU_0711	116.86	−11.02	<0.01	*Dermateaceae*
	Picea	OTU_0175	155.69	10.72	<0.01	*Athelia bombacina*
	Tilia	OTU_0109	75.27	24.3	<0.01	*Mycena haematopus*
		OTU_0701	64.14	22.8	<0.01	*Phialocephala repens*
Bacteria	*Acer*	OTU_00681	2849.25	1.82	<0.01	*Bradyrhizobium*
		OTU_00740	244.13	5.82	0.05	*Haliangium*
		OTU_00763	94.65	22.36	<0.01	*Actinospica*
		OTU_00826	60.32	−9.41	<0.01	*Diplorickettsiaceae*
		OTU_00900	52.83	9.19	0.02	*Dokdonella*
		OTU_00927	71.84	9.64	0.01	*Myxococcaceae*
		OTU_01032	61.98	9.42	<0.01	*Micropepsaceae*
	Betula	OTU_00757	38.09	21.39	<0.01	*Methylorosula*
		OTU_00912	24.21	22.12	<0.01	*Burkholderiales*
	Carpinus	OTU_00763	20.97	22.28	<0.01	*Actinospica*
		OTU_00892	32.94	22.9	<0.01	*Diplorickettsiaceae*
		OTU_00899	39.84	23.17	<0.01	*Vermiphilaceae*
		OTU_00972	23.49	−22.43	<0.01	*Acetobacteraceae*
	Fagus	OTU_00767	141.92	24.46	<0.01	*Klebsiella*
		OTU_00790	39.63	−23.75	<0.01	*Diplorickettsiaceae*
		OTU_00841	37.67	−22.1	<0.01	*Aquicella*
	Larix	OTU_00901	104.75	−24.88	<0.01	*Legionella*
	Picea	OTU_00700	1799.37	3.94	<0.01	*Acidothermus*
		OTU_01099	43.56	−23.46	<0.01	Candidatus *Amoebophilus*
	Populus	OTU_01231	18.15	−22.36	<0.01	*Gammaproteobacteria*
	Pseudotsuga	OTU_00725	327.11	−9.65	0.04	*Halomonas*
		OTU_00716	1025.62	−5.49	0.04	*Delftia*
	Quercus	OTU_00719	200.91	4.02	0.01	*Rhodanobacter*
		OTU_00770	221.0	−26.07	<0.01	*Aeribacillus*
		OTU_01078	43.57	−23.85	<0.01	*Actinoplanes*
		OTU_01183	31.97	−23.44	<0.01	*Tetrasphaera*
	Tilia	OTU_00849	38.96	8.7	<0.01	*Acidicapsa*
		OTU_00901	31.63	−23.2	<0.01	*Legionella*
		OTU_00970	20.00	21.8	<0.01	*Acidobacteriales*
		OTU_00998	32.97	22.5	<0.01	*Parasegetibacter*
		OTU_01030	28.86	−23.1	<0.01	SM2D12_ge

gymnosperms. One possible explanation could be that the increased mass loss was an effect of enhanced N availability associated with the degradation of cellulose and hemicellulose by wood-decomposing fungi (more N for polysaccharide cleaving enzyme proteins), which in turn increased the relative content of lignin in the logs exposed to artificial N deposition. We interpret the increase in laccase activity not least in the context of an increased attack on lignin to detoxify fragments formed in the course of radical-mediated oxidation events (caused e.g. by peroxidases) that lead to the fission of bonds between lignin, hemicelluloses, and cellulose, making the latter two easier to metabolize by the microbial community, which in turn leads to more lignin remaining in logs exposed to elevated N deposition. Laccases are primarily enzymes that force coupling reactions between complex radical species so that this may be accompanied by increases in respiration rates pointing to intensified anabolic activity (i.e. sugar consumption) of the microbial communities. Still, we did not observe significant increases in the activity of endoxylanase and endocellulase under elevated N deposition, which is another commonly observed effect in experiments in soil and leaf litter (Carreiro et al., 2000). An explanation could be the high spatial variability of extracellular enzymatic activities even within a single deadwood log as shown by Arnstadt et al. (2016a). Indeed, in this study, few samples attained appreciable extracellular enzyme activities above 20 mU g^{-1} DM, and if so, many were observed in angiosperm logs. We observed the typical acidification of deadwood logs over time in both angiosperm and gymnosperm deadwood, however, there were no significant differences between treatment and control logs (Fig. S4). This might be unexpected, as acidification is one of the most cited effects of elevated N deposition in soils (Aber et al., 2003; Forsius et al., 2021). Decreases in pH are common in decomposing deadwood due to an increase in organic acids derived from the activity of wood-decaying fungi (both white- and brown-rot fungi cause substantial acidification; Arnstadt et al., 2016b) and further, pH plays a significant role in shaping bacterial communities in deadwood (Moll et al., 2018).

Compared to fungi, bacteria in deadwood have a higher demand for N due to being less able to permeate woody biomass and having fewer enzymes available to degrade it (Rajala et al., 2012). Therefore, we assumed that substantial changes in nutrient availability in deadwood would result in changes in bacterial community composition, however, we only observed minimal shifts. Some of the bacterial OTUs that yielded significant results in the DAA and increased under elevated N deposition possess hydrolytic capabilities (i.e. OTU_00970 *Acidobateriales* in *Tilia*, Dedysh and Oren, 2020) or are associated to nitrogen fixation (i.e. OTU_00912 *Burkholderiales* in *Betula,* OTU_00681 *Bradyrhizobium,* order *Rhizobiales* in *Acer,* Johnston et al., 2016). It is possible that these shifts in abundance are the result of alleviated nutrient constraints and the higher amount of available N in the substrate. However, given the large amount of bacterial OTUs in the whole data set and the comparatively small number of OTUs that did show

significant changes in abundance, we consider those to be more coincidental than expressions of a community shift.

4.2. Methodological constraints

Regarding the experimental setup, we want to address two methodological points that we would consider in the case of a follow-up study. First, we consider the boxes, while necessary for analyses of ^{15}N etc., to inhibit migration of fungal species between logs and soil, which would likely have an impact on the strength and direction of any community shifts due to significant changes in nutrient availability. Second, the doses of applied N deposition the experiment used would now be considered too high to be realistic (Bebber, 2021), as models and observational data forecast atmospheric N deposition above 25 kg ha^{-1} $year^{-1}$ only for heavily populated and industrialized regions of southeast China or India (Schwede et al., 2018). Although we are aware of these methodological constraints, we believe our study does add to current knowledge since it provides a first long-term insight into the effects of N deposition on a broad phylogenetic range of deadwood. In Central Europe, exceedance of critical loads of anthropogenic N deposition has become a political topic, i.e. in the Netherlands, where the government seeks to implement measures to reduce N output from agriculture and traffic in short-term (Stokstad, 2019).

Kahl et al. (2017) demonstrated that decay rates and subsequently residence time of deadwood can vary substantially between tree species. Compared to half-life calculations (Fig. S4) from Edelmann et al. (2023), who investigated the same set of tree species, some logs in our experiment (especially those of gymnosperm tree species) have longer half-lives, which can be attributed to the lack of soil contact. From the differences in the amount of biomass left after nine years, we conclude that we captured deadwood logs from various decay stages when the experiment was terminated. Since decomposition of deadwood is a successional process (Rajala et al., 2012; Lepinay et al., 2021), it is reasonable to assume that the effects of N addition on the decomposition process and subsequent activities of the various enzymes involved depend not only on tree species identity, but also on the decomposition stage and the active community at that stage.

4.3. Comparison to previous studies and outlook

In the early phase of this experiment, Purahong et al. (2018) found no effects of N addition on mass loss, respiration rate or enzymatic activities. One reason for this discrepancy could be the time period in which the treatment started to change the physicochemical conditions in the substrate. Two years after the start of N addition, N content had only significantly increased in *Betula* logs, although a ^{15}N-labelling experiment showed that the additional N had indeed been translocated into the logs (Purahong et al., 2018), whereas we observed an overall change in N availability. Nevertheless, we think that a large share of additionally supplied N was probably lost by leaching or gaseous emission over the years.

Comparing metabarcoding results regarding fungal communities (Purahong et al. did not sequence bacterial communities) directly between studies is not possible due to significant changes in methodology (i.e. changes in sequencing technique and bioinformatics pipelines). We recorded several highly abundant fungal taxa that are characteristic for intermediate (e.g. *Phlebia*, *Calocera*, Stokland et al., 2012) or late stages of wood decomposition (e.g. *Mycena*, Stokland et al., 2012), which were partly present during early decomposition (Purahong et al., 2018).

As Purahong et al. (2018) mention in their publication, it is also likely that due to comparatively high ambient deposition in the Hainich region (Schwarz et al., 2014), microbial communities are already adapted to high N availability, confounding the effects the treatment might have had given less ambient deposition. This would partially explain the overall limited response of the microbial communities we observed as well.

Given the broad phylogenetic range of deadwood from 13 different tree species included in our experiment, we can suggest that the accumulative effect of long-term elevated N deposition has implications for forest C cycling in regions where the most affected tree species dominate (e.g., *Picea abies* in boreal forests) and anthropogenic N deposition remains at high levels compared to pre-industrial levels (Schmitz et al., 2019; Zak et al., 2019). Boreal and temperate forests account for 43 % of forest ecosystems worldwide with approximately 43.4 t ha^{-1} deadwood stocks on average and gymnosperm tree species among the main contributors to the growing stock (e.g. *Pinus, Larix, Picea* for Europe excluding the Russian Federation, *Picea, Pinus, Pseudotsuga* for North America, FAO, 2020). Therefore, we assume that temperate forests in Central and Northern Europe and North America are subject to relevant changes with respect to deadwood decomposition processes, as most of them have been exposed to long-term increases in atmospheric N deposition and still receive significant amounts of reactive N compared to pre-industrial levels (Wang et al., 2017). Moreover, effects in remote boreal forests could be more intense considering the higher N limitation and subsequently increased sensitivity of those ecosystems compared to our experimental site in Central Germany, which received high ambient N deposition.

5. Conclusions

Our results show that N deposition has a moderate long-term impact on deadwood decomposition processes that has mostly been overlooked so far. In contrast to studies investigating soil and litter decomposition and the associated microbial communities, we found no substantial shifts in community composition in fungi and bacteria and therefore interpret the increases in mass loss, respiration rate and laccase activity as a physiological reaction towards long-term increased N availability accompanied by shifts in abundances of only a few fungal and bacterial taxa.

While anthropogenic N deposition levels are declining in the northern hemisphere, the accumulation of bioavailable compounds will have a lasting effect on one of the largest C pools in forests and subsequently forest C cycling. With the strong effect on mass loss and in gymnosperm species, we think that the consequences of atmospheric N deposition on deadwood decomposition should be explored further, since the impacts on forest C cycling in regions with high shares of coniferous forests might be substantial.

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Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

This work is based on data elaborated by the BELongDead Experiment of the Biodiversity Exploratories program (DFG Priority Program 1374). Data for mass loss from t_0 to t_6 (ID: 21809) and respiration of t_8 (31332) as well as mass loss, enzymes and elements of t_8 (31380) can be downloaded at: https://www.bexis.uni-jena.de/ddm/publicsearch/index. OTU tables are available under IDs 31523 and 31524 under the same link.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165868.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2022. Full Mothur UNITE+INSD Dataset 1. Version 16.10.2022. UNITE Community.

Aber, J.D., Goodale, C.L., Ollinger, S.V., Smith, M.-L., Magill, A.H., Martin, M.E., Hallett, R.A., Stoddard, J.L., 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience 53 (4), 375. https://doi.org/10.1641/0006-3568(2003)053[0375:INDATN]2.0.CO;2.

Allison, S.D., Hanson, C.A., Treseder, K.K., 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biol. Biochem. 39 (8), 1878–1887. https://doi.org/10.1016/j.soilbio.2007.02.001.

Arnstadt, T., Hoppe, B., Kahl, T., Kellner, H., Krüger, D., Bässler, C., Bauhus, J., Hofrichter, M., 2016a. Patterns of laccase and peroxidases in coarse woody debris of *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris* and their relation to different wood parameters. Eur. J. For. Res. 135 (1), 109–124. https://doi.org/10.1007/s10342-015-0920-0.

Arnstadt, T., Hoppe, B., Kahl, T., Kellner, H., Krüger, D., Bauhus, J., Hofrichter, M., 2016b. Dynamics of fungal community composition, decomposition and resulting deadwood properties in logs of *Fagus sylvatica*, *Picea abies* and *Pinus sylvestris*. For. Ecol. Manag. 382, 129–142. https://doi.org/10.1016/j.foreco.2016.10.004.

Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., Tonon, G., Brusetti, L., 2018. The role of microbial community in the decomposition of leaf litter and deadwood. Appl. Soil Ecol. 126, 75–84.

Bantle, A., Borken, W., Matzner, E., 2014. Dissolved nitrogen release from coarse woody debris of different tree species in the early phase of decomposition. For. Ecol. Manag. 334, 277–283. https://doi.org/10.1016/j.foreco.2014.06.035.

Barton, K., 2022. MuMIn: Multi-Model Inference. R Package Version 1.47.1. https://CRAN.R-project.org/package=MuMIn.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using Lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Bebber, D.P., 2021. The gap between atmospheric nitrogen deposition experiments and reality. Sci. Total Environ. 801, 149774 https://doi.org/10.1016/j.scitotenv.2021.149774.

Bebber, D.P., Watkinson, S.C., Boddy, L., Darrah, P.R., 2011. Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. Oecologia 167 (4), 1177–1184.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.-W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20 (1), 30–59. https://doi.org/10.1890/08-1140.1.

Brandt, M.I., Trouche, B., Quintric, L., Günther, B., Wincker, P., Poulain, J., Arnaud-Haond, S., 2021. Bioinformatic pipelines combining denoising and clustering tools allow for more comprehensive prokaryotic and eukaryotic metabarcoding. Mol. Ecol. Resour. 21 (6), 1904–1921. https://doi.org/10.1111/1755-0998.13398.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A., Holmes, S.P., 2016. DADA2: high resolution sample interference from Illumina amplicon data. Nat. Methods 7 (13), 581–583.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81 (9), 2359–2365. https://doi.org/10.1890/0012-9658(2000)081[2359:MESELD]2.0.CO;2.

de Vries, W., Du, E., Butterbach-Bahl, K., 2014. Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. Curr. Opin. Environ. Sustain. 9–10, 90–104. https://doi.org/10.1016/j.cosust.2014.09.001.

Dedysh, S.N., Oren, A., 2020. Acidobacteriales. In: Bergey's Manual of Systematics of Archaea and Bacteria. John Wiley & Sons, Ltd., pp. 1–2. https://doi.org/10.1002/9781118960608.obm00001.pub2

Du, E., De Vries, W., Galloway, J.N., Hu, X., Fang, J., 2014. Changes in wet nitrogen deposition in the United States between 1985 and 2012. Environ. Res. Lett. 9 (9), 095004 https://doi.org/10.1088/1748-9326/9/9/095004.

Edelmann, P., Weisser, W.W., Ambarlı, D., Bässler, C., Buscot, F., Hofrichter, M., Hoppe, B., Kellner, H., Minnich, C., Moll, J., Persoh, D., Seibold, S., Seilwinder, C., Schulze, E.-D., Wöllauer, S., Borken, W., 2023. Regional variation in deadwood decay of 13 tree species: effects of climate, soil and forest structure. For. Ecol. Manag. 541, 121094 https://doi.org/10.1016/j.foreco.2023.121094.

Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D., Pregitzer, K.S., 2011. Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood Forest. PLoS One 6 (6), e20421. https://doi.org/10.1371/journal.pone.0020421.

Entwistle, E.M., Zak, D.R., Argiroff, W.A., 2018. Anthropogenic N deposition increases soil C storage by reducing the relative abundance of lignolytic fungi. Ecol. Monogr. 88 (2), 225–244. https://doi.org/10.1002/ecm.1288.

FAO, 2020. Global Forest Resources Assessment 2020: Main Report. Rome. https://doi.org/10.4060/ca9825en.

Filipiak, M., 2018. Nutrient dynamics in decomposing dead wood in the context of wood eater requirements: The ecological stoichiometry of Saproxylophagous insects. In: Ulyshen, M.D. (Ed.), Saproxylic Insects, vol. 1. Springer International Publishing, pp. 429–469. https://doi.org/10.1007/978-3-319-75937-1_13.

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K., Schulze, E.-D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: the biodiversity exploratories. Basic Appl. Ecol. 11, 473–485.

Forsius, M., Posch, M., Holmberg, M., Vuorenmaa, J., Kleemola, S., Augustaitis, A., Beudert, B., Bochenek, W., Clarke, N., de Wit, H.A., Dirnböck, T., Frey, J., Grandin, U., Hakola, H., Kobler, J., Krám, P., Lindroos, A.-J., Löfgren, S., Pecka, T., Váňa, M., 2021. Assessing critical load exceedances and ecosystem impacts of anthropogenic nitrogen and sulphur deposition at unmanaged forested catchments in Europe. Sci. Total Environ. 753, 141791 https://doi.org/10.1016/j.scitotenv.2020.141791.

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B. A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P. H., Wickings, K., 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121 (2), 305–316. https://doi.org/10.1007/s10533-014-0004-0.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S. P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A. F., Porter, J.H., Townsend, A.R., Vosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70 (2), 153–226. https://doi.org/10.1007/s10533-004-0370-0.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320 (5878), 889–892.

Groß, C., Hossen, S., Hartmann, H., Noll, M., Borken, W., 2022. Biological nitrogen fixation and nifH gene abundance in deadwood of 13 different tree species. Biogeochemistry 161, 353–371. https://doi.org/10.1007/s10533-022-00986-w.

Harmon, M.E., Franklin, J.F., Swanson, F.J., Sollins, P., Gregory, S.V., Lattin, J.D., Cummins, K.W., 1986. Ecology of coarse woody debris in temperate ecosystems. Adv. Ecol. Res. 15, 133–302.

Hoppe, B., Krüger, D., Kahl, T., Arnstadt, T., Buscot, F., Bauhus, J., Wubet, T., 2015. A pyrosequencing insight into sprawling bacterial diversity and community

dynamics in decaying deadwood logs of *Fagus sylvatica* and *Picea abies*. Sci. Rep. 5 (1), 1–9.
Hoppe, B., Purahong, W., Wubet, T., Kahl, T., Bauhus, J., Arnstadt, T., Hofrichter, M., Buscot, F., Krüger, D., 2016. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in central European forests. Fungal Divers. 77, 367–379.
Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region—Evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82 (3), 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x.
Isbell, F., Tilman, D., Polasky, S., Binder, S., Hawthorne, P., 2013. Low biodiversity state persists two decades after cessation of nutrient enrichment. Ecol. Lett. 16 (4), 454–460. https://doi.org/10.1111/ele.12066.
Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.-D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3 (5), 315–322. https://doi.org/10.1038/ngeo844.
Johnston, S.R., Boddy, L., Weightman, A.J., 2016. Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiol. Ecol. 92 (11), fiw179. https://doi.org/10.1093/femsec/fiw179.
Kahl, T., Baber, K., Otto, P., Wirth, C., Bauhus, J., 2015. Drivers of CO2 emission rates from dead wood logs of 13 tree species in the initial decomposition phase. Forests 6 (7), 2484–2504.
Kahl, T., Arnstadt, T., Baber, K., Bässler, C., Bauhus, J., Borken, W., Buscot, F., Floren, A., Heibl, C., Hessenmöller, D., Hofrichter, M., Hoppe, B., Kellner, H., Krüger, D., Linsenmair, K.E., Matzner, E., Otto, P., Purahong, W., Seilwinder, C., Gossner, M.M., 2017. Wood decay rates of 13 temperate tree species in relation to wood properties, enzyme activities and organismic diversities. For. Ecol. Manag. 391, 86–95. https://doi.org/10.1016/j.foreco.2017.02.012.
Kellner, H., Luis, P., Pecyna, M.J., Barbi, F., Kapturska, D., Krüger, D., Zak, D.R., Marmeisse, R., Vandenbol, M., Hofrichter, M., 2014. Widespread occurrence of expressed fungal secretory peroxidases in forest soils. PLoS One 9 (4), e95557. https://doi.org/10.1371/journal.pone.0095557.
Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86 (12), 3252–3257.
Krieglsteiner, GJ., 2000. Die Großpilze Baden-Württembergs. Band 1. Verlag Eugen Ulmer GmbH & Co, Stuttgart.
Leonhardt, S., Hoppe, B., Stengel, E., Noll, L., Moll, J., Bässler, C., Dahl, A., Buscot, F., Hofrichter, M., Kellner, H., 2019. Molecular fungal community and its decomposition activity in sapwood and heartwood of 13 temperate European tree species. PLoS One 14 (2), 1–21.
Lepinay, C., Jiráska, L., Tláskal, V., Brabcová, V., Vrška, T., Baldrian, P., 2021. Successional development of fungal communities associated with decomposing deadwood in a natural mixed temperate forest. J. Fungi 7 (6), 412.
Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I., Hobbie, E.A., 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. Environ. Pollut. 246, 148–162. https://doi.org/10.1016/j.envpol.2018.11.074.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15 (12), 550.
Lovett, G.M., Arthur, M.A., Weathers, K.C., Fitzhugh, R.D., Templer, P.H., 2013. Nitrogen addition increases carbon storage in soils, but not in trees, in an eastern US deciduous forest. Ecosystems 16 (6), 980–1001.
Lüdecke, D., 2022. sjPlot: Data Visualization for Statistics in Social Science. R package version 2.8, p. 12. https://CRAN.R-project.org/package=sjPlot.
Lundell, T.K., Mäkelä, M.R., de Vries, R.P., Hildén, K.S., 2014. Genomics, lifestyles and future prospects of wood-decay and litter-decomposing basidiomycota. In: Advances in Botanical Research, 70. Academic Press, pp. 329–370.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.J. 17 (1), 1 https://doi.org/10.14806/ej.17.1.200.
McMurdie, P.J., Holmes, S.P., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 4 (8).
Merganicova, K., Merganic, J., Svoboda, M., Bace, R., Seben, V., 2012. Deadwood in Forest ecosystems. In: Blanco, J.A., Lo, Y.-H. (Eds.), Forest Ecosystems - More than Just Trees. InTech, pp. 81–108.
Moll, J., Kellner, H., Leonhardt, S., Stengel, E., Dahl, A., Bässler, C., Buscot, F., Hofrichter, M., Hoppe, B., 2018. Bacteria inhabiting deadwood of 13 tree species are heterogeneously distributed between sapwood and heartwood. Environ. Microbiol. 20 (10), 3744–3756. https://doi.org/10.1111/1462-2920.14376.
Moll, J., Roy, F., Bässler, C., Heilmann-Clausen, J., Hofrichter, M., Kellner, H., Krabel, D., Schmidt, J.H., Buscot, F., Hoppe, B., 2021. First evidence that nematode communities in deadwood are related to tree species identity and to co-occurring fungi and prokaryotes. Microorganisms 9, 1454. https://doi.org/10.3390/microorganisms9071454.
Moore, J.A.M., Anthony, M.A., Pec, G.J., Trocha, L.K., Trzebny, A., Geyer, K.M., Diepen, L.T.A., Frey, S.D., 2021. Fungal community structure and function shifts with atmospheric nitrogen deposition. Glob. Chang. Biol. 27 (7), 1349–1364. https://doi.org/10.1111/gcb.15444.
Morrison, E.W., Pringle, A., van Diepen, L.T.A., Frey, S.D., 2018. Simulated nitrogen deposition favors stress-tolerant fungi with low potential for decomposition. Soil Biol. Biochem. 125, 75–85. https://doi.org/10.1016/j.soilbio.2018.06.027.
Okasanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H., Szoescs, E., Wagner, H., 2019. Vegan: Community Ecology Package.
Olson, J.S., 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44 (2), 322–331.
Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science. https://doi.org/10.1126/science.1201609.
Pardo, L.H., Fenn, M.E., Goodale, C.L., Geiser, L.H., Driscoll, C.T., Allen, E.B., Baron, J.S., Bobbink, R., Bowman, W.D., Clark, C.M., Emmett, B., Gilliam, F.S., Greaver, T.L., Hall, S.J., Lilleskov, E.A., Liu, L., Lynch, J.A., Nadelhoffer, K.J., Perakis, S.S., Dennis, R.L., 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. Ecol. Appl. 21 (8), 3049–3082. https://doi.org/10.1890/10-2341.1.
Põlme, S., Abarenkov, K., Nilsson, H.R., Lindahl, B., Clemmensen, K., 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Divers. 105 (1), 1–16. https://doi.org/10.1007/s13225-020-00466-2.
Purahong, W., Wubet, T., Kahl, T., Arnstadt, T., Hoppe, B., Lentendu, G., Baber, K., Rose, T., Kellner, H., Hofrichter, M., Bauhus, J., Krüger, D., Buscot, F., 2018. Increasing N deposition impacts neither diversity nor functions of deadwood-inhabiting fungal communities, but adaptation and functional redundancy ensure ecosystem function. Environ. Microbiol. 20 (5), 1693–1710. https://doi.org/10.1111/1462-2920.14081.
Qiao, N., Xu, X., Hu, Y., Blagodatskaya, E., Liu, Y., Schaefer, D., Kuzyakov, Y., 2016. Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. Sci. Rep. 6 (1), 19865.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.
Rajala, T., Peltoniemi, M., Pennanen, T., Mäkipää, R., 2012. Fungal community dynamics in relation to substrate quality of decaying Norway spruce (Picea abies [L.] karst.) logs in boreal forests. FEMS Microbiol. Ecol. 81 (2), 494–505. https://doi.org/10.1111/j.1574-6941.2012.01376.x.
Rinne, K.T., Rajala, T., Peltoniemi, K., Chen, J., Smolander, A., Mäkipää, R., 2017. Accumulation rates and sources of external nitrogen in decaying wood in a Norway spruce dominated forest. Funct. Ecol. 31 (2), 530–541. https://doi.org/10.1111/1365-2435.12734.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75 (23), 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Schmitz, A., Sanders, T.G.M., Bolte, A., Bussotti, F., Dirnböck, T., Johnson, J., Peñuelas, J., Pollastrini, M., Prescher, A.-K., Sardans, J., Verstraeten, A., de Vries, W., 2019. Responses of forest ecosystems in Europe to decreasing nitrogen deposition. Environ. Pollut. 244, 980–994. https://doi.org/10.1016/j.envpol.2018.09.101.
Schwarz, M.T., Bischoff, S., Blaser, S., Boch, S., Schmitt, B., Thieme, L., Wilcke, W., 2014. More efficient aboveground nitrogen use in more diverse central European forest canopies. For. Ecol. Manag. 313, 274–282.
Schwede, D.B., Simpson, D., Tan, J., Fu, J.S., Dentener, F., Du, E., deVries, W., 2018. Spatial variation of modelled total, dry and wet nitrogen deposition to forests at global scale. Environ. Pollut. 243, 1287–1301. https://doi.org/10.1016/j.envpol.2018.09.084.
Seibold, S., Rammer, W., Hothorn, T., Seidl, R., Ulyshen, M.D., Lorz, J., Cadotte, M.W., Lindenmayer, D.B., Adhikari, Y.P., Aragón, R., Bae, S., Baldrian, P., Barimani Varandi, H., Barlow, J., Bässler, C., Beauchêne, J., Berenguer, E., Bergamin, R.S., Birkemoe, T., Müller, J., 2021. The contribution of insects to global forest deadwood decomposition. Nature 597 (7874), 77–81. https://doi.org/10.1038/s41586-021-03740-8.
Stokland, J.N., Siitonen, J., Jonsson, B.G., 2012. Biodiversity in Dead Wood. Cambridge University Press.
Stokstad, E., 2019. Nitrogen crisis threatens Dutch environment—and economy. Science 366 (6470), 1180–1181. https://doi.org/10.1126/science.366.6470.1180.
Sutton, M.A., Oenema, O., Erisman, J.W., Leip, A., van Grinsven, H., Winiwarter, W., 2011. Too much of a good thing. Nature 472 (7342), 159–161. https://doi.org/10.1038/472159a.
Tláskal, V., Zrůstová, P., Vrška, T., Baldrian, P., 2017. Bacteria associated with decomposing dead wood in a natural temperate forest. FEMS Microbiol. Ecol. 93 (12), fix157.
Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol. Lett. 11 (10), 1111–1120. https://doi.org/10.1111/j.1461-0248.2008.01230.x.
Větrovský, T., Voříšková, J., Šnajdr, J., Gabriel, J., Baldrian, P., 2011. Ecology of coarse wood decomposition by the saprotrophic fungus *Fomes fomentarius*. Biodegradation 22 (4), 709–718. https://doi.org/10.1007/s10532-010-9390-8.
Vorob'ev, A.V., de Boer, W., Folman, L.B., Bodelier, P.L.E., Doronina, N.V., Suzina, N.E., Trotsenko, Y.A., Dedysh, S.N., 2009. Methylovirgula ligni gen. nov., sp. nov., an obligately acidophilic, facultatively methylotrophic bacterium with a highly divergent mxaF gene. Int. J. Syst. Evol. Microbiol. 59, 2538–2545. https://doi.org/10.1099/ijs.0.010074-0.

Waldrop, M.P., Zak, D.R., 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9 (6), 921–933. https://doi.org/10.1007/s10021-004-0149-0.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biol. Biochem. 36 (9), 1443–1451. https://doi.org/10.1016/j.soilbio.2004.04.023.

Wang, C., Feng, X., Guo, P., Han, G., Tian, X., 2010. Response of degradative enzymes to N fertilization during litter decomposition in a subtropical forest through a microcosm experiment. Ecol. Res. 25 (6), 1121–1128. https://doi.org/10.1007/s11284-010-0737-8.

Wang, R., Goll, D., Balkanski, Y., Hauglustaine, D., Boucher, O., Ciais, P., Janssens, I., Penuelas, J., Guenet, B., Sardans, J., Bopp, L., Vuichard, N., Zhou, F., Li, B., Piao, S., Peng, S., Huang, Y., Tao, S., 2017. Global forest carbon uptake due to nitrogen and phosphorus deposition from 1850 to 2100. Glob. Chang. Biol. 23, 4854–4872. https://doi.org/10.1111/gcb.13766.

Weedon, J.T., Cornwell, W.K., Cornelissen, J.H., Zanne, A.E., Wirth, C., Coomes, D.A., 2009. Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? Ecol. Lett. 12 (1), 45–56. https://doi.org/10.1111/j.1461-0248.2008.01259.x.

Weißbecker, C., Schnabel, B., Heintz-Buschart, A., 2020. Dadasnake, a Snakemake implementation of DADA2 to process amplicon sequencing data for microbial ecology. GigaScience 9 (12), giaa135. https://doi.org/10.1093/gigascience/giaa135.

Wickham, H., 2016. Ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42 (D1), D643–D648. https://doi.org/10.1093/nar/gkt1209.

Zak, D.R., Pregitzer, K.S., Burton, A.J., Edwards, I.P., Kellner, H., 2011. Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. Fungal Ecol. 4 (6), 386–395. https://doi.org/10.1016/j.funeco.2011.04.001.

Zak, D.R., Freedman, Z.B., Upchurch, R.A., Steffens, M., Kögel-Knabner, I., 2017. Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. Glob. Chang. Biol. 23 (2), 933–944. https://doi.org/10.1111/gcb.13480.

Zak, D.R., Argiroff, W.A., Freedman, Z.B., Upchurch, R.A., Entwistle, E.M., Romanowicz, K.J., 2019. Anthropogenic N deposition, fungal gene expression, and an increasing soil carbon sink in the northern hemisphere. Ecology 100 (10), e02804.