Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2022:18

Tree species effects on litter decomposition and soil carbon

Effekter av treslag på strønedbrytning og jordkarbon

Yngvild Ransedokken

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Ås (2022)

Thesis number 2022:18
ISSN 1894-6402
ISBN 978-82-575-1893-6

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Acknowledgements

To my great team of supervisors: Line, thanks for taking me on as a PhD candidate, for giving me freedom in my work, and for your great support through the years. I have appreciated our many conversations and that you are only a phone call away, even if we have been countries apart. Johan, thanks for always have time for me when I come knocking on your door, and for keeping me sane through struggles with coding in R. Mikael, I admire your ability to see the big picture and weave the threads together, especially when I am lost in all the small details. And thanks for taking me on as a master student way back when, which introduced me to the field of soil carbon research. Janne, thanks for sharing your enthusiasm and knowledge about soil science with me. To all of you, thanks for your guidance and the good company along the way!

To all the collaborators and co-authors for my thesis: Håvard, Luis, and Sunil, thanks for all the fun travels, field trips, and field courses, and for opening my eyes to the wonderful world of fungi. To Rune, thanks for sharing your knowledge of ordination methodology. To Jan, thanks for all the time you have put into processing climatic data.

Many thanks should also be given to Annie and Claus. The staggering number of chemical analyses performed for this thesis, would not have been possible without you.

To all my fellow PhD friends (former and current) and colleagues at MINA, thanks for all the laughs during lunches, field courses, social gatherings, and random hallway conversations, you are too many to thank individually. And a special thanks to the eco-group that I am so glad to have been a part of!

To my family and friends, thanks for your heartfelt love and support! Mom and Dad, thanks for kindling the spark in me to explore and enjoy nature at an early age. And thanks Mom, for the steady stream of food supplies for my family and me, and for letting me use Dad as a field assistant whenever I needed him. He is all yours now. Dad, thanks for the good times in the field!

To Marius, my rock, thanks for being by my side and cheering me on through this journey, and actively taking part through field work, discussions, proofreading, and most importantly commenting on my use of sometimes overly long sentences.

To Hugo, my little bundle of joy and energy – I am so grateful for being your mom.

Summary

Boreal forests soils play an important role in mitigating climate change as they are the largest terrestrial carbon pool. The accumulation of soil carbon is regulated by the balance of carbon and nitrogen input from plant production and output from decomposition. Thus, the type of tree species has an impact on soil C by way of three key factors that regulate decomposition rates: litter quality, associated decomposer communities, and microclimatic conditions. Plant phenolic compounds, such as condensed tannins, have been suggested by previous studies to play important roles during decomposition. But their regulatory effect on litter decomposition has remained uncertain. Tree species also influence the input through their variable contents of recalcitrant compounds and fungal biomass, which represents a substantial proportion of the stable soil carbon in boreal forests. Although previous studies have exposed variances in soil carbon under various dominant tree species, the effects that tree species have on soil carbon are complex and far from fully understood.

This thesis explores the effects that tree species have on early-stage litter decomposition and soil carbon in various forests across Norway. In Paper I, we examined the decomposition of three different litter types during a one-year reciprocal litterbag experiment along a gradient from mesic spruce-dominated to xeric pine-dominated forests. We found a species-specific pattern of temporal change in mass loss, and a synchronous shift in the composition of phenolic compounds and fungal community. This paper offers new insights into the importance of litter quality, especially phenolic compounds, for the decomposition process.

In Paper II, we investigated the litter decomposition in native birch-dominated and planted spruce stands through a one-year reciprocal litterbag transplant experiment and tested the home-field advantage hypothesis. The study included measurements of microclimatic conditions. We found a net negative home-field advantage since both litter types decomposed faster in the birch stands compared to in the spruce stands, and since spruce litter had a greater advantage than birch litter had of decomposing in the birch stands conditions relative to the spruce stands conditions. Our results points to important differences in condensed tannin-dynamics between the birch and spruce litter.

In Paper III, we studied biochemical properties and fungal biomass along fine-scaled soil profiles of beech and spruce forests, in an area where beech has its northern distribution limit. We found no significant difference between the forests when comparing estimates of total carbon stocks. However, we found vertical differences in soil carbon between beech and spruce forests, which is likely driven by differences in fungal biomass along the soil profile.

To sum up, this thesis highlights the importance of litter quality on regulating the early-stage decomposition of plant litter, especially the role of phenolic compounds on the decomposition process. Furthermore, we show that a change in the dominant tree species alter both litter decomposition rates and the vertical distribution of soil chemical characteristics.

Sammendrag

Boreal skogsjord spiller en viktig rolle i å redusere klimaendringer, siden de utgjør det største terrestriske karbonlageret. Akkumuleringen av jordkarbon reguleres av balansen mellom tilførsel av karbon- og nitrogen fra planteproduksjon og tap fra nedbrytning. Dermed har treslagstype en påvirkning på jordkarbon gjennom tre nøkkelfaktorer som regulerer nedbrytningshastigheten: strøkvalitet, tilhørende nedbrytersamfunn og mikroklimatiske forhold. Fenolforbindelser i planter, som kondenserte tanniner, har blitt foreslått av tidligere studier å spille viktige roller under nedbrytning. Men deres regulatoriske effekt på nedbrytning av strø har vært usikker. Trearter påvirker også tilførselen av gjenstridige kondenserte tanniner og soppbiomasse, som representerer en betydelig andel av det stabile jordkarbonet i boreale skoger. Selv om tidligere studier har avdekket variasjoner i jordkarbon under ulike dominerende treslag, er effektene som treslag har på jordkarbon komplekse og langt fra fullstendig forstått.

Denne avhandlingen utforsker effektene treslag har på tidlig nedbrytning av strø og jordkarbon i ulike skoger på ulike steder i Norge. I Paper I undersøkte vi nedbrytningen av tre forskjellige strøtyper gjennom et ettårig repliserbart strøpose-eksperiment langs en gradient fra fuktig gran-dominert til tørr furu-dominert skog. Vi fant et artsspesifikt mønster av tidsmessig endring i massetap, og et synkront skifte i sammensetningen av fenolforbindelser og soppsamfunn. Denne artikkelen gir ny innsikt om viktigheten av strøkvalitet, spesielt fenolforbindelser, for nedbrytningsprosessen.

I Paper II undersøkte vi strønedbrytningen i naturlig bjørkedominert skog og plantet granskog gjennom et ettårig repliserbart transplantasjonseksperiment med strøposer og testet hypotesen om fordelen av hjemmebane for nedbrytning. Studien inkluderte målinger av mikroklimatiske forhold. Vi fant en netto negativ hjemmebanefordel siden begge strøtypene ble brutt ned raskere i bjørkebestandene sammenlignet med granbestandene, og siden granstrøet hadde en relativt større fordel enn bjørkestrøet av å brytes ned i bjørkebestandsforholdene sammenliknet med i granbestandsforholdene. Resultatene våre avdekker forskjeller i kondensert tannin-dynamikk mellom bjørkestrø og granstrø.

I Paper III studerte vi biokjemiske egenskaper og soppbiomasse langs finskalerte jordprofiler av bøk- og granskog, i et område hvor bøk har sin nordlige utbredelsesgrense. Vi fant ingen signifikant forskjell mellom skogene når vi sammenlignet estimater av totale karbonlagre. Imidlertid fant vi vertikale forskjeller i jordkarbon mellom bøk- og granskog, som sannsynligvis er drevet av forskjeller i soppbiomasse langs jordprofilen.

For å oppsummere så belyser denne avhandlingen viktigheten av strøkvalitet for reguleringen av tidlig nedbrytning av plantestrø, spesielt rollen fenoler har på nedbrytningsprosessen. Videre viser vi at en endring i de dominerende treslagene endrer både nedbrytningshastigheten for strø og den vertikale fordelingen av jordkjemiske egenskaper.

List of papers

Paper I

Synchronic shifts in phenolic compounds and fungal communities during litter decomposition in boreal forests

Yngvild Ransedokken, Luis N. Morgado, Håvard Kauserud, Johan Asplund, Sunil Mundra, Mikael Ohlson, Rune Halvorsen & Line Nybakken

Submitted manuscript

Paper II

Faster initial litter decomposition under native birch than planted spruce

Yngvild Ransedokken, O. Janne Kjønaas, Johan Asplund, Mikael Ohlson, Jan Světlík & Line Nybakken

Manuscript

Paper III

Vertical distribution of soil carbon in boreal forest under European beech and Norway spruce

Yngvild Ransedokken, Johan Asplund, Mikael Ohlson & Line Nybakken

European Journal of Forest Research 138: 353–361 (2019)

https://doi.org/10.1007/s10342-019-01176-4

Additional work

Shift in tree species leads to dramatic changes in the belowground biota in boreal forests

Sunil Mundra, Håvard Kauserud, Tonje Økland, Jørn-Frode Nordbakken, Yngvild Ransedokken & O. Janne Kjønaas

In review

Soil depth matters: shift in composition and inter-kingdom co-occurrence patterns of microorganisms in forest soils

Sunil Mundra, O. Janne Kjønaas, Luis N. Morgado, Anders Kristian Krabberød, Yngvild Ransedokken & Håvard Kauserud

FEMS Microbiology Ecology 97: fiab022 (2021)

https://doi.org/10.1093/femsec/fiab022

Synopsis

1 Introduction

Boreal forests play an important role in mitigating climate change as the largest terrestrial carbon (C) pool. This mighty forest belt embraces the land area of the entire Northern Hemisphere, forming a ring around the North Pole. The boreal coniferous forest represents approximately 32 % of the global carbon stock in terrestrial ecosystems (Pan et al. 2011). About 85 % of the total amount of C in the boreal forest biome resides in the soil (DeLuca & Boisvenue 2012). This implies that the carbon in boreal forest ecosystems is primarily regulated by soil biological processes.

Dominant tree species affect chemical and biological characteristics of the soil (Augusto et al. 2015). Litter decomposition is a key process in the forest ecosystem functioning that drives C and nutrient cycling, and the rate of litter decay is highly impacted by the dominant tree species. The chemical composition of plant litter varies greatly between species, and the litter quality, associated decomposer community, and the climatic conditions are the main factors regulating the decomposition of plant litter (Coûteaux et al. 1995). In the early stages of decomposition there is a loss of labile and readily degradable compounds, while highly recalcitrant compounds are decomposed at later decay stages, until only undegradable litter remains. Furthermore, the species-specific rate of litter decomposition is thought to explain the effect of dominant tree species on soil C (Hansen et al. 2009, Vesterdal et al. 2008). Thus, a shift in dominant tree species will have an impact on decomposition rates and the distribution of soil C in boreal forest ecosystems.

Boreal plant species produce a broad range of secondary metabolites for chemical defence, such as phenolic compounds (Thoss et al. 2004). Phenolic compounds, including both low molecular weight phenolic compounds and condensed tannins, are suggested to have important roles during decomposition (Chomel et al. 2016). Condensed tannins consist of large molecules that are known to have a general resistance to degradation (Haase & Wantzen 2008), both through their negative effects on decomposers and through their capacity to form complexes with biological polymers (Mutabaruka et al. 2007, Adamczyk et al. 2019). Moreover, the recalcitrant condensed tannins left after decomposition contributes to the stable soil C in boreal forests (Kraus et al. 2003).

The main decomposers of plant litter in boreal forests are fungi, due to their ability to produce a wide range of extracellular enzymes (Lindahl et al. 2021). Fungal identity and community composition varies with dominant tree species, time of decomposition, and ultimately determine litter decomposition rates (Voříšková & Baldrian, 2013). Although aboveground plant litter have been assumed to be the main origin of soil C, recalcitrant fungal biomass contributes substantially to the stable soil C pool (Clemmensen et al. 2015).

2 Objectives

Tree species affect the C and nutrient cycling in boreal forest ecosystems. To understand the differences between forest types, it is essential to gain more knowledge about the underlying mechanisms and factors, such as plant litter quality and fungal biomass, that contribute to the effect of tree species on soil C. The main aim of this thesis was to contribute to the ecological understanding of how tree species affect litter decomposition and soil C. First, we examined three litter types that decomposed at different time intervals covering one year across a gradient from mesic spruce-dominated to xeric pine-dominated forest (**Paper I**). The study focused on changes in composition of litter phenolic compounds and fungal community. Then, for the second paper we investigated the litter decomposition in native birch-dominated and planted spruce stands including differences in microclimatic conditions and tested the home-field advantage hypothesis (**Paper II**). Lastly, biochemical properties and fungal biomass were studied along fine-scaled soil profiles of beech and spruce forests, in an area where beech has its northern distribution limit (**Paper III**). In the two first papers the input of C and N to the soil via litter were quantified after the first year of decomposition, while in the third paper we looked at the fate of these elements in the soil. In sum, the papers attempt to answer the following overarching research questions:

1. How do plant phenolic compounds and fungal community composition change over time with decomposition across an environmental gradient in boreal forests? (**Paper I**)
2. Do planted spruce stands cause changes in the litter decomposition rates in native birch stands? (**Paper II**)
3. Do beech and spruce differ in stocks and vertical distribution of carbon? (**Paper III**)

3. Materials and methods

3.1 Study sites

This thesis consists of three separate studies conducted at different locations in Norway. The plant species included in these studies were bilberry (*Vaccinium myrtillius* L.), Scots pine (*Pinus sylvestris* L.; hereafter pine), Norway spruce (*Picea abies* (L.) Karst.; hereafter spruce), downy birch (*Betula pubescens* Ehrh.; hereafter birch), and European beech (*Fagus sylvatica* L.; hereafter beech). Thus, covering the most common tree species (birch, spruce, and pine) across Norway (Granhus et al. 2012), and one species (beech) that is expected to increase its distribution northwards in the future (Hickler et al. 2012).

3.1.1 Solhomfjell (Paper I)

The study was conducted in boreal forests of the Solhomfjell and Kvenntjønnane nature reserve in southeast Norway, covering variation from mesic spruce-dominated to xeric pine-dominated forests (Fig. 3.1). Along this gradient the dominant tree species shift from spruce at low drought risk to pine at high drought risk (Fig. 3.2). The landscape is hilly, dominated by shallow soils (depths typically < 50 cm), and peatlands cover extensive areas. Here, 8 transects with 100 permanently marked plots have been regularly studied with respect to vegetation and environment since 1988 (Økland & Eilertsen 1993). For our study, we selected 18 plots along three transects covering the variation along the environmental gradient (Fig. 3.1). Detailed descriptions of the study site are given in **Paper I**.

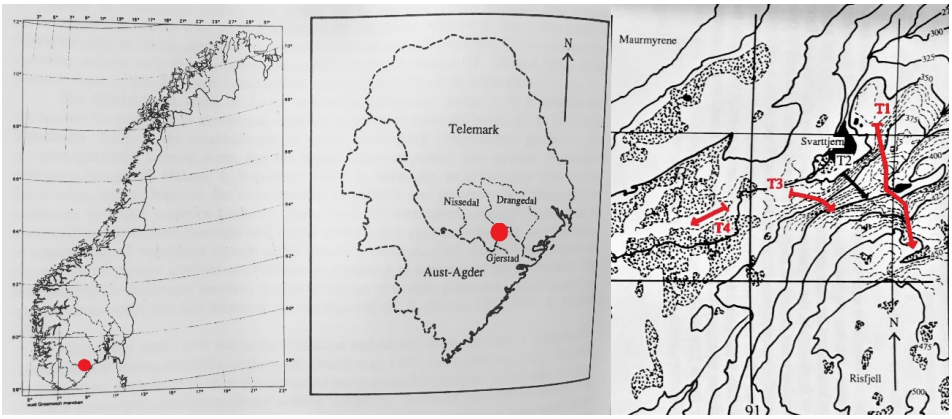

Figure 3.1. Map of the study site at Solhomfjell, and the three transects at the study site is marked in red colour. (Retrieved and modified from Økland & Eilertsen 1993, Fig. 1–2)

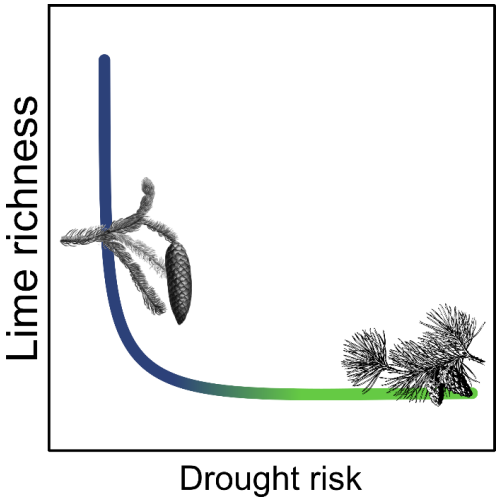

Figure 3.2. The plots are placed along two major environmental gradients. Along the gradient "drought risk" there is a shift in dominant tree species from Norway spruce (blue; *Picea abies*) to Scots pine (green; *Pinus sylvestris*). At low drought risk (dominated by spruce), there is an additional gradient in lime richness. The gradients are derived from an ordination of the ground vegetation and represent a shift in community composition from species thriving on mesic soils (e.g., *Vaccinium myrtillus*) to species less susceptible to drought (e.g., *V. vitis-idaea*). Drought risk and lime richness are assessed by use of ordinal scales with levels 1–8 and 1–9, respectively (Halvorsen et al. 2020).

3.1.2. Western Norway (Paper II)

The study was conducted at four locations (Stranda, Ørsta, Jølster I, and Jølster II) in western Norway (Fig. 3.3). Experimental plots of paired stands of adjacent mature native birch and planted spruce (aged 45–60 years) had previously been established at each location (Kjønaas et al. 2021). All locations were located on hillsides, were varying slopes, altitudes, and aspects contributed to some variation in the local climate. The understory vegetation in the birch stands were more diverse and abundant than in the spruce stands. At each location, three paired macro-plots (144m^2) in parallel birch and spruce stands were established with six randomly positioned 0.5 × 0.5 m subplots within each stand. Detailed descriptions of the study sites are given in **Paper II**.

Figure 3.3. Map of the locations (Stranda, Ørsta, Jølster I, and Jølster II) of the paired stands of native birch and planted spruce in Western Norway. The location Molde was excluded from assessments of a species change due to the spruce stand being subject to nutrient rich water from a well. (Retrieved from Kjønaas et al. 2021, Appendix, Supplementary Figures, Figure S1)

3.1.3. Brånakollene (Paper III)

The study was conducted in three distinct forest stands, selected due to their similar environmental conditions, in the same forest landscape in southeast Norway (Fig. 3.4). The present northern margin of the beech forest range is overlapping the southern margin of the boreal spruce forest in this area. A natural beech forest (Be) is located within Brånakollane nature reserve. The reserve has a clear boundary to the surrounding spruce forest (SpBe), which was planted after a clear-cut of the previous beech forest in 1956. The other spruce forest (Sp) is located about 1.5 km south of the reserve. The preceding spruce forest was clear-cut and re-planted in 1981. Understory vegetation at each site is generally sparse. Soil depths at the study sites are relatively shallow, and the thickness of the organic soil was similar for beech and spruce forests (~ 6 cm). Detailed descriptions of the study sites are given in **Paper III**.

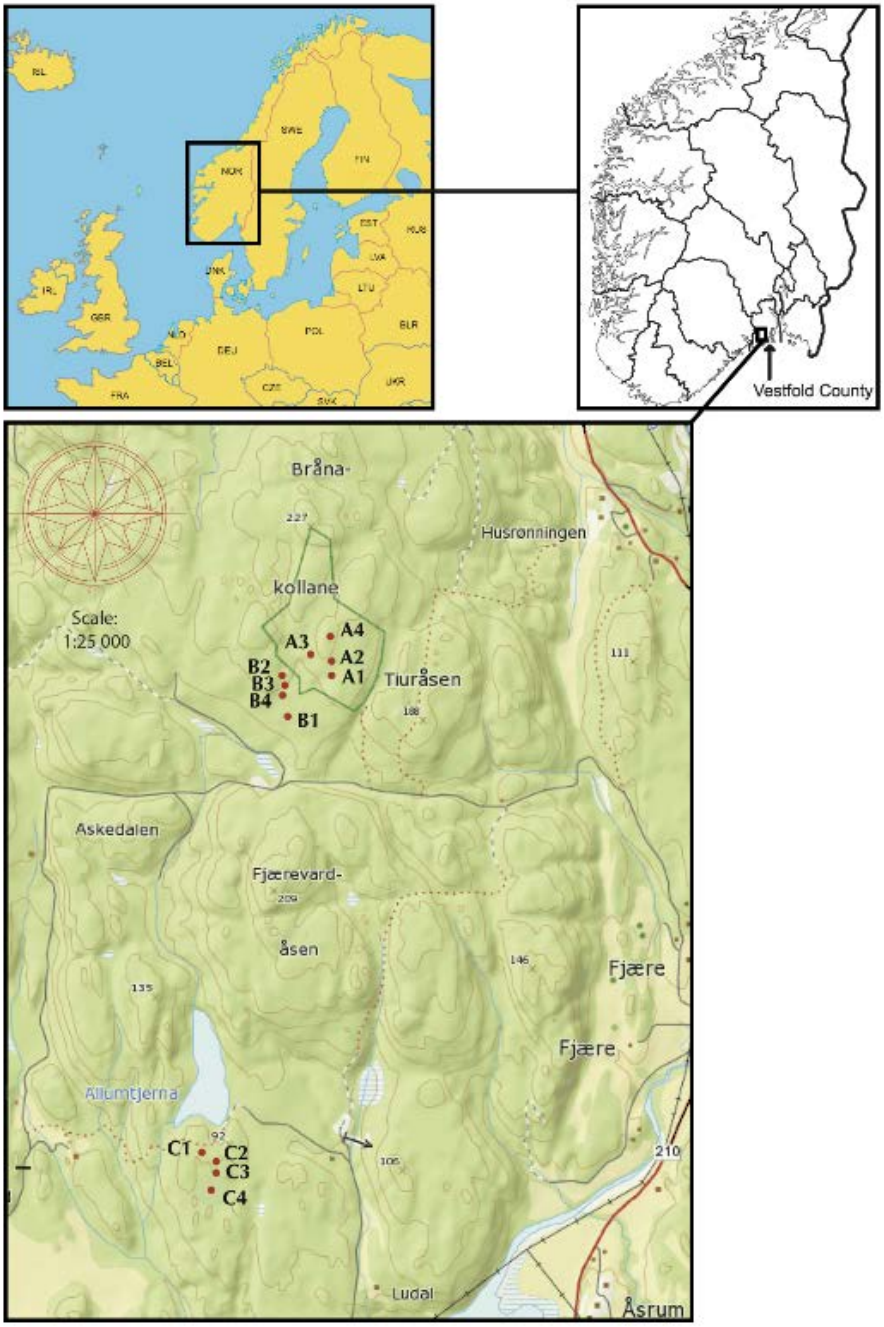

Figure 3.4. Map of the location of plots in the beech forest (A), previous beech forest (B), and spruce forest (C) in southeast Norway. (Retrieved from Ransedokken 2016, Fig. 3)

3.2 Study design

The papers in this thesis involved reciprocal litterbag experiments (**Papers I** and **II**) and soil sampling (**Paper III**). The litterbag approach was used to determine mass remaining after different decomposition intervals. Both litter material and soil were analysed for various chemical and fungal analyses (Table 3.1).

Table 3.1. Summary of study design and various measurements in the three different studies included in this thesis.

	Study design	**Measurements**
PAPER I	Litterbag experiment along an environmental gradient	C, N, C:N ratio, LMP, MeOH-soluble CT, MeOH-insoluble CT, ergosterol, fungal community composition
PAPER II	Litterbag transplant experiment including microclimatic conditions	C, N, C:N ratio, MeOH-soluble CT, MeOH-insoluble CT, ergosterol, microclimatic conditions
PAPER III	Soils samples along fine-scaled soil profiles	C, N, C:N ratio, acetone-soluble CT, ergosterol, pH

3.2.1 Litterbag experiments

In **Paper I,** senesced leaves from bilberry, pine, and spruce were collected from localities off-site to exclude impact of interspecific variation in litter quality. At each of the 18 plots, 4 litterbags of each of the three litter types were placed at 5 cm soil depth in September 2017, leaving a total of 216 litterbags (Fig. 3.5). Litterbags were retrieved after 14, 61, 250, and 370 days of incubation. In **Paper II**, senesced leaves of birch and spruce were collected at each of the locations in October 2018 (Fig. 3.6). A total of 288 litterbags, 144 of each litter type, were placed on the soil surface in 72 birch and 72 spruce subplots in October 2018 and retrieved after one year (Fig. 3.7). The understory vegetation biomass had previously been harvested, leaving similar conditions in ground position between the stand types. Details on the methodologies are given in **Papers I** and **II**.

Figure 3.5. Pictures of litterbags filled with pine litter (left), and distribution of litterbags in the field (**Paper I**).

Figure 3.6. Picture of 1g of spruce (left) and birch litter (**Paper II**).

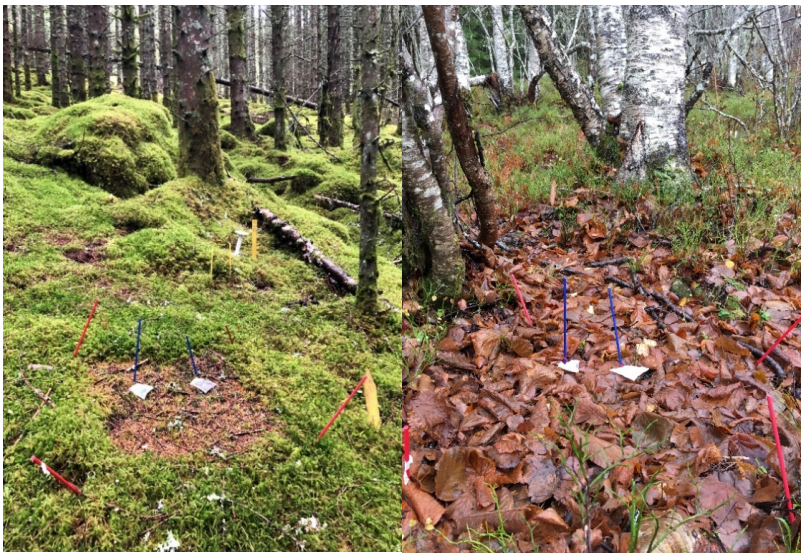

Fig.3.7. Litterbags of birch and spruce litter distributed in spruce (left) and birch stands (**Paper II**). One of the sensors recording surface temperature and soil temperature and moisture at each subplot is visible in the back of the picture from the spruce stand.

3.2.2 Soil sampling

For **Paper III**, soil samples were collected at four randomly located plots within each site in September 2015. At each plot, soil samples were collected from the organic soil layer using a soil corer. Soil samples along the mineral soil profile were collected by using a steel cylinder with a 2.5 cm diameter, from the top of the mineral soil profile (0 cm of depth) and down to 47.5 cm of depth (or down to 42.5 cm in plots with shallower soil depths) (Fig. 3.8). This left a 2.5 cm interval of soil between each sample taken. The complete dataset consisted of 129 soil samples (9 plots × 11 samples, 3 plots × 10 samples). Samples were stored in 20-ml vials to allow for estimation of C and N stocks. Details on the methodologies are given in **Paper III**.

3.3 Chemical analyses

Concentration of C and N were quantified for litter (**Papers I** and **II)** and soil samples (**Paper III)**. To calculate the litter N release in **Papers I** and **II**, the total mass × N concentration after incubation were subtracted from the initial mass × N concentration and expressed as the proportion of the initial mass × N concentration before incubation (Wardle 2002). In **Paper III**, the C and N concentration were used for estimation of total

Figure 3.8. Soil cores taken from the organic soil layer (top), and samples taken along the mineral soil profile in beech forest (left) and spruce forest planted on previous beech forest (**Paper III**). In the foreground of the bottom left picture, the steel cylinder we used to collect soil samples with is visible.

C and N stocks. To measure secondary metabolites, we used high-performance liquid chromatography (HPLC). **In Paper I,** I measured the concentration and composition of low molecular weight phenolic compounds (LMP). Individual phenolic compounds were identified and classified into four groups: acetophenones, flavonoids, phenolic acids, and stilbenes. In **Papers I** and **II,** condensed tannins were extracted from the litter by use of methanol (MeOH) and measurements of MeOH-soluble and MeOH-insoluble condensed tannins were determined. While for the soil samples in **Paper III**, measurements of condensed tannins were determined by use of acetone extraction. The loss of MeOH-soluble and MeOH-insoluble condensed tannins after decomposition in **Papers I**

and **II**, and LMP loss in **Paper I**, were calculated by the method used for N release. Analysis of ergosterol was used as a proxy to estimate fungal biomass in **Papers I, II,** and **III**. The label *total ergosterol* used in **Paper III** refer to the same method used in all three papers. For **Papers I** and **II**, we performed the described analyses on both litter after decomposition and prior to decay to quantify initial concentrations in the litter. In **Paper III**, acetone-soluble condensed tannins and ergosterol were converted to mg cm^{-3} by using the volumetric mass of each sample (20 ml), and acetone-soluble condensed tannins:C and ergosterol:C ratios were determined. The soil pH was analysed of the soil samples in **Paper III**. Detailed descriptions of all chemical analyses are given in **Papers I, II,** and **III**.

3.4 Fungal community composition

In **Paper I,** fungal community composition was analysed through DNA metabarcoding for bilberry, pine, and spruce litter after the four time-intervals of decomposition. The final data set used for statistical analyses consisted of 1051 Operational Taxonomic Units (OTUs) based on 184 samples for all three litter types, with the corresponding numbers were 504 OTUs in 57 samples, 560 OTUs in 62 samples, and 541 OTUs in 65 samples for bilberry, pine, and spruce litter, respectively. Detailed descriptions of DNA sequencing and bioinformatics are given in **Paper I**.

3.5 Microclimatic measurements

In **Paper II**, surface temperature and soil moisture and temperature (at 6 cm soil depth) were recorded by a sensor at each subplot every 15 minutes for one year (Fig. 3.7). From these measurements, average surface temperature and soil moisture and temperature were calculated, together with the sum of growing degree days (GDD) above 0 °C and 5 °C during the one-year study period to assess potential differences in temperature sums related to microbial- and plant activity, respectively.

3.6 Statistical analyses

For **Paper I**, we used linear mixed-effects models to test for the effect of time (14, 61, 250, and 370 days), litter type (bilberry, pine, and spruce), lime richness, and drought risk on

mass loss (%), carbon (%), nitrogen (%), C:N ratio, ergosterol (mg g^{-1}), acetophenones (mg g^{-1}), phenolic acids (mg g^{-1}), flavonoids (mg g^{-1}), stilbenes (mg g^{-1}), low molecular weight phenol (LMP) loss (mg g^{-1}), MeOH-soluble condensed tannins (CTs) (mg g^{-1}), MeOH-insoluble condensed tannins (CTs) (mg g^{-1}), and total amount of phenols (mg g^{-1}). Plot nested within transect were used as random factor, and model selection were based on lowest AIC. Overlap among 95% confidence intervals for significant litter-type effects were assessed. Similar analyses were performed for individual phenolic compounds for each litter type. To test for the effect of litter type on initial litter concentration, simple linear regression models followed by one-way ANOVA were used. To illustrate differences in phenolic compounds and fungal community composition for each species with time we performed global non-metric multidimensional scaling (GNMDS) following options and settings by Liu et al. (2008).

For **Paper II**, we performed linear mixed effects model, with subplot nested within plot nested within location as random factor, to examine the effect of litter type (birch, spruce), stand type (birch, spruce), location (Stranda, Ørsta, Jølster I, Jølster II), and climatic parameters on mass loss (%), carbon (%), nitrogen release, C:N ratio, ergosterol concentration (mg $g^{-1)}$ and loss of MeOH-soluble and MeOH-insoluble condensed tannins (%) after one year of decomposition. Temperature parameters were highly correlated, revealed by Pearson correlations, we therefore ran separate models for each climatic parameter and compared their AIC values to determine which variables to include in the final models based on lowest AIC. Similar analyses were used to test for the effect of stand type and location on climatic factors. Whereas simple linear regression models were used to test for the effect for stand type and location on initial litter properties. To examine if the plant litter decomposed faster when it decomposed at home versus away, we calculated the home-field advantage index (HFAI) following Ayres et al. (2009).

For **Paper III**, we tested the effect of forest type and soil depth on carbon (mg cm^{-3}), nitrogen (mg cm^{-3}), C:N ratio, acetone-soluble condensed tannins (mg cm^{-3}), ergosterol (mg cm^{-3}), pH values, and acetone-soluble condensed tannins:C and ergosterol:C ratios, with linear mixed-effects models using plot as random factor. To examine the relationship between the soil property data we used the Kendall's tau correlation test and matrix. To determine whether there were any significant differences between the sites in estimates

of total C and N stocks, we performed simple linear regression models followed by one-way ANOVA.

All statistical analyses were performed in R (R Core Team 2021), and graphical illustrations were generated in Veusz (Sanders 2020).

4 Main results and discussion

4.1 Synchronic shifts in composition of phenolic compounds and fungal communities

In **Paper I**, we present a time course in phenolic compound and fungal community compositions of boreal forest litter that to our knowledge is not earlier studied. We found litter type to be the main factor influencing mass loss, underling the importance of litter quality on early-stage decay in boreal forest ecosystems. Bilberry litter had a rapid mass loss compared to that of pine and spruce litter in the initial phase, and the remaining mass of bilberry litter were found to be lower than that of pine litter at the end of the one-year decomposition experiment (Fig. 4.1**a**). Initial concentration of N, low molecular weight phenolic compounds, and condensed tannins correlated with mass loss. Thus, differences in mass loss between litter types reflected their initial N concentration (Cornwell et al. 2008, Makkonen et al. 2012).

The differences between litter types were most pronounced after 14 days of decomposition, a phase characterized by leaching of soluble compounds, as well as opportunistic microorganisms using low molecular weight phenols as an energy source (Swift et al. 1979, Chomel et al. 2016, Fierer et al. 2001, Schimel et al. 1998). The tree litter types did not differ in N release during the decomposition period (Fig. 4.1**c**). However, the three litter types produced different concentrations and compositions of low molecular phenolic compounds that changed with time of decay (Fig. 4.2**a-d**). Bilberry produced large quantities of low molecular weight phenolic compounds, especially phenolic acids and flavonoids, that were quickly released (Fig. 4.1**d**). In comparison, pine and spruce produced lower quantities of low molecular weight phenolic compounds, mainly flavonoids and stilbenes, some still present in small amounts at the end of the one-year decomposition. Similar to low molecular weight phenolic compounds, MeOH-soluble condensed tannins were rapidly lost from all litter types (Fig. 4.1**e**). Leaf toughness may be part of the explanation for the strong initial loss of phenolics from the deciduous bilberry compared with the conifer species (Gallardo & Merino, 1993). Our results show that phenolic compounds may be a large part of the mass lost during the first stage of decomposition. After the two first weeks of decomposition, the difference in

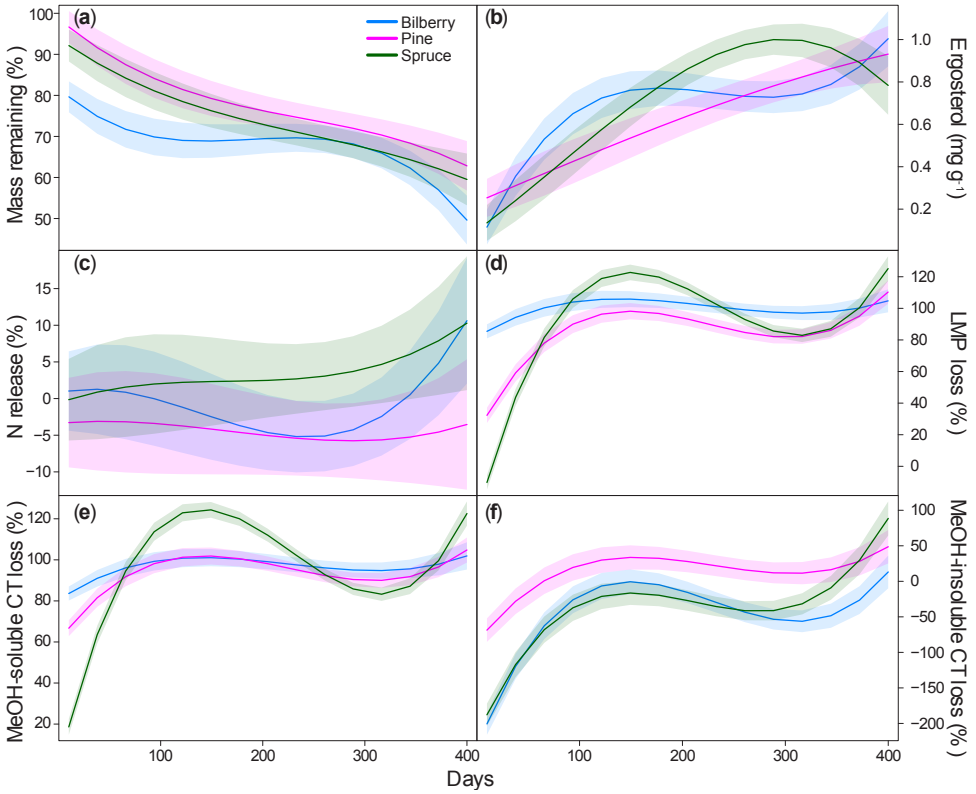

Figure 4.1. Predicted values of (**a**) mass remaining (%), (**b**) ergosterol (mg g^{-1}), (**c**) nitrogen release (%), (**d**) low molecular weight phenol (LMP) loss (%), (**e**) MeOH-soluble condensed tannin (CT) loss (%), and (**f**) MeOH-insoluble CT loss (%) of bilberry, pine, and spruce litter after 0, 14, 61, 250, and 370 days of soil incubation. Predictions are based on simple linear models (using litter type × time). Shaded areas represent 95% confidence intervals.

mass loss among the litter types were reduced. The remaining phenolic compounds consisted mainly of the MeOH-insoluble condensed tannins fraction throughout the rest of the decomposition period (Fig. 4.2**d**). This suggests an increasing role of condensed tannins in controlling mass loss rates at later stages (Chomel et al. 2016). Moreover, the distinct increase in MeOH-insoluble condensed tannins after the initial decomposition stage may be a result of the MeOH-soluble fraction changing into forms that are MeOH-insoluble or that parts of the MeOH-insoluble fraction are present in undetectable forms before decay (Fig. 4.1**f**).

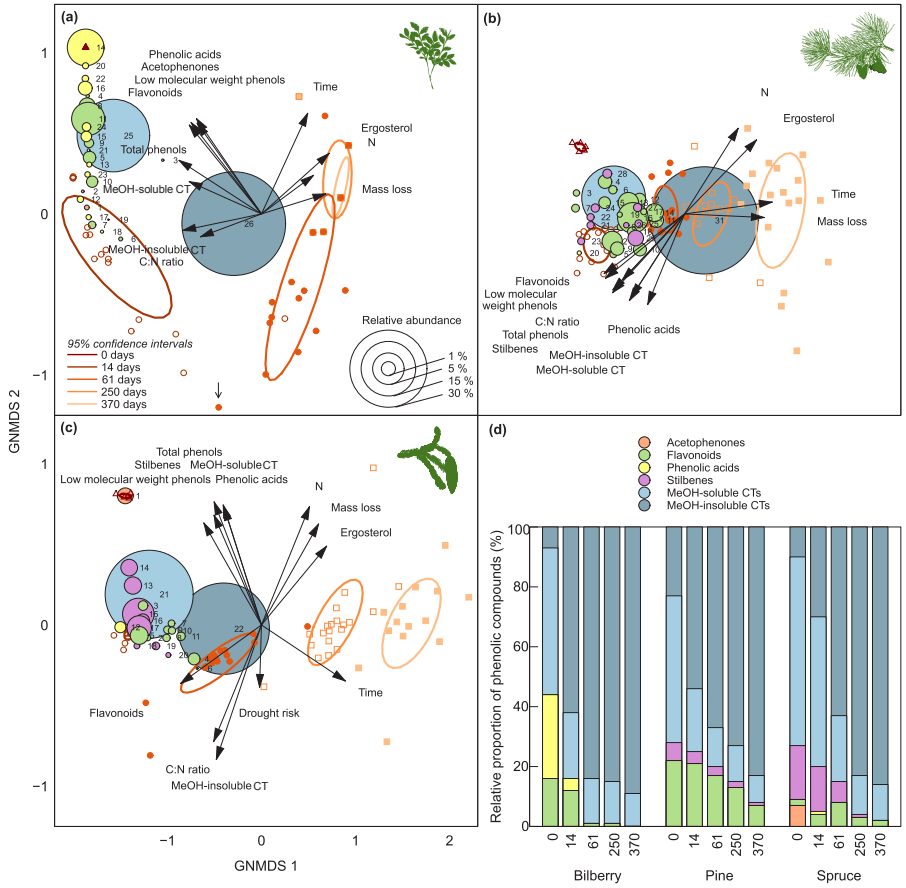

Figure 4.2. Two-dimensional global non-metric multidimensional scaling (GNMDS) ordination plots of the phenolic compound composition in **(a)** bilberry, **(b)** pine, and **(c)** spruce litter after 0, 14, 61, 250, and 370 days after incubation in the soil, based on all identified phenolic compounds (26, 31, and 22, respectively). Stress values were 0.07, 0.06, and 0.03, respectively. Ellipses represents 95% confidence intervals (based on SD) for time-point centroids, with symbols in the corresponding colours representing samples. Arrows indicate the direction of maximum increase for the variable represented by the vector in question, and vector length is proportional to the correlation between the variables and the ordination axes (only vectors significant at the $\alpha < 0.05$ level are shown). Each dot represents one phenolic compound, dot colour shows affiliation to phenolic group, and dot size is proportional to the relative abundance of the compound. Numerical prefixes identify all phenolic compounds (listed in Supporting Information Table S1–S3). Relative abundance of phenolic compounds **(d)** in bilberry, pine, and spruce litter after incubation in the soil for 0, 14, 61, 250, and 370 days.

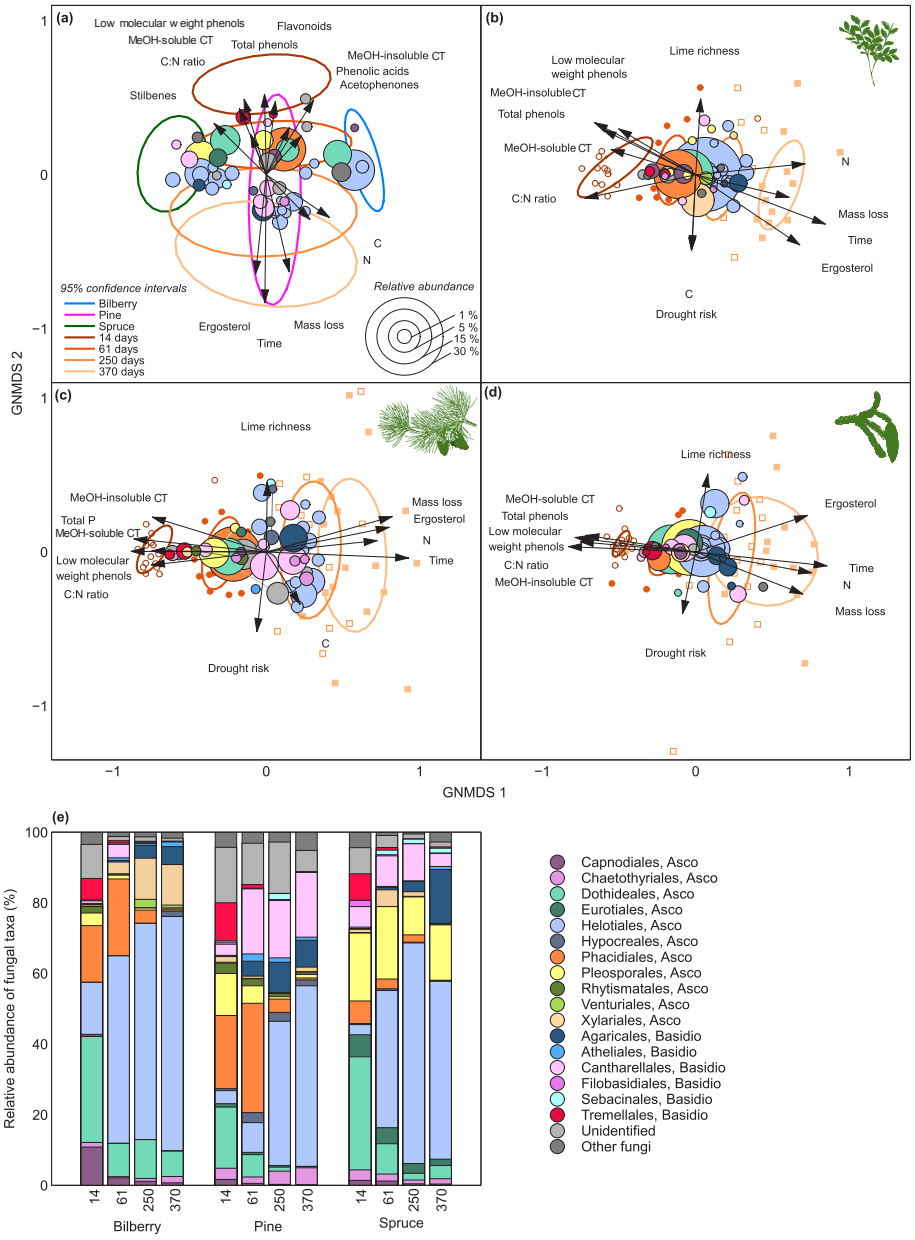

Figure 4.3. Two-dimensional global non-metric multidimensional scaling (GNMDS) ordination plots of fungal community composition in **(a)** all litter types, **(b)** bilberry, **(c)** pine, and **(d)** spruce litter after 14, 61, 250, and 370 days after incubation in the soil. Stress values were 0.20, 0.12, 0.13, and 0.12, respectively. Ellipses represents 95% confidence intervals (based on SE) for time-point centroids, with symbols in the corresponding colours representing samples. Arrows indicate the direction of maximum increase for the variable represented by the vector in question,

and vector length is proportional to the correlation between the variables and the ordination axes (only vectors significant at the $\alpha < 0.05$ level are shown). Low weight molecular phenol groups are only included as vectors in the ordination plot for all species (a). Each dot represents one OTU, dot colour shows affiliation to taxonomic group, and dot size is proportional to the number of reads, and only the 50 most common OTUs are visualized. Numerical prefixes that identify all OTUs are presented in Supporting Information Figure S1. Relative abundance of fungal taxa (based on number of reads) **(e)** in bilberry, pine, and spruce litter after incubation in the soil after 14, 61, 250, and 370 days.

We observed shifts in composition of fungal community during litter decomposition that were synchronous with the changes in phenolic compound concentration (Fig. 4.3**a-e**). We found that ascomycetes were present throughout the decomposition process, while basidiomycetes increased towards later stages. Moreover, a more or less similar increase in fungal biomass (ergosterol) with time of decomposition were found (Fig.4.1**b**). The impact of pathogenic and endophytic fungi together with large differences in the initial litter quality among the litter types, may explain the differences found in fungal community composition. Interestingly, the environmental gradient of lime richness and drought risk did not influence litter mass loss. The results of this study give new insights in the importance of litter quality for the decomposition process, especially with regards to the importance of phenolic compounds.

4.2 Differences in early-stage decomposition rates

In **Paper II**, we found that litter type, i.e., tree species, was the most important driver of differences in litter mass loss between stands of native birch and planted spruce. After one year of decomposition, the mass loss of birch litter was higher than that of spruce litter and both litter types had a lower mass loss in spruce compared to birch stands (Fig. 4.4**a**). This coincides with previous studies that have found broadleaved leaf litter to decompose faster than conifer needle litter in the early decay phase (Cornwell et al. 2008, Prescott et al. 2000). The difference in mass loss between litter types was reflected in higher initial concentrations of N and condensed tannins of birch litter, which is in line with the common assumption of faster decomposition of N-rich litter (Melillo et al. 1982). Differences in leaf toughness and initial concentration of water-soluble compounds may have contributed strongly to the mass loss in the early decay stage since water-soluble

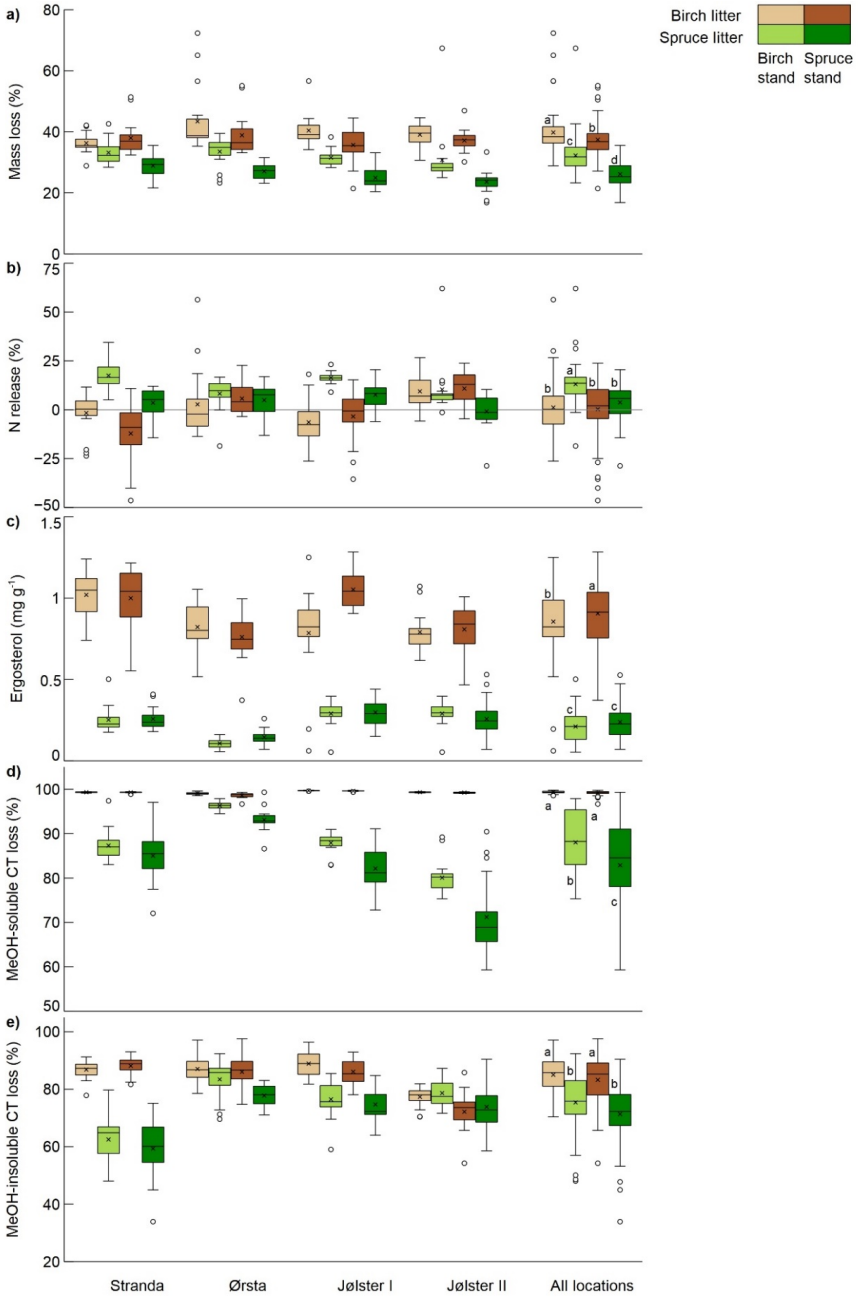

Figure 4.4. Boxplots of **(a)** mass loss (%), **(b)** nitrogen release (%), **(c)** ergosterol concentration (mg g^{-1}), **(d)** MeOH-soluble condensed tannins (CT) loss (%), and **(e)** MeOH-insoluble CT loss (%) in litterbags of birch and spruce litter after one year of decomposition in birch and spruce stands at all locations (Stranda, Ørsta, Jølster I, Jølster II), as well as for all locations combined. Boxplots marked with contrasting letters indicate significant differences (Tukey, $P < 0.05$) for all locations combined.

compounds are released faster from leaf litter compared to needle litter (Johansson 1995, Nykvist 1963).

The early stage of litter decomposition is often N limited (Berg 2000, Parton et al. 2007). However, we found a general trend of N release after one year of decomposition (Fig. 4.4**b**). Fungal biomass was significantly higher in birch litter after the one-year decomposition experiment, indicating higher fungal activity in birch litter compared to spruce litter (Fig. 4.4**c**). The MeOH-soluble condensed tannin loss was almost total for birch litter in both stand types, while the loss was lower for spruce litter with higher loss in birch stands (Fig. 4.4**d**). Thus, loss of MeOH-soluble condensed tannins may explain a large part of the difference in mass loss between litter types. In contrast, concentration of the MeOH-insoluble fraction of condensed tannins was lower with higher loss in birch compared to spruce litter (Fig. 4.4**e**). This suggests that some condensed tannins are more recalcitrant than others. Moreover, the structures and complexities of condensed tannins from spruce may be different from that of birch litter. Condensed tannin-dynamics may be important for differences in litter decomposition, thus more insight into the chemistry of tannins of different species is needed.

Although the birch litter had faster litter decomposition in birch stands, the greater advantage of spruce litter decomposing in birch compared to spruce stands resulted in a net negative home-field advantage (HFA) (Fig. 4.5). Our findings oppose the dominant trend of HFA in similar studies (Ayres et a. 2009), but others have also found lower litter mass loss HFA effects for conifer species compared to broadleaves and faster decomposition of conifer litter in broadleaved forests (Wang et al. 2013, Prescott et al. 2000). Interestingly, we found that litter type and stand type overshadowed small scale variation in climatic variables. Our results indicate that a tree species change from native birch-dominated forest to planted spruce forest will result in slower decomposition rates. This is consistent with previous studies in the current study system that has found the shift from native birch to planted spruce to cause changes in fungal community composition and higher C stocks in the forest floor of spruce compared to birch stands (Kjønaas et al. 2021, Mundra et al. in review).

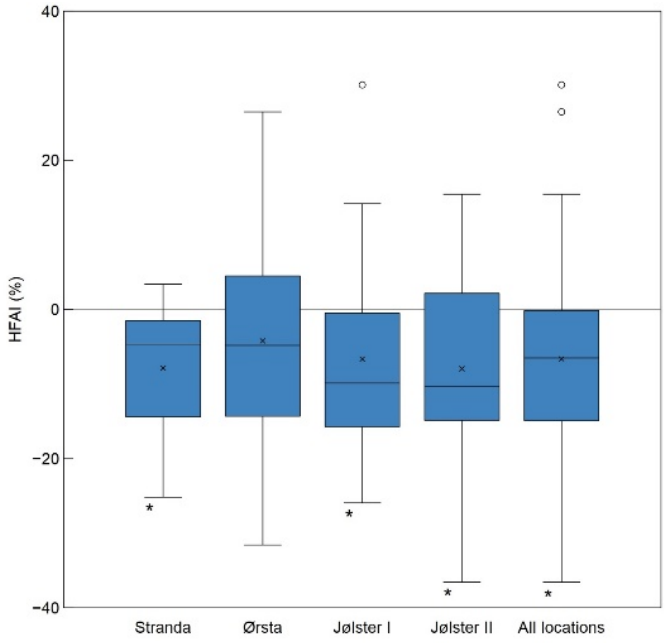

Figure 4.5. Home-field advantage index (HFAI) calculated for all locations (Stranda, Ørsta, Jølster I, Jølster II, and all locations combined), significant values are indicated (*).

4.3 Vertical distribution differences of soil properties

In **Paper III**, we found that estimates of total C and N stocks were similar for the organic soil layer, the mineral soil layer, and the whole soil profile in the three forests (Fig. 4.6). As soils under beech in central Europe have been found to store less C compared to spruce (Cremer et al. 2016), this suggest that beech forest soils at its northern distribution limit are different from areas where beech has its main distribution. The distribution of C and N roughly declined with increasing soil depth throughout the soil profile for all three forests. However, the vertical distribution of C and N varied significantly between the beech forest and the spruce forest planted on former beech forest, while the spruce forest was intermediate (Fig. 4.7**a-b**). Concentration of ergosterol and condensed tannins generally declined with increasing soil depth, while soil pH mainly increased with increasing depth in all three forests (Fig. 4.7**d-f**). Moreover, the distribution of fungal biomass along the soil profile in the beech forest differed significantly from the two other forests. Fungal biomass has been found to be one of the major contributors to the

accumulation of C in the boreal forest soils (Clemmensen et al. 2013), our results may therefore indicate that fungal biomass drives the observed differences in soil C among the forests. The findings imply that an eventual transformation from beech to spruce forest is likely to change the vertical distribution of soil C in boreal forest soils.

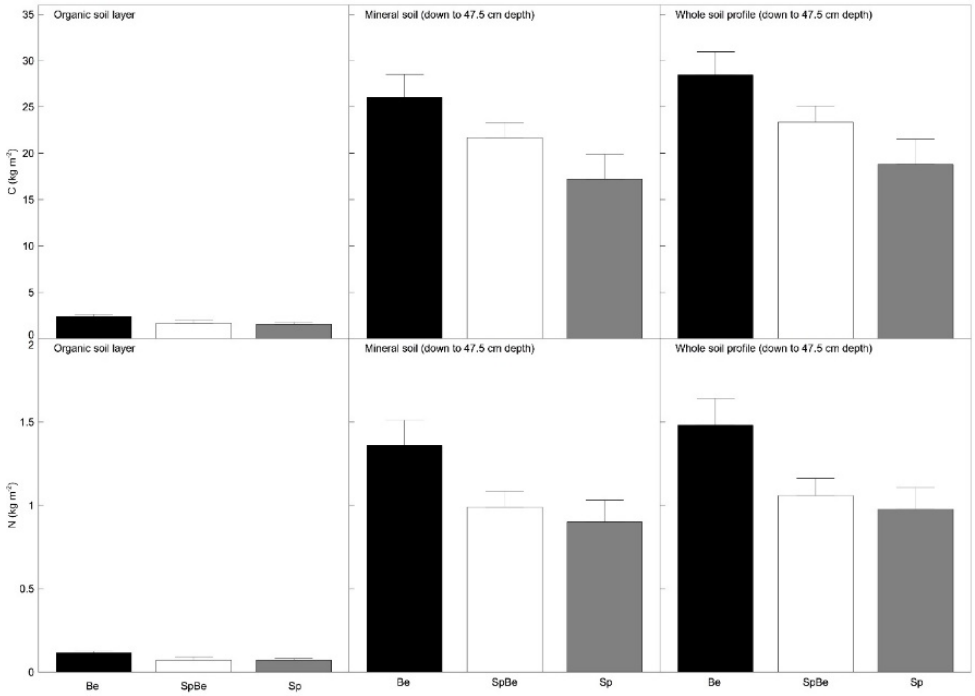

Figure 4.6. Mean (± 1 SE) for estimates of total carbon (C) and nitrogen (N) stocks at beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp) in the organic soil layer, the mineral soil, and the whole soil profile.

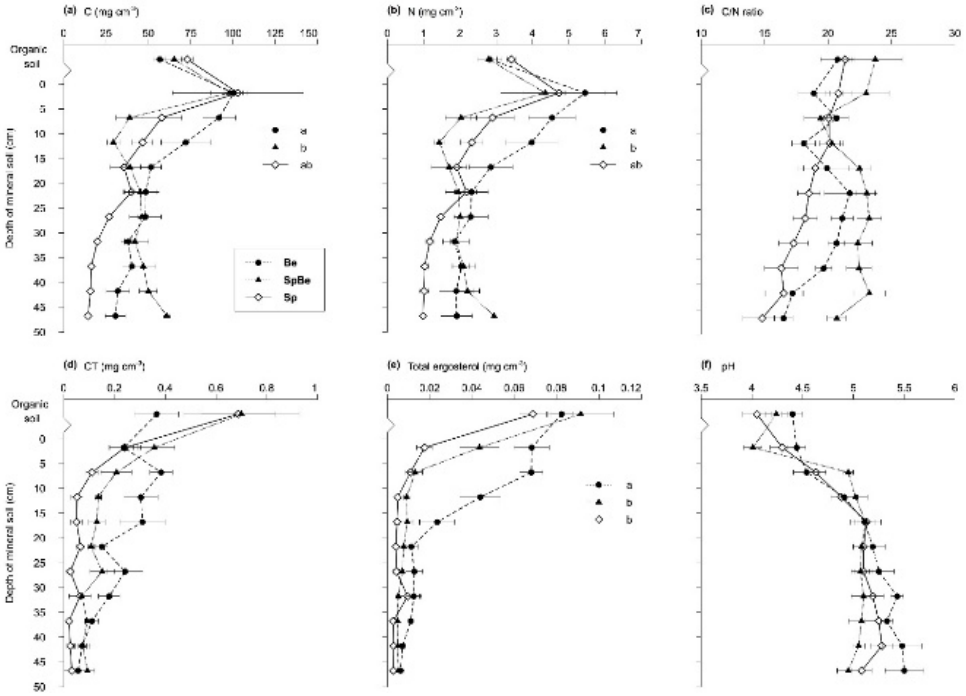

Figure 4.7. Mean (± 1 SE) for **(a)** carbon (C), **(b)** nitrogen (N), **(c)** C/N ratio, **(d)** condensed tannins (CT), **(e)** total ergosterol, and f pH values displayed at different soil depths (organic soil, mineral soil 0–47.5 cm) at beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp). n = 4, except for SpBe for which n = 2 and Sp for which n = 3 at the deepest soil depth (45–47.5 cm). For each graph, lines marked with contrasting letters indicate significant differences between forests (Tukey, $P < 0.05$).

5 Concluding remarks and implications

This thesis has revealed differences in tree species effects on litter decomposition and soil carbon. The main conclusion that can be drawn from the studies in this thesis is that litter quality is important in regulating the early-stage decomposition of plant litter (**Papers I** and **II**). We provide detailed analyses of phenolic compounds in decomposing litters, which to our knowledge only a few studies have done before. Furthermore, a change in the dominant tree species can be expected to change the rates of litter decomposition (**Paper II**), as well as alter the vertical distribution of soil chemical characteristics (**Paper III**).

The early-stage decomposition investigated in **Papers I** and **II** give insights into the nutrient release at the early stage when a substantial fraction of the carbon is lost from the plant litter. The concentration of C in the litter was relatively constant, and the loss of C therefore followed the same pattern as mass loss with increasing time of decay. In line with previous studies (Averill & Warning 2017), litter with high initial N concentration decomposed more rapidly. In general, we found a minor release of N after the one-year decomposition in **Papers I** and **II**. Moreover, results from both **Papers I** and **II** indicate that initial phenolic compounds also are positively related to early mass loss rates. Low molecular weight phenolic compounds were quickly released from the litter, although a few individual phenolic compounds were still present in small amounts at the end of the one-year decomposition in **Paper I**. The two fractions of condensed tannins were found to play opposing roles in the decomposition process. MeOH-soluble condensed tannins were quickly lost in the initial decay phase, while the loss of the MeOH-insoluble fraction was slower, and a higher amount remained in the litter after one year of decomposition in **Papers I** and **II**. This suggests an increasing role of condensed tannins in regulating decay rates once the importance of other drivers (e.g., N, and low molecular weight phenolics) declines (Chomel et al. 2016). The separation of the two fractions tells us that some condensed tannins are easily released from litter, while others are more recalcitrant. Condensed tannins are difficult to characterize down to single compounds and are therefore commonly analysed in bulk assays like in this thesis. Thus, there is a need for more detailed studies of condensed tannins to understand the significance of these compounds for the differences found among species in litter decomposition. In

addition, we found a notable increase in MeOH-soluble condensed tannins in the initial decomposition stage in **Paper I.** The general trend in loss of chemical components measured in our decomposition studies is presented in Fig. 6.1.

However, the relatively short time span of the litter decomposition experiments enables us to examine only the early stages of the decomposition processes. Thus, long-term decomposition studies are necessary to evaluate the role of condensed tannins at later stages of decomposition. In **Paper III**, we show that condensed tannins remain in the soil and are found throughout the soil profile, which illustrates the need for detailed studies of condensed tannins to understand tree species influence on soil C. In addition, patterns of early-stage mass loss do not necessarily reflect similar patterns for final mass remaining after litter decomposition (Prescott & Vesterdal 2021). The importance of litter quality in early stage decomposition is further supported by our findings that litter type effects on litter mass loss overshadowed the effects of the environmental gradient in **Paper I**, and the effects of the microclimatic conditions in **Paper II**.

In the decomposition experiments, fungal biomass in the litter increased with time of decay for all litter types (**Papers I** and **II**), indicating higher fungal activity. While we found no difference in fungal biomass concentration among litter types after one year of

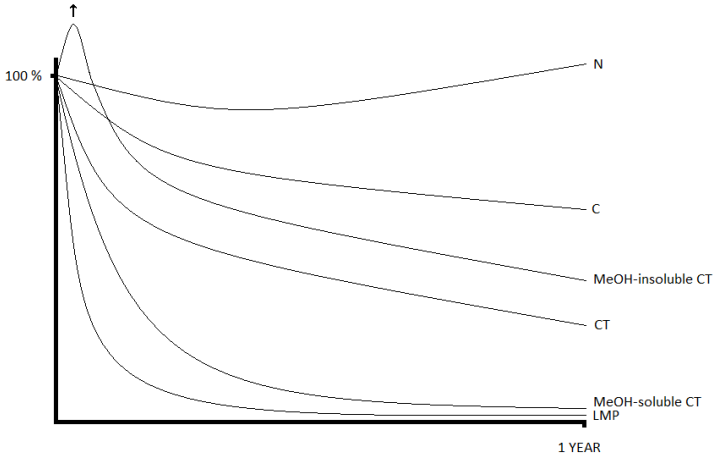

Figure 6.1. Illustration of the general trend in changes of carbon (C), nitrogen (N), low molecular weight phenolic compounds (LMP), MeOH-soluble condensed tannins (CT), MeOH-insoluble CT, and CT (both fractions combined) at the early-stage decomposition (first year). The arrow indicates an increase of more than 100% in MeOH-insoluble CT at the initial decomposition stage.

decomposition in **Paper I**, we found a significant difference between the litter types in **Paper II**. Thus, the findings are context and site-specific. It is also worth mentioning that the difference in placement of litterbags in the soil may have affected the amount left of fungal biomass after decomposition (5 cm into the soil *vs* on the soil surface in **Papers I and II**, respectively). Further, the importance of fungal biomass for C accumulation in forest soils were highlighted in **Paper III,** where differences in fungal biomass likely explain the tree species effects on vertical soil C distribution. Moreover, we observed a synchronous shift in species-specific low molecular weight phenolic compounds and fungal community composition with time of decay in **Paper I**. This finding calls for further exploration of the direct effects that groups of phenolic compounds have on soil organisms.

Tree species have been found to primarily influence the vertical distribution of soil carbon (Vesterdal et al. 2013), which corresponds with our findings in **Paper III.** Moreover, our results support the idea that the species-specific rate of litter decomposition explain the effects that tree species appear to have on soil C stocks (Hansen et al., 2009, Vesterdal et al. 2008). A slower mass loss of the conifer plant litter compared to the broadleaved species in **Paper II** have also been found in the study system of **Paper III**. In a similar transplanting decomposition experiment to that of **Paper II**, Asplund et al. (2018) found spruce needles to decompose at a slower rate compared to that of beech leaves. Decomposition rates were slower in the spruce forest in both study systems. Thus, the slower decomposition rates of spruce litter correlate with the higher accumulation of soil C found in the forest floor of the spruce forests. However, in the study systems of **Paper II** and **III** it has been found the total C stocks are similar between the compared forest types (beech vs spruce and birch vs spruce, respectively). Future studies are needed to investigate tree species change on soil C as it appears complex and is far from fully understood (Jandl et al. 2007, Vesterdal et al. 2013).

6 References

Adamczyk, B., Sietiö, O.M., Biasi, C. & Heinonsalo, J. 2019. Interaction between tannins and fungal necromass stabilizes fungal residues in boreal forest soils. *New Phytologist* 223: 16–21. https://doi.org/10.1111/nph.15729

Asplund, J., Kauserud, H., Bokhorst, S., Lie, M.H., Ohlson, M. & Nybakken, L. 2018. Fungal communities influence decomposition rates of plant litter from two dominant tree species. *Fungal Ecology* 32: 1–8. https://doi.org/10.1016/j.funeco.2017.11.003

Augusto, L., De Schrijver, A., Vesterdal, L., Smolander, A., Prescott, C. & Ranger, J. 2015. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. *Biological Reviews* 90: 4444–466. https://doi.org/10.1111/brv.12119

Averill, C. & Waring, B. 2017. Nitrogen limitation of decomposition and decay: How can it occur? *Global Change Biology* 24: 1417–1427. https://doi.org/10.1111/gcb.13980

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C. & Wall, D.H. 2009. Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biology & Biochemistry* 41: 606–610. https://doi.org/10.1016/j.soilbio.2008.12.022

Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. *Forest Ecology and Management 133*: 13–22. https://doi.org/10.1016/S0378-1127(99)00294-7

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., Jackson, B.G. & Baldy, V. 2016. Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. *Journal of Ecology*. https://doi.org/10.1111/1365-2745.12644

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., Lindahl, B.D. 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist* 205: 1525–1536. https://doi.org/10.1111/nph.13208

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Bart Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Ian J., Wright, I.J., Aerts, R., Allison, S.D., van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V. & Westoby, M. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11: 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

Coûteaux, M.-M., Bottner, P. & Berg, B. 1995. Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution* 10: 63–66. https://doi.org/10.1016/S0169-5347(00)88978-8

Cremer, M., Kern, N.V. & Prietzel, J. 2016. Soil organic carbon and nitrogen stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. Forest Ecology and Management 367: 30–40. https://doi.org/10.1016/j.foreco.2016.02.020

Deluca, T.H. & Boisvenue, C. 2012. Boreal forest soil carbon: distribution, function and modelling. *Forestry* 85: 161–184. https://doi.org/10.1093/forestry/cps003

Fierer, N., Schimel, J.P., Cates, R.G. & Zou, J. 2001. Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biology and Biochemistry* 33: 1827–1839. https://doi.org/10.1016/S0038-0717(01)00111-0

Gallardo, A. & Merino, J. 1993. Leaf decomposition in two Mediterranean ecosystems of southwest Spain: influence of substrate quality. *Ecology* 74: 152-161. https://doi.org/10.2307/1939510

Granhus, A., Hylen, G. & Nilsen J.-E.N. 2012. Skogen i Norge. Statistikk over skogforhold og skogressurser i Norge registrert i perioden 2005-2009. Norwegian National Forest Inventory. Ressursoversikt fra Skog og landskap :03/2012. (*In Norwegian*)

Halvorsen, R., Skarpaas, O., Bryn, A., Bratli, H., Erikstad, L, Simensen, T. & Lieungh, E. 2020. Towards a systematics of ecodiversity: The EcoSyst framework. Global Ecology and Biogeography 29: 1887–1906. https://doi.org/10.1111/geb.13164

Hansen, K., Vesterdal, L., Schmidt, I.K., Gundersen, P., Sevel, L., Bastrup-Birk, A., Pedersen, L.B. & Bille-Hansen, J. 2009. Litterfall and nutrient return in five tree species in a common garden experiment. *Forest Ecology and Management* 257: 2133–2144. https://doi.org/10.1016/j.foreco.2009.02.021

Haase, K. & Wantzen, K. M. 2008. Analysis and decomposition of condensed tannins in tree leaves. *Environmental Chemistry Letters* 6: 71-75. https://doi.org/10.1007/s10311-008-0140-7

Hickler, T., Vohland, K., Feehan, J., Miller, P.A., Smith, B., Costa, L., Giesecke, T., Fronzek, S., Carter, T.R., Cramer, W., Kühn, I. & Sykes, M.T. 2012. Projecting the future distribution of European potential natural vegetation zones with a generalized, tree species-based dynamic vegetation model. *Global Ecology and Biogeography* 21: 50–63. https://doi.org/10.1111/j.1466-8238.2010.00613.x

Jandl, R., Lindner, M., Bauwens, B., Vesterdal, L., Baritz, R., Hagedorn, F., Johnson, D.W., Minkkinen, K., Byrne & K.A. 2007. How strongly can forest management influence soil carbon sequestration? *Geoderma* 137: 253–268. https://doi.org/10.1016/j.geoderma.2006.09.003

Johansson, M.-B. 1995. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. *Forestry* 68: 49–62. https://doi.org/10.1093/forestry/68.1.49

Kjønaas, O.J., Bárcena, T.G., Hylen, G., Nordbakken, J.-F. & Økland, T. 2021. Boreal tree species change as a climate mitigation strategy: impact on ecosystem C and N stocks and soil nutrient levels. *Ecosphere* 12: e03826. https://doi.org/10.1002/ecs2.3826

Kraus, T.E.C., Dahlgren, R.A. & Zasoski, R.J. 2003. Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil* 256: 44–61. https://doi.org/10.1023/A:1026206511084

Lindahl, B.D., Kyaschenko, J., Varenius, K., Clemmensen, K.E., Dahlberg, A., Karltun, E. & Stendahl, J. 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. *Ecology Letters* 47: 1341–1351. https://doi.org/10.1111/ele.13746

Liu, H., Økland, T., Halvorsen, R., Gao, J., Liu, Q., Eilertsen, O. & Bratli, H. 2008. Gradients analyses of forests ground vegetation and its relationships to environmental variables in five subtropical forest areas, S and SW China. *Sommerfeltia* 32: 3–196. https://doi.org/10.2478/v10208-011-0012-6

Makkonen, M., Berg, M.P., Handa, I.T., Hättenschwiler, S., van Ruijven, J., van Bodegom, P.M. & Aerts, R. 2012. Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecology Letters* 15: 1033–1041. https://doi.org/10.1111/j.1461-0248.2012.01826.x

Melillo, J.M., Aber, J.D. & Muratore, J.F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63: 621–626. https://doi.org/1936780

Mundra, S., Kauserud, H., Økland, T., Nordbakken, J.-F., Ransedokken, Y. & Kjønaas, O.J. Shift in tree species leads to dramatic changes in the belowground biota in boreal forests. *In review.*

Mutabaruka, R., Hairiah, K. & Cadisch, G. 2007. Microbial degradation of hydrolysable and condensed tannin polyphenol–protein complexes in soils from different land-use histories. *Soil Biology and Biochemistry* 39: 1479–1492. https://doi.org/10.1016/j.soilbio.2006.12.036

Nykvist, N. 1963. Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. *Studia forestalia Suecica* 3: 33.

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, D.,Piao, S., Rautiainen, A., Sitch, S. & Hayes, D. 2011. A Large and Persistent Carbon Sink in the World's Forests. *Science* 333: 988–993. DOI: 10.1126/science.1201609

Parton, W., Silver, W. L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C. & Fasth, B. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* 315: 361–364. https://doi.org/10.1126/science.1134853

Prescott, C.E. & Vesterdal, L. 2021. Decomposition and transformations along the continuum from litter to soil organic matter in forest soils. *Forest Ecology and Management* 498:119522. https://doi.org/10.1016/j.foreco.2021.119522

Prescott, C.E., Zabek, L.M., Staley, C.L. & Kabzems, R. 2000. Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures. *Canadian Journal of Forest Research* 30: 1742–1750. https://doi.org/10.1139/x00-097

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ransedokken, Y. 2016. Challenging conventional carbon wisdom: a climate driven vegetation shift from spruce to beech may not change the size of the carbon pool in southeast Norwegian forest soils. Master thesis. Norwegian University of Life Sciences, Ås.

Sanders J. 2020. Veusz – a scientific plotting package. https://veusz.github.io/

Schimel, J.P., Cates, R.G. & Ruess, R. 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry* 42: 221–234. https://doi.org/10.1023/A:1005911118982

Swift, M.J., Heal, W. & Anderson, J.M. 1979. Decomposition in Terrestrial Ecosystems, Blackwell Scientific Publications, Oxford.

Thoss, V., Shevtsova, A., Nilsson, M.C. 2004. Environmental manipulation treatment effects on the reactivity of water-soluble phenolics in a subalpine tundra ecosystem. *Plant and Soil* 259: 355–365. https://doi.org/10.1023/B:PLSO.0000020984.17403.82

Vesterdal, L., Clarke, N., Sigurdsson, B.D. & Gundersen, P. 2013. Do tree species influence soil carbon stocks in temperate and boreal forests? *Forest Ecology and Management* 309: 4–18. https://doi.org/10.1016/j.foreco.2013.01.017

Vesterdal, L., Schmidt, I.K., Callesen, I., Nilsson, L.O. & Gundersen, P. 2008. Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management* 255: 35–48. https://doi.org/10.1016/j.foreco.2007.08.015

Voříšková, J. & Baldrian, P. 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. *The ISME Journal* 7: 477–486. https://doi.org/10.1038/ismej.2012.116

Wang, Q., Zhong, M. & He, T. 2013. Home-field advantage of litter decomposition and nitrogen release in forest ecosystems. *Biology and Fertility of Soils* 49, 427–434. https://doi.org/10.1007/s00374-012-0741-y

Wardle, D.A. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton.

Økland, R.H. & Eilertsen, 0. 1993. Vegetation-environment relationships of boreal coniferous
forests in the Solhomfjell area, Gjerstad, S Norway. Sommerfeltia 16: 1–254. Oslo. ISBN 82-
7420-018-7. ISSN 0800-6865.

Paper I

Synchronic shifts in phenolic compounds and fungal communities during litter decomposition in boreal forests

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Submitted manuscript

Summary

- Phenolic compounds in plant litter influences decomposition processes both directly by phenols degree of decomposability and indirectly by affecting decomposer processes. While phenolic characteristics of aboveground plant biomass is well-known, the fate of litter phenolics and their regulatory effects on belowground decomposition remain unclear.
- We conducted a one-year decomposition experiment distributing litterbags of spruce, pine, and bilberry along a gradient from mesic spruce-dominated to xeric pine-dominated forests. Litter material was analysed for mass loss, low molecular weight phenolic compounds, condensed tannins, fungal biomass, and fungal community composition (using DNA metabarcoding).
- Bilberry litter initially decayed more rapidly than the conifer litter, and remaining mass of bilberry litter was significantly lower than that of pine litter after one year. The concentration and composition of phenolic compounds decreased with time independent of litter type, with mainly MeOH-insoluble condensed tannins remaining at the end of the experiment. As fungal biomass increased with time, all litter types underwent rapid changes in fungal community composition.
- The species-specific pattern of temporal change in mass loss, as well as in the synchronic shift in composition of phenolic compounds and fungal community, give new insights in the importance of litter quality for the decomposition process.

Key words: condensed tannins, decomposition, fungal communities, litter quality, phenolic compounds, plant litter

Introduction

Litter decomposition plays a fundamental role in carbon (C) and nutrient cycling in boreal forests. In general, short growing seasons with cold temperatures lead to low decomposition rates, which in turn enables C sequestration and long-term C storage (Prescott, 2010; Clemmensen *et al*., 2015). While climate is an important factor of decay rates at the global scale, litter quality and soil organisms are important drivers at the ecosystem scale (Aerts, 1997; Bradford *et al*., 2016; Wickings *et al*., 2012).

Litterfall supplies organic matter to the soil surface. The foliar plant litter breakdown process is complex, determined mainly by litter chemistry, microbial decomposer communities, and environmental conditions (Bradford *et al*., 2016; Gessner *et al*., 2010). Boreal forest plants are typically nutrient conservative and in addition to high lignin content, they allocate a significant proportion of their C to produce a broad range of secondary metabolites for chemical defence (Thoss *et al*., 2004). Phenolic compounds, an ecologically important class of such defence metabolites, are found in high concentrations in boreal forest plants, and are thought to play important roles during decomposition (Hättenschwiler & Vitousek 2000; Chomel *et al*., 2016). However, decomposition of phenolic compounds in plant litter and their interaction with decomposer organisms are highly multifaceted processes (Hättenschwiler *et al*., 2005), and there is a general need for more knowledge about how, and to what extent, different groups of phenolic compounds regulate decomposition rates in boreal forests under variable environmental conditions.

In a recent review, Chomel *et al*. (2016) proposed the following time course for loss of major chemical constituents of plants litter: In the early decomposition process, low molecular weight phenolic compounds, like phenolic acids, flavonoids, and stilbenes, will typically be released along with highly labile and readily degradable compounds (e.g., soluble carbohydrates). Subsequently, recalcitrant phenolics, such as condensed tannins with relatively high molecular weights, are released together with other recalcitrant compounds (e.g., lignin) until only undegradable litter remains. Thus, condensed tannins are expected to be present longer in the litter than low molecular weight phenolics. Tannins affect soil microbial processes by various mechanisms that slow down decomposition rates, including complexing with proteins or metals, affecting C cycling and immobilizing N, altering enzyme activity, and inhibiting of microorganisms (Adamczyk *et al*., 2018; Kraus *et al*., 2003; Smolander *et al*., 2012). In woody species, condensed tannin concentrations commonly range from 15 to 25% of dry foliar weight

(Kraus *et al*., 2003), potentially contributing to stable soil C accumulation. Moreover, tannins have been found to potentially form complexes with fungal proteins, chitin, and necromass, and may as such stabilize fungal-derived C in boreal forest soils (Adamczyk *et al*., 2019).

Fungi, the primary decomposers of plant litter in boreal forest soils, succeed each other during the decomposition process (Purahong *et al*., 2016; Tang *et al*., 2005). The fungal community composition shift from generalist fungi consuming freshly, abounding soluble litter compounds to more specialist fungi mining recalcitrant compounds during the decomposition process (Moorhead & Sinsabaugh, 2006). While saprotrophic ascomycetes dominate in early stages of litter decomposition, basidiomycetes become dominant with increasing decay due to their ability to produce lignin-modifying enzymes (Vivelo *et al*., 2019; Voriskova & Baldrian, 2013). Furthermore, fungal communities present in the phyllosphere before the litter shedding can impact the litter decomposition rates. This may be by altering the composition of decomposer community or by endophytes that grow in the living plant tissue turning into saprotrophs in the early phase of decomposition (Fanin *et al*., 2021; Purahong & Hyde, 2011).

Although plant phenolic compounds are suggested to have important roles during decomposition (Hättenschwiler & Vitousek, 2000; Chomel *et al*.; 2016), the degree to which different compounds in plant litter are recalcitrant during decomposition, or how their presence corresponds with change in litter C- and N pools, and succession of fungal communities, are poorly known. The main aim of this study was to investigate how plant phenolic compounds and fungal community composition change over time with decomposition across an ecological gradient in boreal forests. To accomplish this, we conducted a one-year litterbag experiment where we incubated foliar litter from bilberry (*Vaccinium myrtillius* L.), Scots pine (*Pinus sylvestris* L.; hereafter pine), and Norway spruce (*Picea abies* (L.) Karst.; hereafter spruce) in the forest floor and harvested the litterbags at four different time points. Spruce, pine, and bilberry leaves contain different concentrations and compositions of phenolics (Nybakken *et al*., 2013; 2018; Turtola *et al*. ,2006). We predicted that (i) the concentration and composition of phenolic compounds will change with decay stage and vary among litter types. More precisely, low molecular weight phenolic compounds are expected to be quickly leached, while condensed tannins are retained longer in the plant litter (Chomel *et al*., 2016). Accordingly, we predict that (ii) litter type, reflecting differences in litter quality (e.g., initial C:N ratio and phenolic compounds), and position along the local environmental gradient will be the main determinants of mass loss rates. Further, the variation in litter quality during the decomposition

is likely to impact decomposer organisms. Therefore, we expect (iii) the fungal biomass to increase and fungal community composition of plant litter to shift in pace with changes in phenolic compound composition during decomposition. More specifically, differences in litter quality among litter types are likely to influence the timing of fungal community shifts.

Materials and Methods

Study site

The study was conducted in boreal forests of the Solhomfjell and Kvenntjønnane nature reserve (Gjerstad, Agder, Norway 58°57'N, 8°50'E, 350-475 m a.s.l.), covering variation from mesic spruce-dominated to xeric pine-dominated forests. The landscape is hilly, dominated by shallow soils (depths typically < 50 cm), and peatlands cover extensive areas. For a complete description of vegetation and environmental conditions, see Økland & Eilertsen (1993) and Framstad (2018). Average temperature and total precipitation in the area during the one-year study period were 6.0 °C and 1298 mm, respectively (MET 2021). At the study site, 8 transects of different lengths with a total of 100 semi-randomly, permanently marked 16 m^2 macro sample plots have been regularly studied with respect to vegetation and environment since 1988. We selected 18 of the 100 plots along three of the transects, ensuring that the variation along the major environmental gradients lime richness and drought risk in the boreal forest landscape (Økland & Eilertsen, 1993) was included in the subset. Along this gradient the dominant tree species shifts from spruce at low drought risk to pine at high drought risk (Fig. 1).

Litterbag experiment

Senesced pine and spruce needles from living branches were collected in March 2017 in Hobøl and Åsmåsan (Østfold and Akershus, respectively, Norway). Bilberry leaves were collected from living twigs in August 2017 in Siggerud (Akershus, Norway). Harvesting litter from localities off-site allowed us to exclude impact of interspecific variation in litter quality but maintain local decomposers and site conditions. After being air dried (>4 days), approximately 1 g of pine or spruce litter or 0.6 g of bilberry litter was placed in 5 × 5 cm sachets constructed of 50-micron masked nylon. To determine initial mass prior to decomposition, 5 × 1 g of litter from each species was oven dried (70°C, 48h) and the ratio between air and oven dried weights were calculated. In the field, 216 litterbags, 72 for each species, were distributed randomly on the 18 selected plots. For each plant species, four litterbags per plot were placed at 5 cm soil depth on September 10, 2017. The litterbags were retrieved after 14, 61, 250, and 370 days of

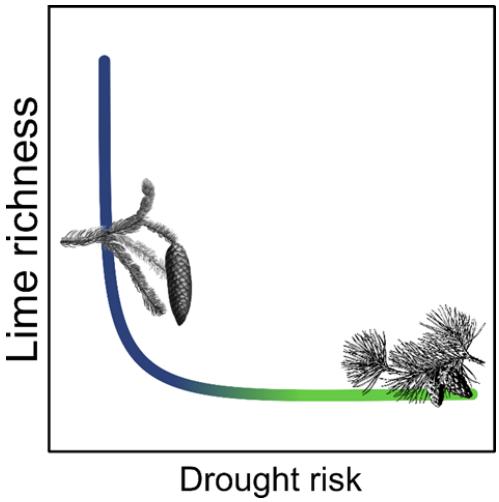


Figure 1. The plots are placed along two major environmental gradients. Along the gradient "drought risk" there is a shift in dominant tree species from Norway spruce (blue; *Picea abies*) to Scots pine (green; *Pinus sylvestris*). At low drought risk (dominated by spruce), there is an additional gradient in lime richness. The gradients are derived from an ordination of the ground vegetation and represent a shift in community composition from species thriving on mesic soils (e.g., *Vaccinium myrtillus*) to species less susceptible to drought (e.g., *V. vitis-idaea*). Drought risk and lime richness are assessed by use of ordinal scales with levels 1–8 and 1–9, respectively (Halvorsen et al. 2020).

incubation (September 25 and November 10, 2017, and May 18 and September 15, 2018, respectively). All samples were frozen immediately after collection and stored at –80°C at the lab until analysis. Nine litterbags were damaged or lost upon retrieval, leaving a total of 207 bags for further analyses. Prior to DNA and chemical analyses, all samples were freeze-dried for 48 h and ground into powder with a ball mill. Five subsamples of the initial litter from each species were used to determine the initial concentration of measured variables. Concentrations of C and N were measured using a vario MICRO cube elemental analyser (Elementar, Hanau, Germany). To calculate the amount of released N, the total mass × N concentration after incubation were subtracted from the initial mass × N concentration and expressed as the proportion of the initial mass × N concentration before incubation (Wardle, 2002).

Phenolic compounds analyses

For analysis of low molecular weight phenolics (LMP), between 50 and 100 mg of prepared litter sample, depending on the predicted LMP concentration for each litter type, was extracted with methanol (MeOH) as described by Nybakken *et al*. (2018). The residues from the extraction were stored at –20°C until further analysis of MeOH-insoluble condensed

tannins (CT). Dried MeOH extracts were re-dissolved in 100–200 µl MeOH and diluted with 100–200 µl of ultra-pure water (USF ELGA Maxima HPLC; Veolia Water Technologies, Saint-Maurice, France), depending on predicted LMP concentration. The extracts were analysed by injecting 20 µl on a 1200 Series HPLC (Agilent Technologies, Waldbronn, Germany) with a G1312A binary pump, a G1329A autosampler, a G1316A thermoregulated column heater, and a G1315D diode array detector. LMP were separated using a stationary phase ODS Thermo Scientific column (50 mm × 4.6 mm; particle size 3 µm). As the mobile phase, we used two solvents that eluted the samples by way of a gradient, as described by Julkunen-Tiitto & Sorsa (2001). Absorption spectra of LMP at 270 and 320 nm, along with respective retention times, were used to identify the LMP and concentrations were calculated by comparison with commercial standards.

Concentrations of both MeOH-soluble and MeOH-insoluble CT were quantified using the acid butanol assay for proanthocyanidins described by Hagerman (2002). Within 24 hours since HPLC analysis, the vials were removed from the auto sampler and 25–100 µl of the supernatant was used to determine the quantities of MeOH-soluble CT. The residues left in the vials after the extraction process were used to examine the amount of MeOH-insoluble CT. Both liquid and dried extractions were added the adequate amount of MeOH for the total volume to be 0.5 ml. After adding further 3 ml of acid butanol (95% butanol, 5% HCI) and 100 µl of iron reagent (2% ferric ammonium sulphate in 2N HCI), duplicate samples (depending on extract amounts) were placed in boiling water for 1 h. Absorbance (550 nm) of cooled samples was detected by a UV spectrophotometer (Shimadzu, Kyoto, Japan). Concentrations were calculated by averaging measurements in duplicate samples, using purified extracts of spruce needles as a standard. Low molecular weight phenolic compounds were grouped into phenolic acids, flavonoids, acetophenones, and stilbenes, while CT were separated into MeOH-soluble and MeOH-insoluble fractions. Concentrations of low molecular weight phenols (all phenolic groups except CT) and the total amount of phenolic compounds were summed for analyses (Supporting Information Table S1-S3). The loss of phenolic compounds was calculated by the method used for released N.

Ergosterol analysis

Total ergosterol (a proxy for fungal biomass) was analysed using a modified version of the protocol of Ransedokken *et al*. (2019). Approximately 100 mg of prepared litter sample was mixed with 7 ml 3M KOH in MeOH, vortexed and sonicated in a 70 °C ultrasonic water bath

in darkness for 90 min. After being vortexed and centrifuged (c. 16 400 rpm, 15 min), the supernatant was mixed with 2 ml purified water in new tubes. Ergosterol was extracted twice by adding 5 ml hexane, vigorous vortexing (approx. 1 min), and the hexane phase was collected after the liquid separated into two phases. Both extractions were collected in the same vial and evaporated using an Eppendorf Concentrator Plus 5301 (Eppendorf, Hamburg, Germany). Dried extracts were re-dissolved in 500 µl MeOH and analysed for ergosterol concentration using a 1200 Series HPLC (Agilent Technologies, Waldbronn, Germany). Ergosterol was separated using a reversed phase ODS ultra sphere column (250 mm × 4.6 mm; particle size 5 µm). Methanol was used as the mobile phase (flow rate 1.5 ml min^{-1}, total analysis time 12 min). Absorption of ergosterol was detected at 280 nm, and identified by comparing retention time, online UV-spectra, and commercial standard of ergosterol (Sigma, St. Louis, USA).

DNA sequencing

We extracted DNA using a CTAB-Chloroform DNA extraction protocol followed by a column based DNA purification using the E.Z.N.A.® Soil DNA Kit following the manufacturers protocol (Omega Bio-tek, Norcross, USA). Technical replicates and extraction negatives (negative controls) were introduced during DNA extraction, while mock communities (positive controls) were introduced during the PCR step. The primers gITS7 (forward) and ITS4 (reverse) (Ihrmark et al. 2012), tagged with unique molecular identifiers (MIDs), were used for amplifying the ITS2 region. Each PCR reaction consisted of 1 µl DNA template and 24 µl master mix: 15.7 µl dH2O, 2.5 µl Gold Buffer, 2.5 µl Gold MgCl2, 1 µl 20mg/ml BSA, 0.2 µl dNTPs, 0.1 µl AmpliTaq Gold, 1.5 µl 10 µM forward primer and 1.5 µl 10 µM reverse primer. We run PCR reactions for ITS2 with initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec and elongation at 72°C for 1 min. We added a final elongation step at 72°C for 7 min, before cooling down to 4°C. We controlled each PCR product for positive amplification with gel electrophoresis using a 2% agarose gel, before individual clean-up and purification of the amplicons with ZR-96 DNA Clean & Concentrator-5 kit (Zymo Research, California, USA). DNA concentrations for each sample were measured with the Qubit 2 fluorometer dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and pooled to equimolar concentration into libraries. The libraries were sequenced by Fasteris SA (Switzerland) using Illumina MiSeq with a 250 bp paired-end (PE) with V3 chemistry. A ligation protocol, specifically designed to minimize tag-jumping, was used to ligate the amplicons with the Miseq flow-cell adapters.

Bioinformatics

We performed all bioinformatics analyses on the Abel high-performance computer cluster at the University of Oslo. The raw PE reads were demultiplexed separately with simultaneous tags and primers removal using CUTADAPT (Martin, 2011). No miss-match with primer and MID tags were allowed. Further processing of the data was performed with the DADA2 pipeline (Callahan *et al*., 2016) using the statistical environment R version 3.6.0 (R Core Team, 2014). We generated sequence quality profiles and used this information to decide parameters for filtering low quality reads. Maximum expected error was set to 2.5. We then independently corrected forward and reverse reads using the DADA2 machine-learning algorithm that estimates correction parameters from the data itself. The corrected forward and reverse reads were then merged using a minimum overlap of 50 nucleotides. Chimeras were checked and removed with a denovo approach using the DADA2 bimera algorithm with default settings. We then constructed an amplicon sequence variant (ASV) table with the chimera-free reads. We used ITSx (Bengtsson-Palme *et al*., 2013) for extracting the ITS2 region and filtering of non-fungal reads. Due to widespread intraspecific variation in ITS2, an additional clustering step with 97% similarity was performed using VSEARCH (Rognes et al.2016) and singleton sequences were discarded. Finally, to adjust for over-splitting of Operational Taxonomic Units (OTUs), we performed post clustering curation using the LULU algorithm (Froslev *et al*., 2017) for both datasets. For taxonomic annotation, the dataset was query searched using VSEARCH global against UNITE v8.0 (Nilsson *et al*., 2019). The final ITS2 dataset consisted of 184 samples and 1853 OTUs.

Statistical analyses

Linear mixed-effects models were used to test for the effect of time, litter type, lime richness, and drought risk on mass loss and litter metabolites (C, N, ergosterol, and phenolic compounds) with plot nested within transect as random factors. The dredge function in the R MuMIn package (Barton, 2020) was used to conduct model selection based on lowest AIC. Similar analyses were carried out for all individual phenolic compounds of each litter type. The ggpredict function in the ggeffects package (Lüdecke, 2018) was used to assess overlap between 95% confidence intervals (CIs) for significant litter-type effects, and to generate graphical illustrations. One-way ANOVAs were used to test for the effect of litter type on the initial concentration of the measured litter metabolites. Separate Tukey's post-hoc tests were performed when litter type effects were significant.

Patterns of compositional structure were found separately for phenolic compounds and fungal OTUs by use of global non-metric multidimensional scaling (GNMDS) (Kruskal, 1964; Kruskal *et al*., 1973) based on a Bray-Curtis distance matrix using the monoMDS function in the R vegan package (Oksanen *et al*., 2020). All options and settings followed Liu *et al.* (2008): number of random starting configurations = 100; maximum number of iterations = 2000; convergence criterion = $1.0 \cdot 10^{-7}$. All minimum-stress solutions were obtained from a minimum of two starting configurations. The numbers of samples and phenolic compounds, i.e., the variables, in the ordination of phenolic composition were 68 and 26, 64 and 31, and 67 and 22 for bilberry, pine, and spruce litter, respectively. Data from five time points (0, 14, 61, 250, 370) were used for ordination of phenolic compounds, while the starting time point was left out from the ordination of fungal community composition due to missing data. For the ordination of fungi, only OTUs occurring more than three times were included. A total of 1051 OTUs satisfied this demand in the total data set based on 184 samples for all three litter types, while the corresponding numbers were 504 OTUs in 57 samples, 560 OTUs in 62 samples, and 541 OTUs in 65 samples for bilberry, pine, and spruce litter, respectively. The abundance of fungal OTUs was recorded as the number of reads divided by the maximum recorded abundance for the OTU in question. Fungal abundances were log-transformed prior to analysis to avoid unduly high influence by the abundant taxa on the cost of less abundant taxa (Melo, 2021). Variables were fitted onto all GNMDS ordinations using the function envfit (Oksanen *et al*., 2020) with 999 permutations. Only significant vectors (randomisation test; $P < 0.05$) are shown in the GNMDS ordinations. The function ordiellipse (Oksanen *et al*., 2020) was used to plot 95% CIs of time-point centroids. All statistical analyses were performed in R 4.1.1 (R Core Team, 2021), and graphical illustrations were generated in Veusz 3.3.1 (Sanders, 2020).

Results

Litter quality and mass loss

The initial C concentration was highest in pine litter, followed by bilberry and spruce litter (Table 1). The N concentration, on the other hand, was highest in bilberry litter, followed by spruce and pine litter. Consequently, pine litter had highest initial C:N ratio among the three litter types (Table 1).

Total mass loss (%) after 370 days differed significantly (95% CIs not overlapping, Fig. 2**a**) between bilberry (42.6 ± 1.12; mean ± 1 SE) and pine litter (33.9 ± 1.38), while spruce litter

Table 1. Initial concentrations or ratios (Mean ± 1 SE) of carbon (%), nitrogen (%), C:N ratio, ergosterol (mg g^{-1}), phenolic acids (mg g^{-1}), flavonoids (mg g^{-1}), acetophenones (mg g^{-1}), stilbenes (mg g^{-1}), low molecular weight phenols (LMP) (mg g^{-1}), MeOH-soluble condensed tannins (CT) (mg g^{-1}), MeOH-insoluble condensed tannins (CT) (mg g^{-1}), and total amount of phenols (mg g^{-1}) in the litter of bilberry, pine, and spruce. n = 15. *F* and *P* values derived from one-way ANOVAs to test for the effect of species on litter properties. Statistically significant results ($P < 0.05$) are printed in bold. Degrees of freedom = 2, 12. For each variable, values marked with contrasting letters indicate significant differences between litter types (Tukey, $P < 0.05$).

	Bilberry	Pine	Spruce	*F* (*P*)
Carbon	48.22 ± 0.07^{b}	49.77 ± 0.07^{a}	46.79 ± 0.09^{c}	**321.2 (<0.001)**
Nitrogen	1.13 ± 0.01^{a}	0.78 ± 0.01^{b}	1.04 ± 0.01^{c}	**261.4 (<0.001)**
C:N ratio	42.61 ± 0.39^{c}	63.86 ± 0.90^{a}	45.19 ± 0.26^{b}	**314 (<0.001)**
Ergosterol	0.01 ± 0.00^{c}	0.13 ± 0.01^{a}	0.05 ± 0.00^{b}	**116.6 (<0.001)**
Acetophenones	0.21 ± 0.00^{b}	0.00 ± 0.00^{b}	7.27 ± 0.24^{a}	**705.8 (<0.001)**
Flavonoids	37.15 ± 0.27^{a}	4.78 ± 0.12^{b}	2.38 ± 0.09^{c}	**9450 (<0.001)**
Phenolic acids	64.64 ± 0.64^{a}	0.00 ± 0.00^{b}	0.17 ± 0.00^{b}	**8173 (<0.001)**
Stilbenes	0.00 ± 0.00^{b}	1.32 ± 0.09^{b}	16.35 ± 0.54^{a}	**659.3 (<0.001)**
LMP	102.00 ± 0.89^{a}	6.10 ± 0.17^{c}	26.17 ± 0.73^{b}	**4519 (<0.001)**
MeOH-soluble CT	116.09 ± 2.28^{a}	10.24 ± 0.58^{c}	62.53 ± 0.70^{b}	**1119 (<0.001)**
MeOH-insoluble CT	16.91 ± 0.81^{a}	5.01 ± 0.42^{c}	10.23 ± 1.42^{b}	**29.75 (<0.001)**
Total phenols	235.00 ± 2.59^{a}	21.36 ± 0.88^{c}	98.93 ± 1.92^{b}	**2510 (<0.001)**

(37.8 ± 1.06) was intermediate. Further, the mass loss after 14 and 61 days was significantly higher for bilberry litter than for the two conifer litter types, indicating faster initial decomposition. We did not find any effect of drought risk or lime richness on mass loss (Table 2). The pattern of C concentration during litter decomposition corresponded to mass loss. Nitrogen release did not differ among litter types at any decomposition stage (95% CIs overlapping, Fig. 2**c**). The significant litter type × lime richness interaction term was driven by a slightly higher N release for pine litter at high lime richness compared to the two other litter types (Table 2). Both bilberry and pine litter immobilised N during the incubation period, however, bilberry and spruce released N at the final decomposition stage (Fig. 2**c**). This is reflected in decreasing C:N ratios with increasing decomposition time. The C:N ratio after 370 days of decomposition was 26.9 ± 0.69 (mean ± 1 SE) in bilberry litter, 42.9 ± 1.36 in pine litter, and 31.96 ±1.08 in spruce litter. The concentration of ergosterol increased during decomposition for all three litter types (Fig. 2**b**), indicating that fungal biomass was

accumulated throughout the study period. No overall significant difference in fungal biomass between litter types was observed during decomposition (Table 2).

Litter secondary metabolites

The initial concentration of total phenolic compounds in bilberry litter was twice as large as the concentration in spruce litter, and ten times as large as in pine litter (Table 1). We identified a total of 26 different phenolic compounds in bilberry litter, 31 in pine litter, and 22 in spruce litter. Phenolic compounds were classified into six groups: acetophenones, flavonoids, phenolic acids, stilbenes, and MeOH-soluble and MeOH-insoluble condensed tannins.

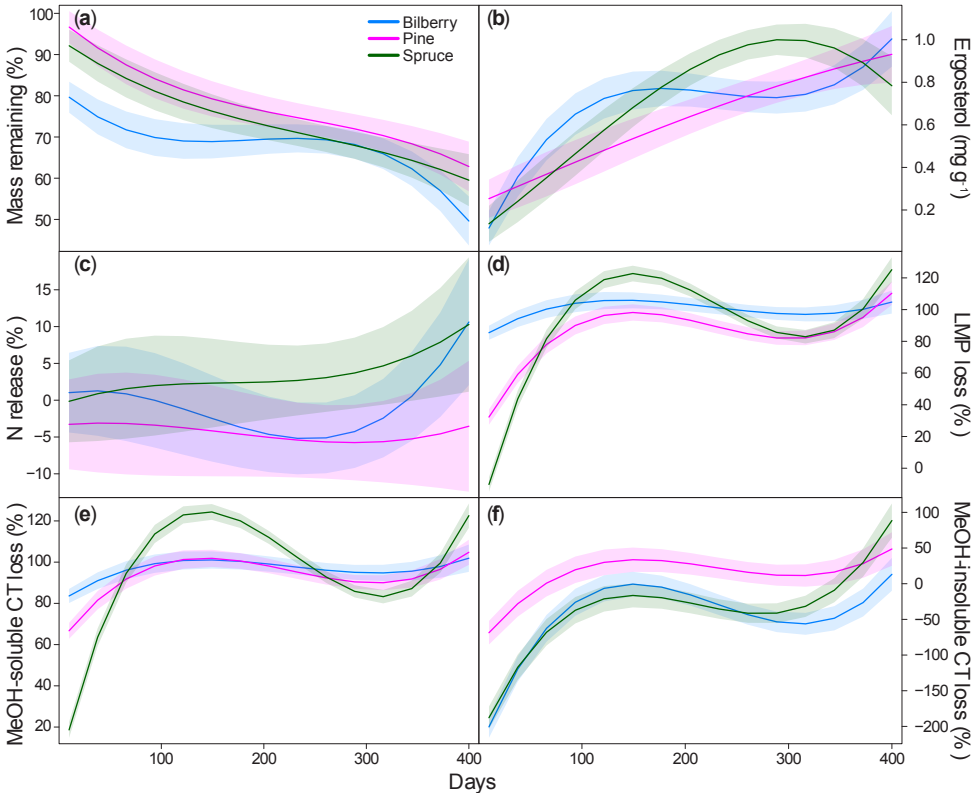


Figure 2. Predicted values of (**a**) mass remaining (%), (**b**) ergosterol (mg g^{-1}), (**c**) nitrogen (N) release (%), (**d**) low molecular weight phenolics (LMP) loss (%), (**e**) MeOH-soluble condensed tannins (CT) loss (%), and (**f**) MeOH-insoluble CT loss (%) of bilberry, pine, and spruce litter after 0, 14, 61, 250, and 370 days of soil incubation. Predictions are based on simple linear models (using litter type × time). Shaded areas represent 95% confidence intervals.

Table 2. Linear mixed effects models, with plot nested within transect as random factors, to test for the effect of time (14, 61, 250, 370), litter type (bilberry, pine, spruce), lime richness, and drought risk on mass loss (%), carbon (%), nitrogen (%), C:N ratio, ergosterol (mg g^{-1}), acetophenones (mg g^{-1}), phenolic acids (mg g^{-1}), flavonoids (mg g^{-1}), stilbenes (mg g^{-1}), low molecular weight phenol (LMP) loss (mg g^{-1}), MeOH-soluble condensed tannins (CT) (mg g^{-1}), MeOH-insoluble condensed tannins (CT) (mg g^{-1}), and total amount of phenols (mg g^{-1}). Significant *F* and *P* values are printed in bold, only significant effects are shown.

Response variable	Selected model	Significant variables	DF	*F* (*P*)
Mass loss	Time * Litter type	Time	2, 12	**398.40 (<0.001)**
		Litter type	2, 12	**93.72 (<0.001)**
		Time × Litter type	4, 12	**21.30 (<0.001)**
Carbon	Time * Litter type *	Time	2, 39	**15.81 (<0.001)**
	Lime richness *	Litter type	2, 39	**307.64 (<0.001)**
	Drought risk	Lime richness	1, 39	**17.96 (<0.001)**
		Litter type × Lime richness	2, 39	**8.11 (0.017)**
		Time × Lime richness × Drought risk	2, 39	**6.10 (0.047)**
Nitrogen release	Time * Litter type *	Litter type	2, 39	**18.00 (<0.001)**
	Lime richness *	Litter type × Lime richness	2, 39	**8.26 (0.016)**
	Drought risk	Time × Drought risk	2, 39	**8.07 (0.018)**
		Litter type × Time × Lime richness	4, 39	**13.59 (0.009)**
C:N ratio	Time * Litter type *	Litter type	2, 39	**1161.00 (<0.001)**
	Lime richness *	Time	2, 39	**337.20 (<0.001)**
	Drought risk	Litter type × Time	2, 39	**49.57 (<0.001)**
		Litter type × Lime richness	4, 39	**7.53 (0.023)**
Ergosterol	Time * Litter type	Time	2, 12	**640.26 (<0.001)**
		Time × Litter type	4, 12	**25.04 (<0.001)**
Acetophenones	Time * Litter type	Time	2, 12	**39.08 (<0.001)**
		Litter type	2, 12	**47.13 (<0.001)**
		Time × Litter type	4, 12	**74.07 (<0.001)**
Flavonoids	Time * Litter type *	Time	2, 39	**150.48 (<0.001)**
	Lime richness *	Litter type	2, 39	**10.57 (0.005)**
	Drought risk	Time × Litter type	4, 39	**46.42 (<0.001)**
		Time × Lime richness	2, 39	**6.27 (0.043)**
		Time × Litter type × Lime richness	4, 39	**12.08 (0.017)**
Phenolic acids	Time * Litter type *	Time	2, 39	**66.30 (<0.001)**
	Lime richness *	Litter type	2, 39	**35.67 (<0.001)**
	Drought risk	Time × Litter type	4, 39	**59.11 (<0.001)**
		Time × Lime richness	2, 39	**6.21 (0.045)**

Table 2. continued

Response variable	Selected model	Significant variables	DF	*F* (*P*)
		Time × Litter type × Lime richness	4, 39	**10.20 (0.037)**
Stilbenes	Time * Litter type *	Time	2, 39	**133.84 (<0.001)**
	Lime richness *	Litter type	2, 39	**152.54 (<0.001)**
	Drought risk	Time × Litter type	4, 39	**224.55 (<0.001)**
LMP loss	Time * Litter type *	Time	2, 39	**335.59 (<0.001)**
	Lime richness *	Litter type	2, 39	**134.87 (<0.001)**
	Drought risk	Time × Litter type	4, 39	**141.24 (<0.001)**
MeOH-soluble CT loss	Time * Litter type *	Time	2, 39	**171.01 (<0.001)**
	Lime richness *	Litter type	2, 39	**45.75 (<0.001)**
	Drought risk	Time × Litter type	4, 39	**71.38 (<0.001)**
MeOH-insoluble CT loss	Time * Litter type *	Time	2, 39	**426.11 (<0.001)**
	Lime richness *	Litter type	2, 39	**177.61 (<0.001)**
	Drought risk	Time × Litter type	4, 39	**44.85 (<0.001)**
Total phenol loss	Time * Litter type *	Time	2, 39	**315.50 (<0.001)**
	Lime richness *	Litter type	2, 39	**87.31 (<0.001)**
	Drought risk	Time × Litter type	4, 39	**59.35 (<0.001)**

The loss of low molecular weight phenolics and MeOH-soluble condensed tannins increased quickly and stabilised within 250 days of decomposition for all species (Fig. 2**d-e**). In contrast, the concentration of MeOH-insoluble condensed tannins in the three litter types had a sharp increase during the first two weeks and then slowly decreased with time, as reflected in the negative loss values for MeOH-insoluble condensed tannins (Fig. 2**f**). The loss of total phenolic compounds after 370 days of soil incubation was 90%, 78%, and 91% for bilberry, pine, and spruce litter, respectively. Concentrations of MeOH-insoluble condensed tannins were highest in pine and spruce litter compared to bilberry litter at the final stage of decomposition (95% CIs not overlapping, Fig. 2**f)**. Although the total amount of phenolic compounds decreased with time for all species (Supporting Information Table S1–S3), the concentration of MeOH-insoluble condensed tannins increased in relative proportion to all phenolic compounds during decomposition and was the most dominant compound group at the end of the incubation period (Fig. 3**d**).

Time had a significant effect on all individual phenolic compounds and, hence, on all phenolic groups, in all litter types (Supporting Information Table S1–S3). When combining phenolic groups of all litter types, both time and litter type had significant effects on all phenolic groups

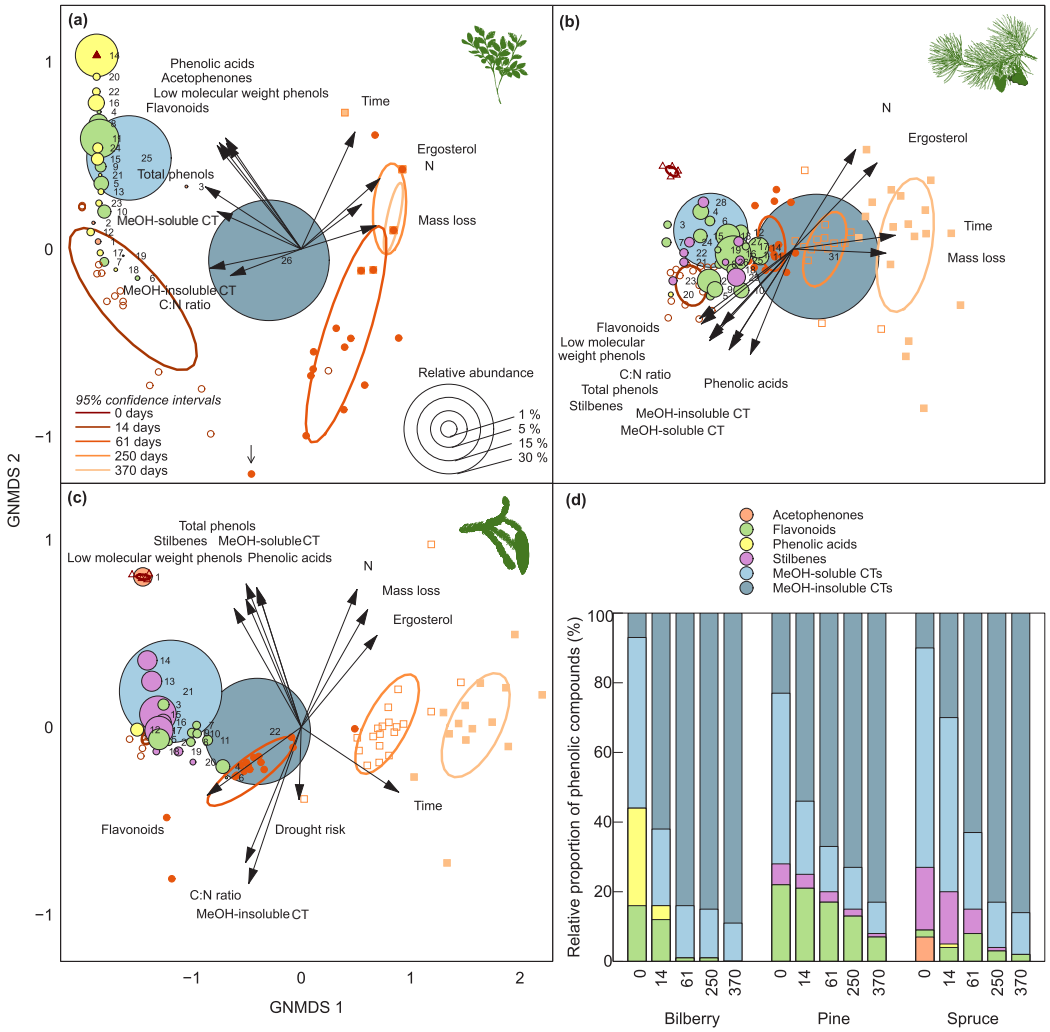


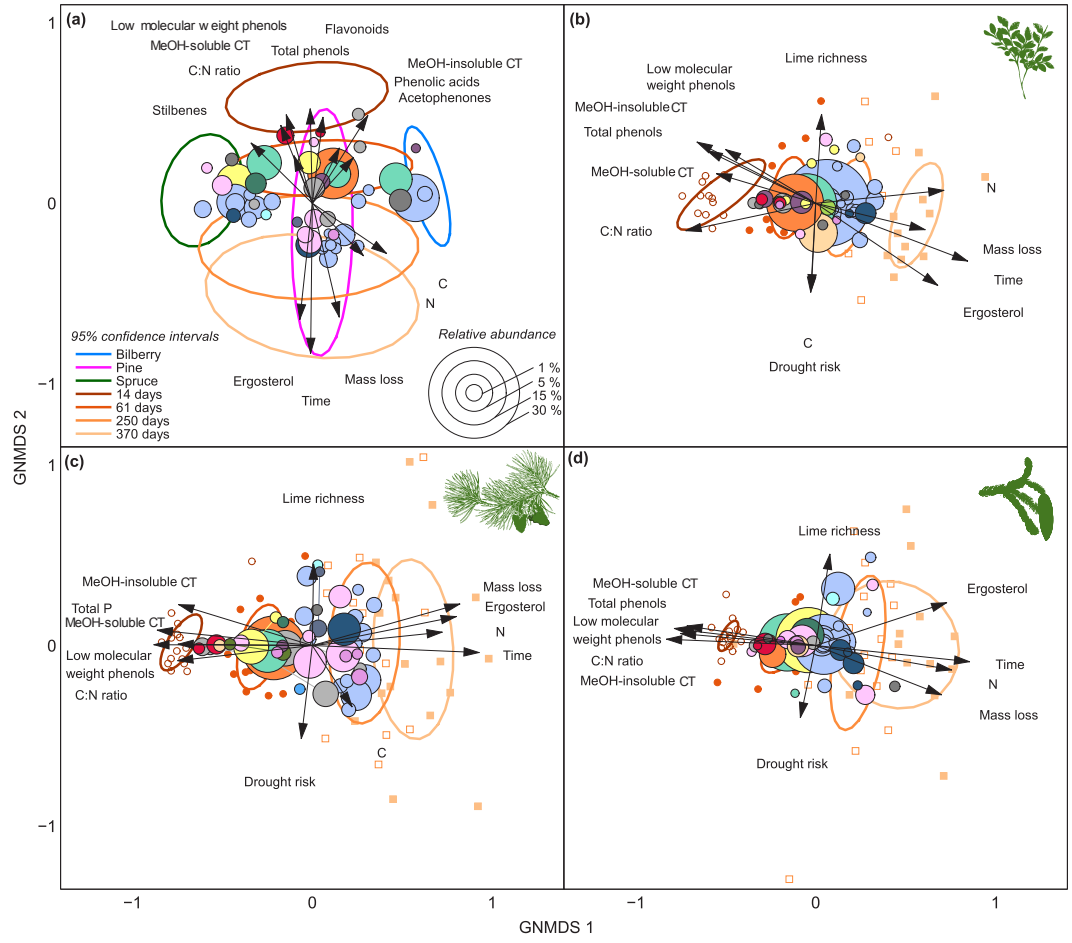
Figure 3. Two-dimensional global non-metric multidimensional scaling (GNMDS) ordination plots of the phenolic compound composition in **(a)** bilberry, **(b)** pine, and **(c)** spruce litter after 0, 14, 61, 250, and 370 days after incubation in the soil, based on all identified phenolic compounds (26, 31, and 22, respectively). Stress values were 0.07, 0.06, and 0.03, respectively. Ellipses represents 95% confidence intervals (based on SD) for time-point centroids, with symbols in the corresponding colours representing samples. Arrows indicate the direction of maximum increase for the variable represented by the vector in question, and vector length is proportional to the correlation between the variables and the ordination axes (only vectors significant at the $\alpha < 0.05$ level are shown). Each dot represents one phenolic compound, dot colour shows affiliation to phenolic group, and dot size is proportional to the relative abundance of the compound. Numerical prefixes identify all phenolic compounds (listed in Supporting Information Table S1–S3). Relative abundance of phenolic compounds **(d)** in bilberry, pine, and spruce litter after incubation in the soil for 0, 14, 61, 250, and 370 days.

(Table 2). A significant litter type × time interaction term was also found for most groups, reflecting slight differences in the patterns displayed by the litter types during decomposition. These interactions are explained in detail below.

The composition of phenolic compounds differed significantly across litter types and decomposition stages (i.e., sampling dates), with the most distinct difference between the start and the end of the experimental period (0 and 370 days of decomposition). This pattern is visualised by GNMDS ordination results, showing that time had the strongest effect on the composition of phenolics and that the 95% CIs of the centroids of the five time-intervals mainly did not overlap (Fig. 3**a-c**). The exception was for bilberry litter at 61, 250, and 370 days of incubation. At the end of the experiment, litters of all types were dominated by condensed tannins (Fig. 3**d**). For bilberry litter, two flavonoids, a quercetin 3-glycoside and a myricetin 3-glycoside, together with the phenolic acid chlorogenic acid were the quantitatively most important low molecular weight phenols (Fig. 3**a**). Various flavonoids, especially monocoumaroyl astragallins, dominated in the pine litter (Fig. 3**b**), while spruce litter was dominated by various stilbenes, especially piceatannol glucoside (Fig. 3**c**). For all three litter types, the chemical groups of acetophenones (not found in pine litter), flavonoids, and phenolic acids were mainly present in the litter initially and after 14 days of incubation, while stilbenes (not found in bilberry litter) were still present around 61 to 250 days of litterbag incubation. Hence, stilbenes are present in the litter longer than the other low molecular weight phenolics.

Litter fungal community

The fungal community composition differed significantly across litter types and decomposition stages (i.e., sampling dates), overlap among 95% CIs of centroids was not observed for the three litter types and mainly not for the four time-intervals (Fig. 4**a-d**). The exceptions were observed for the community composition of all litter types, pine litter, and spruce litter after 250 and 370 days of soil incubation, indicating some form of stabilisation (Fig. 4**a, c, d**). The GNMDS ordination of all litter types combined (Fig. 4**a**) shows that litter types, which were clearly separated along the first axis, had the strongest effect on fungal community composition, whereas the second axis separated fungal communities from different time points, reflecting fungal succession during decay. Several variables with temporal trends were associated with the second GNMDS axis (Fig. 4**a**). In all individual litter-type GNMDSs, the first axis reflected differences among time points while lime richness and drought risk explained compositional shifts along the second axis (Fig. 4**b-d**). The most distinct difference


(a)
Low molecular weight phenols
Flavonoids
MeOH-soluble CT
Total phenols
C:N ratio
MeOH-insoluble CT
Phenolic acids
Acetophenones
Stilbenes
C
N
95% confidence intervals
Bilberry
Pine
Spruce
14 days
61 days
250 days
370 days
Ergosterol
Mass loss
Time
Relative abundance
1 %
5 %
15 %
30 %
(b)
Lime richness
Low molecular weight phenols
MeOH-insoluble CT
Total phenols
MeOH-soluble CT
C:N ratio
N
Mass loss
Time
Ergosterol
C
Drought risk
(c)
Lime richness
MeOH-insoluble CT
Total P
MeOH-soluble CT
Low molecular weight phenols
C:N ratio
Mass loss
Ergosterol
N
Time
C
Drought risk
(d)
Lime richness
MeOH-soluble CT
Total phenols
Low molecular weight phenols
C:N ratio
MeOH-insoluble CT
Ergosterol
Time
N
Mass loss
Drought risk
GNMDS 1
GNMDS 2

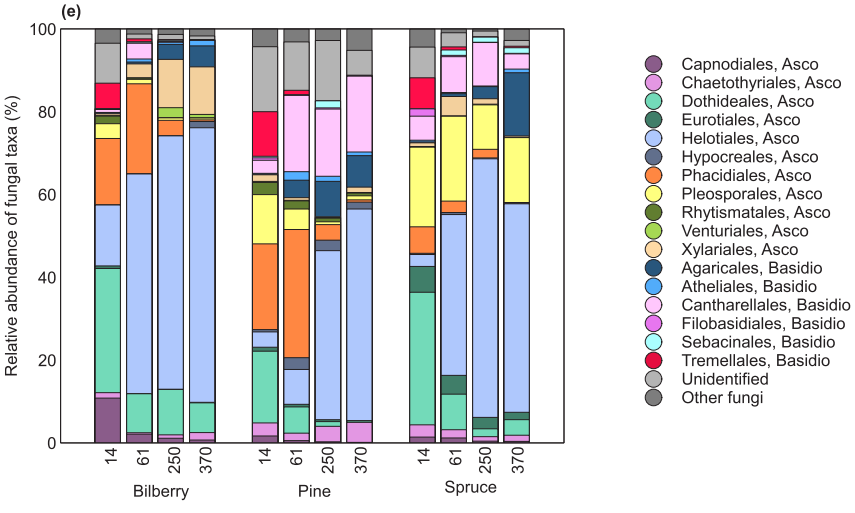

(e)
Relative abundance of fungal taxa (%)
Bilberry
Pine
Spruce
Capnodiales, Asco
Chaetothyriales, Asco
Dothideales, Asco
Eurotiales, Asco
Helotiales, Asco
Hypocreales, Asco
Phacidiales, Asco
Pleosporales, Asco
Rhytismatales, Asco
Venturiales, Asco
Xylariales, Asco
Agaricales, Basidio
Atheliales, Basidio
Cantharellales, Basidio
Filobasidiales, Basidio
Sebacinales, Basidio
Tremellales, Basidio
Unidentified
Other fungi

Figure 4. Two-dimensional global non-metric multidimensional scaling (GNMDS) ordination plots of fungal community composition in **(a)** all litter types, **(b)** bilberry, **(c)** pine, and **(d)** spruce litter after 14, 61, 250, and 370 days after incubation in the soil. Stress values were 0.20, 0.12, 0.13, and 0.12, respectively. Ellipses represents 95% confidence intervals (based on SE) for time-point centroids, with symbols in the corresponding colours representing samples. Arrows indicate the direction of maximum increase for the variable represented by the vector in question, and vector length is proportional to the correlation between the variables and the ordination axes (only vectors significant at the $\alpha < 0.05$ level are shown). Low weight molecular phenol groups are only included as vectors in the ordination plot for all species (a). Each dot represents one OTU, dot colour shows affiliation to taxonomic group, and dot size is proportional to the number of reads, and only the 50 most common OTUs are visualized. Numerical prefixes that identify all OTUs are presented in Supporting Information Figure S1. Relative abundance of fungal taxa (based on number of reads) **(e)** in bilberry, pine, and spruce litter after incubation in the soil after 14, 61, 250, and 370 days.

between time points was observed between 0 and 370 days of litter incubation in the soil. Overall, the three litter types shared 56% of the total OTUs. The fraction of OTUs shared by the three litter types after 14, 61, 250, and 370 days of incubation were 27%, 26%, 21%, and 21%, respectively. The community assemblage varied significantly between litter types and decomposition stages (Fig. 4**e**). The fungal community was dominated by saprotrophic Ascomycota, with increasing contribution from Basidiomycota towards later stages of decomposition. The main trend in community composition for all litter types, was decreasing presence of the early dominant fungi in the orders Dothideales, Phacidiales, and Pleosporales with increasing litter incubation. Fungi in the order Tremellales were also abundant mainly in the early decomposition stage in all three litter types. Meanwhile, fungi in the orders Agaricales, Cantharellales, Helotiales, and Xylariales became increasingly dominant towards later stages of decomposition.

Discussion

Secondary metabolites play important roles in many ecosystem processes, but we lack insight into their role in plant litter decomposition and the interplay with soil organisms. In this study from the boreal forest, we explored the link between phenolic compounds, fungal communities, and mass loss at early decay stages. In accordance with our first hypothesis, the composition and concentration of phenolic compounds in all litter types changed throughout the decomposition period. Further, litter type was the main factor influencing mass loss, supporting our second hypothesis that litter from different plant species decompose at different rates. In contrast to the latter part of the second hypothesis, however, the environmental gradients of lime richness and drought risk did not influence litter mass loss. These results underline the

importance of litter quality on decomposition in these ecosystems and we explore their implications below.

The differences between litter types were most pronounced after 14 days, when bilberry had clearly higher mass loss than the two other species. This early stage of decomposition is characterized by leaching of soluble compounds, as well as opportunistic microorganisms using low molecular weight phenols as an energy source (Swift *et al*., 1979; Chomel *et al*., 2016; Fierer *et al*., 2001; Schimel *et al*., 1998). For instance, bilberry produced large quantities of low molecular weight phenolics like phenolic acids and flavonoids, that were quickly released during the first fortnight. As such, loss of low molecular weight phenolics represented 48% of the total bilberry mass lost by the first harvest. Meanwhile, spruce and especially pine had lower concentrations and other types of low molecular weight phenolics than bilberry at start, some of which also showed a slower release. Loss of low molecular weight phenolics merely represented 14 % and 7 % of the mass lost by spruce and pine, respectively. Different coumaroyl-astragallins and stilbenes, for example, were only present in the conifer litter, and still present in small amounts at later decomposition stages. The stilbenes pinosylvin and pinosylvinmonomethyleter in pine litter, and resveratrols in spruce litter, have important roles in defence against fungi in the living trees (Ganthaler *et al*., 2017; Metsämuuronen & Sirén, 2019). Further, many flavonoids have antibacterial effect (Yuan *et al*., 2021). However, whether such functions explain the relatively slow release of some low molecular weight compounds from litter is not known.

Leaf toughness is also recognized as an important aspect of litter quality during the early leaching stage (Gallardo & Merino, 1993) and may be part of the explanation for the strong initial loss of phenolics from the deciduous bilberry compared with the conifer species. Further, the rapid release of low molecular phenolics in bilberry concurs with its relatively high initial N concentration. This is similar to earlier findings showing that initial litter N concentrations were decisive for phenol loss in subtropical tree species (Ristok *et al*., 2019). Likewise, in accordance with other studies from this early decomposition stage, differences in mass loss between litter types reflected their initial N concentration (Cornwell *et al*., 2008; Makkonen *et al*., 2012).

After the first two weeks, when a large part of the low molecular weight phenolics were lost, the difference in mass loss between the litter types were reduced. At this stage, the larger part

of phenolics left are that of the MeOH-insoluble fraction of condensed tannins. This suggests an increasing role of condensed tannins in controlling mass loss rates at later stages once the importance of other drivers (e.g., N and low molecular weight phenolics) lessens (e.g., Chomel *et al*., 2016). Condensed tannins are large molecules built up of several flavonoid units known to have a general resistance to degradation (Haase & Wantzen, 2008), both through their negative effects on decomposers and through their capacity to form complexes with for example proteins and chitin in soil organic matter (Mutabaruka *et al*., 2007; Adamczyk *et al*., 2019). However, the MeOH-soluble fraction of condensed tannins was to a large extent released during the year our study lasted, which may have several possible explanations. Although not well documented, the differences in solubility between condensed tannin fractions are thought to be caused by the degree of polymerization (Shay *et al*., 2017). Moreover, the distinct increase in MeOH-insoluble condensed tannins after the initial decomposition stage may suggest that during the decay process parts of the MeOH-soluble fraction changes into forms that are MeOH-insoluble. It should also be mentioned that the actual tannin concentrations remaining in the litter are likely to be higher than what we were able to quantify, as tannins can be a part of undetectable complexes (Adamczyk *et al*., 2018; Shay *et al*., 2018). In summary, our results suggest that secondary metabolites may be both an important part of the mass loss itself at initial stages as well as having regulatory effects on the soil food web. This corresponds well with similar studies, although these were performed in tropical or subtropical ecosystems (Loranger *et al*., 2002; Ristok *et al*., 2019). By analysing low molecular weight phenolics down to individual compounds and condensed tannins in two fractions, we also show that chemical groups and even individual compounds may impact differently.

The observed shifts in composition of fungal community during litter decomposition were synchronous with the changes in phenolic compound concentration, thus strongly supporting our third hypothesis that the fungal community composition varies with litter type and quality and undergo rapid successional changes in the litter decomposition process (Treseder *et al*., 2014; Voříšková & Baldrian, 2013). These patterns suggest that the chemical composition of the litter is reflected in the functional role of the microbial community at each stage of decomposition (Chomel *et al*., 2016).

The increase of fungal biomass (ergosterol) with decay, support the second part of our final hypothesis. The most abundant fungi found were in the ascomycete order Helotiales, which

mainly include saprotrophs that are common in early stages of litter decomposition (Boberg *et al*., 2011; Lindahl *et al*., 2006). Our observation that parasitic Tremellales initially dominates in all litter types, accords with results of Voříšková & Baldrian (2013), who found aboveground phyllosphere fungi to be commonly present in decomposing litter, and Winder *et al*. (2013) who found substantial increase of Tremellomycetes when litter was added to the soils. The Tremellales fungi likely represent yeast-like endophytes, present in living tissue and early decay stages. Our taxonomic annotation indicates that members of the yeast-like family Bulleraceae (Tremellales) were abundant. Additionally, many of the fungi in the orders Dothideales, Phacidiales, and Pleosporales that were dominant in the early decomposition stages, are likely plant pathogens and endophytes that were present in the litter before soil incubation. The presence of pathogenic and endophytic fungi in senescent plant litter may affect decomposition rates because some biotrophs have the ability to switch to a saprotrophic mode and break down plant cell wall polymers, including lignin (Fanin *et al*., 2021; Treseder *et al*., 2014). One example of this, present in our dataset, is *Lophodermium piceae*, an endophyte in spruce needles that acts as a saprotroph in the initial decomposition process (Müller *et al*., 2001). The impact of pathogenic and endophytic fungi together with large differences in the initial litter quality among the litter types, may explain the differences found in fungal community composition.

While ascomycetes were present throughout the decomposition process, basidiomycetes increased towards later stages and are likely to account for most of the decomposition of lignocellulose in litter in later decay stages (Voříšková & Baldrian, 2013). Our experiment only lasted for one year; the contribution from ligninolytic basidiomycetes would probably have been more profound in a more long-lasting experiment. However, ascomycetous Xylariales species, which became increasingly abundant in especially bilberry litter towards later stages of decomposition, are also able to decompose lignin (Purahong *et al*. 2016). This may also be the case for other specialized ascomycetes (Osono & Takeda, 2002). In the fungal succession on plant litter, we do know that individual fungal taxa respond differently to tannins and various substrates (McGuire *et al*., 2010). Fungal enzymatic activity is known to be affected by litter quality, and especially tannin-rich soils with high concentration of recalcitrant condensed tannin–protein complexes have been reported to harbour specific fungal community compositions that are highly adapted to the conditions (Mutabaruka *et al*., 2007). However, other phenolic substrate preferences by individual fungal taxa remain mainly unknown.

Conclusion

Our study presents a time course in phenolic compound and fungal community compositions of boreal forest litter that to our knowledge is not earlier studied. We show that phenolic compounds may be a large part of the mass loss during the first stage of decomposition, but also strong differences between plant species, underlining the importance of litter quality. Synchronous shifts in fungal communities and chemical composition in litter suggests important roles of phenolics in belowground processes, and future studies should explore the direct effects of phenolic groups on soil organisms. The longevity of the MeOH-insoluble fraction of condensed tannins in the plant litter highlights their potential in regulating later stage decomposition, once more prominent drivers (e.g., N concentration) have played out their role. This calls attention to the importance of long-term decomposition studies for evaluating the role of condensed tannins.

Acknowledgements

We thank Professor Peter Kennedy for supportive ideas on the study design and securing the funding, and Dabao Sun Lu for help in the field and with DNA extractions. Annie Aasen and Claus D. Kreibich contributed significantly to the chemical analyses. This study was funded in part by a Norwegian Centennial Chair Grant and the Research Council of Norway (project 240859). The authors declare no conflict of interest.

Author Contribution

R.H. established the plot system at the study site and recorded gradient positions of the plots. All authors took part in planning and design of the study. H.K., L.N.M., S.M., J.A., L.N., and Y.R. did the fieldwork. L.N.M. and S.M. performed the DNA metabarcoding, and L.M. performed the bioinformatics. Y.R. prepared the litterbags, performed chemical analyses, analysed the data, and led the writing of the manuscript. All authors contributed to writing and revision of the manuscript and gave final approval for publication.

Data Availability

Sequence data, including raw sequences as well as processed files, will be submitted to NMBU Open Research Data (www.dataverse.no)

References

Adamczyk B, Adamczyk S, Smolander A, Kitunen V, Simon, J. 2018. Plant Secondary Metabolites—Missing Pieces in the Soil Organic Matter Puzzle of Boreal Forests. *Soil Systems* **2**: 2. https://doi.org/10.3390/soils2010002

Adamczyk B, Sietiö OM, Biasi C, Heinonsalo J 2019. Interaction between tannins and fungal necromass stabilizes fungal residues in boreal forest soils. *New Phytologist* **223**: 16–21. https://doi.org/10.1111/nph.15729

Aerts 1997. Climate, Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems: A Triangular Relationship. *Oikos* **79**: 439–449. https://doi.org/10.2307/3546886

Barton K. 2020. MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F, Amend AS, Jumpponen A, Unterseher M, Kristiansson E 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* **4**: 914–919. https://doi.org/10.1111/2041-210x.12073

Boberg JB, Ihrmark K, Lindahl BD. 2011. Decomposing capacity of fungi commonly detected in *Pinus sylvestris* needle litter. *Fungal Ecology* **4**: 110–114. https://doi.org/10.1016/j.funeco.2010.09.002

Bradford MA, Berg B, Maynard DS, Wieder WR, Wood SA. 2015. Understanding the dominant controls on litter decomposition. *Journal of Ecology* **104**: 229–238. https://doi.org/10.1111/1365-2745.12507

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 543–545. https://doi.org/10.1038/Nmeth.3869

Chomel M, Guittonny-Larchevêque M, Fernandez C, Gallet C, DesRochers A, Paré D, Jackson BG, Baldy V. 2016. Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. *Journal of Ecology* **104**: 1527–1541. https://doi.org/10.1111/1365-2745.12644

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist* **205**: 1525–1536. https://doi.org/10.1111/nph.13208

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Bart Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Ian J., Wright, I.J., Aerts, R., Allison, S.D., van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V. & Westoby, M. 2008. Plant species traits are the predominant control on

litter decomposition rates within biomes worldwide. *Ecology Letters* **11**: 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

Fanin N, Lin D, Freschet GT, Keiser AD, Augusto L, Wardle DA, Veen GF. 2021. Home-field advantage of litter decomposition: from the phyllosphere to the soil. *New Phytologist* **231**: 1353–1358. https://doi.org/10.1111/nph.17475

Fierer N, Schimel JP, Cates RG, Zou J. 2001. Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biology and Biochemistry* **33**: 1827–1839. https://doi.org/10.1016/S0038-0717(01)00111-0

Framstad E. 2019. *Terrestrisk naturovervåking i 2018: Markvegetasjon, epifytter, smågnagere og fugl. Sammenfatning av resultater*. NINA Rapport 1692. Norsk institutt for naturforskning.

Froslev TG, Kjoller R, Bruun HH, Ejrnaes R, Brunbjerg AK, Pietroni C, Hansen AJ. 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications* **8**: 1188. https://doi.org/10.1038/s4146 7-017-01312-x

Gallardo A, Merino J. 1993. Leaf decomposition in two Mediterranean ecosystems of southwest Spain: influence of substrate quality. *Ecology* **74**: 152-161. https://doi.org/10.2307/1939510

Ganthaler A, Stöggl W, Kranner I, Mayr S. 2017. Foliar Phenolic Compounds in Norway Spruce with Varying Susceptibility to *Chrysomyxa rhododendri*: Analyses of Seasonal and Infection-Induced Accumulation Patterns. *Frontiers of Plant Science* **8**: 1173. https://doi.org/10.3389/fpls.2017.01173

Gessner MO, Swan C, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S. 2010. Diversity meets decomposition. *Trends in Ecology and Evolution* **25**: 372–380. https://doi.org/10.1016/j.tree.2010.01.010

Haase K, Wantzen KM. 2008. Analysis and decomposition of condensed tannins in tree leaves. *Environmental Chemistry Letters* **6**: 71-75. https://doi.org/10.1007/s10311-008-0140-7

Hagerman AE. 2002. The Tannin Handbook. Oxford: Miami University.

Halvorsen, R., Skarpaas, O., Bryn, A., Bratli, H., Erikstad, L, Simensen, T. & Lieungh, E. 2020. Towards a systematics of ecodiversity: The EcoSyst framework. Global Ecology and Biogeography **29**: 1887–1906. https://doi.org/10.1111/geb.13164

Hättenschwiler S, Tiunov AV, Scheu S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics* **36**: 191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

Hättenschwiler S, Vitousek PM. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution* **15**: 238–243. https://doi.org/10.1016/S0169-5347(00)01861-9

Kraus TEC, Dahlgren RA, Zasoski RJ. 2003. Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil* **256**: 44–61. https://doi.org/10.1023/A:1026206511084

Kruskal JB. 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* **29**:1–27. https://doi.org/10.1007/BF02289565

Kruskal JB, Young FW, Seery JB. 1973. How to use KYST, a very flexible program to do multidimensional scaling and unfolding. Murray Hill, NJ, USA: Bell Labs.

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2006. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* **173**: 611–620. https://doi.org/10.1111/j.1469-8137.2006.01936.x

Liu H, Økland T, Halvorsen R, Gao J, Liu Q, Eilertsen O, Bratli H. 2008. Gradients analyses of forests ground vegetation and its relationships to environmental variables in five subtropical forest areas, S and SW China. *Sommerfeltia* **32**: 3–196. https://doi.org/10.2478/v10208-011-0012-6

Loranger G, Ponge JF, Imbert D, Lavelle P. 2002. Leaf decomposition in two semi-evergreen tropical forests: influence of litter quality. *Biology and Fertility of Soils* **35**: 247–252. https://doi.org/10.1007/s00374-002-0467-3

Lüdecke D. 2018. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software* **3**: 772. https://doi.org/10.21105/joss.00772

Makkonen M, Berg MP, Handa IT, Hättenschwiler S, van Ruijven J, van Bodegom PM, Aerts R. 2012. Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecology Letters* **15**: 1033–1041. https://doi.org/10.1111/j.1461-0248.2012.01826.x

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet Journal* **17**: 10. https://doi.org/10.14806/ ej.17.1.200

McGuire KL, Bent E, Borneman J, Majumder A, Allison SD, Treseder KK. 2010. Functional diversity in resource use by fungi. *Ecology* **91**: 2324–2332. https://doi.org/10.1890/09-0654.1

Melo AS. 2021. Heavy-weighting rare species in dissimilarity indices improve recovery of multivariate groups. *Ecological Complexity* **46**: https://doi.org/10.1016/j.ecocom.2021.100925

MET 2021. Norwegian Meteorological Institute. https://seklima.met.no/

Metsämuuronen S, Sirén H. 2019. Bioactive phenolic compounds, metabolism and properties: a review on valuable chemical compounds in Scots pine and Norway spruce. *Phytochemistry Reviews* **18**: 623–664. https://doi.org/10.1007/s11101-019-09630-2

Moorhead DL, Sinsabaugh RL. 2006. A theoretical model of litter decay and microbial interaction. *Ecological Monographs* **76**: 151–174. https://doi.org/10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2

Müller MM, Valjakka R, Suokko A, Hantula J. 2001. Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. *Molecular Ecology* **10**: 1801–1810. https://doi.org/10.1046/j.1365-294X.2001.01304.x

Mutabaruka R, Hairiah K, Cadisch G. 2007. Microbial degradation of hydrolysable and condensed tannin polyphenol–protein complexes in soils from different land-use histories. *Soil Biology and Biochemistry* **39**: 1479–1492. https://doi.org/10.1016/j.soilbio.2006.12.036

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. 2019. Mycobiome diversity: High-throughput sequencing and identification of fungi. *Nature Reviews Microbiology* **17**: 95–109. https://doi.org/10.1038/s4157 9-018-0116-y

Nybakken L, Lie MH, Julkunen-Tiitto R, Asplund J, Ohlson M. 2018. Fertilization changes chemical defense in needles of mature Norway spruce (*Picea abies*). *Frontiers in Plant Science* **9**: 770. https://doi.org/10.3389/fpls.2018.00770

Nybakken L, Selås V, Ohlson M. 2013. Increased growth and phenolic compounds in bilberry (*Vaccinium myrtillus* L.) following forest clear-cutting. *Scandinavian Journal of Forest Research* **28**: 319–330. https://doi.org/10.1080/02827581.2012.749941

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Osono T, Takeda H. 2002. Comparison of litter decomposing ability among diverse fungi in a cool temperate deciduous forest in Japan. *Mycologia* **94**: 421–427. https://doi.org/10.1080/15572536.2003.11833207

Pérez-Harguindeguy N, Díaz S, Cornelissen JHC, Vendramini, F, Cabido M, Castellanos A. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. *Plant and Soil* **218**: 21–30. https://doi.org/10.1023/A:1014981715532

Prescott CE. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils?. *Biogeochemistry* **101**: 133–149. https://doi.org/10.1007/s10533-010-9439-0

Purahong W, Hyde KD. 2011. Effects of fungal endophytes on grass and non-grass litter decomposition rates. *Fungal Diversity* **47**: 1–7. https://doi.org/10.1007/s13225-010-0083-8

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F. 2016. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology* **25**: 4059–4074. https://doi.org/10.1111/mec.13739

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ransedokken Y, Asplund J, Ohlson M, Nybakken, L. 2019. Vertical distribution of soil carbon in boreal forest under European beech and Norway spruce. *European Journal of Forest Research* **138**: 353–361. https://doi.org/10.1007/s10342-019-01176-4

Ristok C, Leppert KN, Scherer-Lorenzen, Niklaus PA, Bruelheide H. 2019. Soil macrofauna and leaf functional traits drive the decomposition of secondary metabolites in leaf litter. *Soil Biology and Biochemistry* **135**: 429–437. https://doi.org/10.1016/j.soilbio.2019.06.007

Rognes T, Flouri T, Nichols B, Quince C, Mahe F. 2016. VSEARCH: A versatile open source tool for metagenomics. *Peerj* **4**: e2584. https://doi.org/10.7717/peerj.2584

Sanders J. 2020. Veusz – a scientific plotting package. https://veusz.github.io/

Schimel JP, Cates RG, Ruess R. 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry* **42:** 221–234. https://doi.org/10.1023/A:1005911118982

Shay PE, Trofymow JA, Constabel CP. 2017. An improved butanol-HCl assay for quantification of water-soluble, acetone:methanol-soluble, and insoluble proanthocyanidins (condensed tannins). *Plant Methods* **13**: 63. https://doi.org/10.1186/s13007-017-0213-3

Shay PE, Constabel CP, Trofymow JA. 2018. Evidence for the role and fate of water-insoluble condensed tannins in the short-term reduction of carbon loss during litter decay. *Biogeochemistry* **137**: 127–141. https://doi.org/10.1007/s10533-017-0406-x

Swift MJ, Heal W, Anderson JM. 1979. Decomposition in Terrestrial Ecosystems, Blackwell Scientific Publications, Oxford.

Tang AM, Jeewon R, Hyde KD. 2005. Succession of microfungal communities on decaying leaves of *Castanopsis fissa*. *Canadian Journal of Microbiology* **51**: 967–74. https://doi.org/10.1139/w05-086

Thoss V, Shevtsova A, Nilsson MC. 2004. Environmental manipulation treatment effects on the reactivity of water-soluble phenolics in a subalpine tundra ecosystem. *Plant and Soil* **259**: 355–365. https://doi.org/10.1023/B:PLSO.0000020984.17403.82

Treseder KK, Bent E, Borneman J, McGuire KL. 2014. Shifts in fungal communities during decomposition of boreal forest litter. *Fungal Ecology* **10**: 58–69. https://doi.org/10.1016/j.funeco.2013.02.002

Turtola S, Sallas L, Holopainen JK, Julkunen-Tiitto R, Kainulainen P. 2006. Long-term exposure to enhanced UV-B radiation has no significant effects on growth or secondary compounds of outdoor-grown Scots pine and Norway spruce seedlings. *Environmental Pollution* **144**: 166–171. https://doi.org/10.1016/j.envpol.2005.12.025

Vivelo S, Bhatnaga JM. 2019. An evolutionary signal to fungal succession during plant litter decay. *FEMS Microbiology Ecology* **95**. https://doi.org/10.1093/femsec/fiz145

Voříšková J, Baldrian P. 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. *The ISME Journal* **7**: 477–486. https://doi.org/10.1038/ismej.2012.116

Wardle DA. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton.

Wickings K, Grandy AS, Reed SC, Cleveland CC. 2012. The origin of litter chemical complexity during decomposition. *Ecology letters* **15**: 1180–1188. https://doi.org/10.1111/j.1461-0248.2012.01837.x

Winder RS, Lamarche J, Constabel CP, Hamelin RC. 2013. The effects of high-tannin leaf litter from transgenic poplars on microbial communities in microcosm soils. *Frontiers in Microbiology* **4**: https://doi.org/10.3389/fmicb.2013.00290

Yuan G, Guang Y, Yi H, Lai S, Sun Y, Cao S. 2021. Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. *Scientific reports***11**: https://doi.org/10.1038/s41598-021-90035-7

Økland RH, Eilertsen 0. 1993. Vegetation-environment relationships of boreal coniferous forests in the Solhomfjell area, Gjerstad, S Norway. *Sommerfeltia* **16**: 1–254. Oslo. ISBN 82-7420-018-7. ISSN 0800-6865.

Supporting Information Table 1. Concentrations (mg g^{-1}) (mean values ±1 SE) of phenolic compounds in bilberry litter and results of linear mixed-effects models, with plot nested within transect as random factors, to test for the effect of time (14, 61, 250, 370), lime richness (LR), and drought risk (DR). Significant *F* and *P* values are printed in bold, only significant effects are shown.

	Time								
	0	14	61	250	370	Selected model	Significant variables	DF	*F* (*P*)
ACETOPHENONES									
1. Acetophenone 1	0.09 ± 0.04	0.25 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**37.54 (<0.001)**
2. Acetophenone 2	0.04 ± 0.02	0.09 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**33.59 (<0.001)**
3. Acetophenone 3	0.09 ± 0.04	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**22.47 (<0.001)**
Sum Acetophenones	*0.21 ± 0.00*	*0.37 ± 0.07*	*0.01 ± 0.00*	*0.01 ± 0.00*	*0.01 ± 0.00*	*Time*	*Time*	*2, 6*	***36.77 (<0.001)***
FLAVONOIDS									
4. Dihydromyricetin	0.33 ± 0.15	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	*Time*	*Time*	2, 6	**53.38 (<0.001)**
5. Flavonoid	2.67 ± 1.19	0.99 ± 0.24	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	*Time*	*Time*	2, 6	**59.58 (<0.001)**
6. Kaempfeol aglycon	0.00 ± 0.00	0.15 ± 0.04	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	*Time*	*Time*	2, 6	**44.75 (<0.001)**
7. Kaempferol3glyc	0.00 ± 0.00	0.62 ± 0.15	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	*Time*	*Time*	2, 6	**54 (<0.001)**
8. Myricetin3glyc 1	6.21 ± 2.78	1.13 ± 0.27	0.05 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	*Time*	*Time*	2, 6	**51.49 (<0.001)**
9. Myricetin3glyc 2	1.15 ± 0.69	0.54 ± 0.13	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	*Time*	*Time*	2, 6	**57.16 (<0.001)**
10. Quercetin aglycon	1.52 ± 0.68	0.97 ± 0.23	0.06 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	*Time*	*Time*	2, 6	**65.91 (<0.001)**
11. Quercetin3glyc	24.86 ± 11.12	5.20 ± 1.26	0.15 ± 0.04	0.07 ± 0.02	0.03 ± 0.01	Time * LR * DR	Time	2, 15	**51.66 (<0.001)**
							Time × LR	2, 15	**6.55 (<0.001)**
Sum flavonoids	*37.15 ± 0.27*	*9.63 ± 1.36*	*0.39 ± 0.03*	*0.15 ± 0.01*	*0.06 ± 0.01*	*Time * LR * DR*	*Time*	*2, 15*	***54.87 (<0.001)***
							Time × LR	*2, 15*	***6.80 (0.040)***
PHENOLIC ACIDS									
12. Gallic acid	0.01 ± 0.00	0.48 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**21.53 (<0.001)**
13. Chl der	0.35 ± 0.16	0.20 ± 0.05	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**53.65 (<0.001)**
14. Chlorogenic acid	52.15 ± 23.32	0.25 ± 0.06	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	Time	Time	2, 6	**39.57 (<0.001)**
15. HCA 1	2.06 ± 0.92	0.67 ± 0.16	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**43.88 (<0.001)**
16. HCA 2	5.44 ± 2.43	0.66 ± 0.16	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**38.25 (<0.001)**

Supporting Information Table 1. continued

	Time								
	0	14	61	250	370	Selected model	Significant variables	DF	*F* (*P*)
17. HCA 3	0.00 ± 0.00	0.33 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**45.08 (<0.001)**
18. HCA 4	0.00 ± 0.00	0.10 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**47.37 (<0.001)**
19. HCA 5	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**29.64 (<0.001)**
20. Phenolic acid 1	1.35 ± 0.60	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**40.15 (<0.001)**
21. Phenolic acid 2	0.13 ± 0.06	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**37.96 (<0.001)**
22. Phenolic acid 3	1.39 ± 0.62	0.09 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**37.77 (<0.001)**
23. Phenolic acid 4	0.33 ± 0.15	0.25 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**42.01 (<0.001)**
24. Phenolic acid 5	1.46 ± 0.65	0.40 ± 0.10	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**46.64 (<0.001)**
Sum phenolic acids	*64.64 ± 0.64*	*3.53 ± 0.63*	*0.13 ± 0.01*	*0.07 ± 0.01*	*0.04 ± 0.00*	*Time * LR * DR*	*Time*	*2, 15*	***39.29 (<0.001)***
SUM LOW-MOLECULAR PHENOLICS	**102.00 ± 0.89**	**13.53 ± 2.04**	**0.53 ± 0.04**	**0.22 ± 0.02**	**0.10 ± 0.01**	Time * LR * DR	Time	2, 15	**50.25 (<0.001)**
							Time × Lime richness	2, 15	**6.45 (0.040)**
CONDENSED TANNINS									
25. MeOH-soluble	116.09 ± 2.28	17.98 ± 2.35	5.34 ± 0.38	3.93 ± 0.26	2.75 ± 0.25	Time * LR * DR	Time	2, 15	**36.70 (<0.001)**
26. MeOH-insoluble	16.91 ± 0.81	48.64 ± 1.72	28.83 ± 1.40	23.38 ± 0.71	21.74 ± 1.00	Time * LR * DR	Time	2, 15	**148.66 (<0.001)**
							Time × LR × DR	2, 15	**6.90 (0.032)**
SUM PHENOLIC COMPOUNDS	**235.00 ± 2.59**	**80.15 ± 4.49**	**34.79 ± 1.46**	**28.13 ± 0.64**	**24.42 ± 1.48**	Time * LR * DR	Time	2, 15	**73.36 (<0.001)**

Supporting Information Table 2. Concentrations (mg g^{-1}) (mean values ±1 SE) of phenolic compounds in pine litter and results of linear mixed-effects models, with plot nested within transect as random factors, to test for the effect of time (14, 61, 250, 370), lime richness (LR), and drought risk (DR). Significant *F* and *P* values are printed in bold, only significant effects are shown.

	Time								
	0	14	61	250	370	Selected model	Significant variables	DF	*F* (*P*)
FLAVONOIDS									
1. Dihydromyrectin	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**125.21 (<0.001)**
2. Dihydroquercitin	1.33 ± 0.08	0.38 ± 0.05	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**61.36 (<0.001)**
3. Flavonoid 1	0.00 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**102.08 (<0.001)**
4. Flavonoid 2	0.42 ± 0.03	0.14 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**86.36 (<0.001)**
5. Flavonoid 3	0.00 ± 0.00	0.07 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**62.98 (<0.001)**
6. Monocoumaroylastragallin 1	0.07 ± 0.00	0.07 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**90.28 (<0.001)**
7. Monocoumaroylastragallin 2	0.00 ± 0.00	0.07 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**104.93 (<0.001)**
8. Monocoumaroylastragallin 3	0.02 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**124.5 (<0.001)**
9. Monocoumaroylastragallin 4	0.15 ± 0.00	0.14 ± 0.02	0.10 ± 0.01	0.05 ± 0.01	0.02 ± 0.00	Time	Time	2, 6	**82.08 (<0.001)**
10. Monocoumaroylastragallin 5	0.23 ± 0.01	0.14 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.02 ± 0.00	Time	Time	2, 6	**112.6 (<0.001)**
11. Monocoumaroylastragallin 6	0.16 ± 0.00	0.16 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.03 ± 0.00	Time	Time	2, 6	**256.82 (<0.001)**
12. Monocoumaroylastragallin 7	0.30 ± 0.01	0.12 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0.00	Time	Time	2, 6	**58.98 (<0.001)**
13. Monocoumaroylastragallin 8	0.31 ± 0.01	0.38 ± 0.01	0.17 ± 0.02	0.11 ± 0.01	0.06 ± 0.01	Time	Time	2, 6	**144.12 (<0.001)**
14. Monocoumaroylastragallin 9	0.22 ± 0.01	0.25 ± 0.03	0.17 ± 0.01	0.10 ± 0.01	0.04 ± 0.00	Time	Time	2, 6	**92.75 (<0.001)**
15. Monocoumaroylastragallin 10	0.11 ± 0.00	0.14 ± 0.02	0.06 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	Time	Time	2, 6	**52.45 (<0.001)**
16. Monocoumaroylastragallin 11	1.15 ± 0.05	0.90 ± 0.05	0.34 ± 0.02	0.19 ± 0.02	0.08 ± 0.01	Time	Time	2, 6	**168.46 (<0.001)**
17. Pinocembrin der	0.00 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**164.87 (<0.001)**
18. Quercetin3gluciside	0.24 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**52.38 (<0.001)**
19. Rhamnetin	0.05 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**63.77 (<0.001)**
Sum flavonoids	*4.78 ± 0.12*	*3.24 ± 0.10*	*1.29 ± 0.05*	*0.72 ± 0.05*	*0.31 ± 0.03*	*Time*	*Time*	*2, 6*	***222.51 (<0.001)***

Supporting Information Table 2. continued

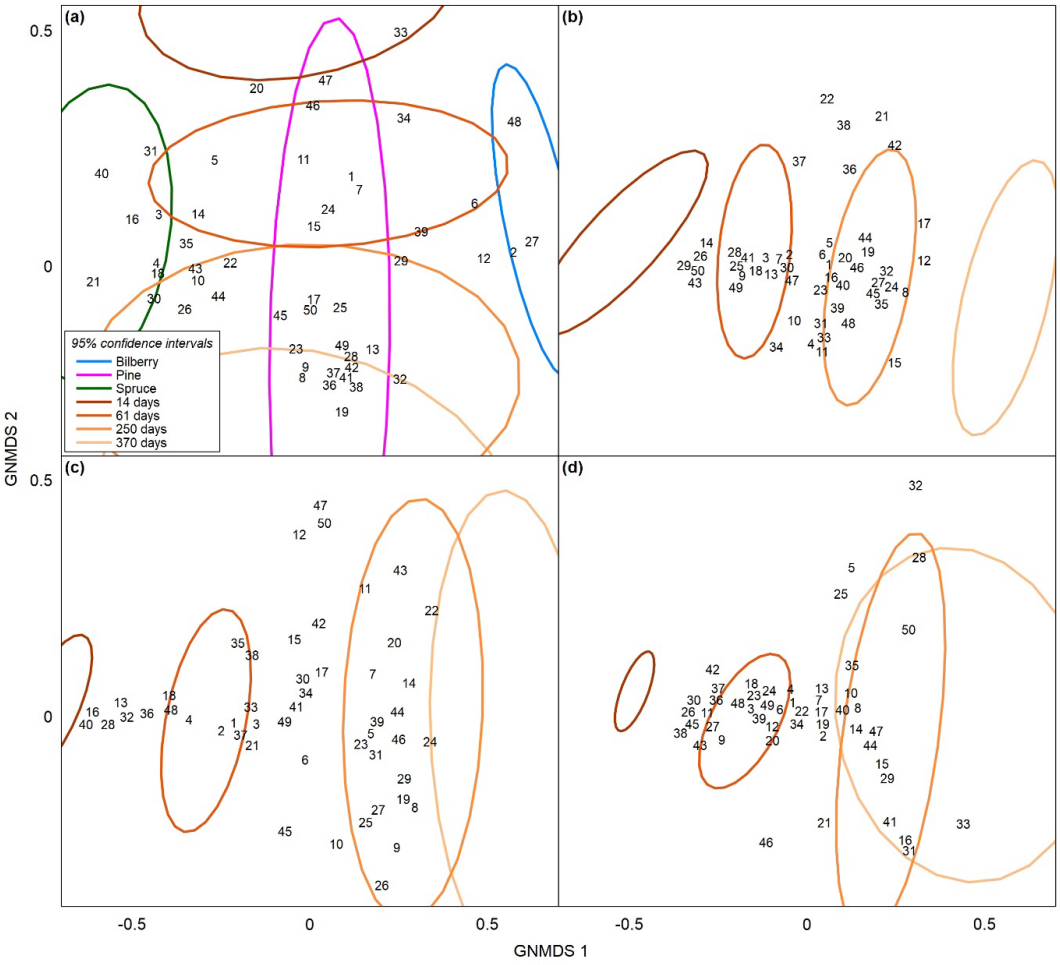
	Time								
	0	**14**	**61**	**250**	**370**	**Selected model**	**Significant variables**	**DF**	***F* (*P*)**
PHENOLIC ACIDS									
20. Phenolic acid	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**42.25 (<0.001)**
STILBENES									
21. Pinosylvin	0.05 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**136.59 (<0.001)**
22. Pinosylvinmonomethyleter	0.02 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**74.00 (<0.001)**
23. Resveratrol	0.00 ± 0.00	0.09 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**39.20 (<0.001)**
24. Stilbene 1	0.04 ± 0.00	0.07 ± 0.01	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**159.84 (<0.001)**
25. Stilbene 2	0.12 ± 0.01	0.07 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**206.43 (<0.001)**
26. Stilbene 3	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**103.43 (<0.001)**
27. Stilbene 4	0.03 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**39.20 (<0.001)**
28. Stilbene 5	0.06 ± 0.00	0.09 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**97.64 (<0.001)**
29. Stilbene 6	0.95 ± 0.07	0.08 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	Time	Time	2, 6	**47.70 (<0.001)**
Sum stilbenes	*1.32 ± 0.09*	*0.59 ± 0.03*	*0.21 ± 0.02*	*0.12 ± 0.01*	*0.05 ± 0.01*	*Time*	*Time*	*2, 6*	***154.76 (<0.001)***
SUM LOW-MOLECULAR PHENOLICS	**6.10 ± 0.17**	**3.86 ± 0.13**	**1.50 ± 0.07**	**0.84 ± 0.06**	**0.36 ± 0.03**	Time	Time	2, 6	**213.75 (<0.001)**
CONDENSED TANNINS									
30. MeOH-soluble	10.24 ± 0.58	3.16 ± 0.37	0.99 ± 0.06	0.68 ± 0.04	0.42 ± 0.02	Time * LR	Time	2, 9	**70.70 (<0.001)**
							Time × LR	2, 9	**8.07 (0.018)**
31. MeOH-insoluble	5.01 ± 0.42	8.14 ± 0.25	5.18 ± 0.41	4.13 ± 0.16	3.68 ± 0.22	Time	Time	2, 6	**95.54 (<0.001)**
SUM PHENOLIC COMPOUNDS	**21.36 ± 0.88**	**15.20 ± 0.58**	**7.89 ± 0.41**	**5.56 ± 0.16**	**4.75 ± 0.20**	Time * LR * DR	Time	2, 15	**166.74 (<0.001)**

Supporting Information Table 3. Concentrations (mg g^{-1}) (mean values ±1 SE) of phenolic compounds in spruce litter and results of linear mixed-effects models, with plot nested within transect as random factors, to test for the effect of time (14, 61, 250, 370), lime richness (LR), and drought risk (DR). Significant *F* and *P* values are printed in bold, only significant effects are shown.

	Time								
	0	14	61	250	370	Selected model	Significant variables	DF	*F* (*P*)
ACETOPHENONES									
1. Picein	7.27 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00				-
FLAVONOIDS									
2. Apigenin der	0.15 ± 0.00	0.21 ± 0.01	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**223.07 (<0.001)**
3. Apigenin7glucoside	0.23 ± 0.02	0.62 ± 0.02	0.11 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**177.58 (<0.001)**
4. Dicoumaroylastragallin der 1	0.46 ± 0.01	0.59 ± 0.01	0.39 ± 0.05	0.08 ± 0.01	0.02 ± 0.00	Time	Time	2, 6	**305.89 (<0.001)**
5. Dicoumaroylastragallin der 2	1.06 ± 0.04	1.24 ± 0.04	0.78 ± 0.15	0.31 ± 0.08	0.10 ± 0.01	Time	Time	2, 6	**84.26 (<0.001)**
6. Dihydromyricetin	0.00 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**68.22 (<0.001)**
7. Flavonoid 1	0.00 ± 0.00	0.13 ± 0.01	0.20 ± 0.04	0.04 ± 0.01	0.01 ± 0.00	Time	Time	2, 6	**40.81 (<0.001)**
8. Flavonoid 2	0.00 ± 0.00	0.12 ± 0.01	0.33 ± 0.05	0.04 ± 0.01	0.01 ± 0.00	Time	Time	2, 6	**30.81 (<0.001)**
9. Kaempferol der 1	0.12 ± 0.01	0.27 ± 0.02	0.14 ± 0.02	0.03 ± 0.01	0.00 ± 0.00	Time	Time	2, 6	**209.61 (<0.001)**
10. Kaempferol der 2	0.13 ± 0.00	0.24 ± 0.01	0.17 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**450.76 (<0.001)**
11. Monocoumaroylastragallin	0.23 ± 0.01	0.32 ± 0.03	0.18 ± 0.02	0.04 ± 0.00	0.02 ± 0.00	Time	Time	2, 6	**127.97 (<0.001)**
Sum flavonoids	*2.38 ± 0.09*	*3.78 ± 0.09*	*2.35 ± 0.34*	*0.56 ± 0.11*	*0.17 ± 0.01*	*Time*	*Time*	*2, 6*	***227.59 (<0.001)***
PHENOLIC ACIDS									
12. Chlorogenic acid	0.17 ± 0.00	0.97 ± 0.04	0.04 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**117.41 (<0.001)**
STILBENES									
13. Isorhapontin	4.14 ± 0.10	1.30 ± 0.06	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**108.29 (<0.001)**
14. Piceatannol aglycon	0.83 ± 0.03	1.81 ± 0.10	0.32 ± 0.28	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**61.541 (<0.001)**
15. Piceatannol glucoside	10.41 ± 0.40	4.84 ± 0.21	0.55 ± 0.13	0.01 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**142.6 (<0.001)**
16. Resveratrol	0.40 ± 0.02	1.21 ± 0.04	0.22 ± 0.10	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**132.62 (<0.001)**
17. Resveratrol aglycon	0.36 ± 0.01	4.30 ± 0.12	0.63 ± 0.39	0.03 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**109.51 (<0.001)**
18. Stilbene 1	0.16 ± 0.04	0.41 ± 0.02	0.12 ± 0.03	0.00 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**203.1 (<0.001)**

Supporting Information Table 3. continued

	Time								
	0	**14**	**61**	**250**	**370**	**Selected model**	**Significant variables**	**DF**	***F* (*P*)**
19. Stilbene 2	0.04 ± 0.00	0.42 ± 0.01	0.12 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	Time	Time	2, 6	**264.66 (<0.001)**
20. Stilbene 3	0.00 ± 0.00	0.12 ± 0.01	0.11 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	Time	Time	2, 6	**81.74 (<0.001)**
Sum stilbenes	*16.35 ± 0.54*	*14.41 ± 0.49*	*2.11 ± 0.91*	*0.09 ± 0.01*	*0.03 ± 0.00*	*Time * LR * DR*	*Time*	*2, 15*	***120.83 (<0.001)***
SUM LOW-MOLECULAR PHENOLICS	**26.17 ± 0.73**	**19.17 ± 0.59**	**4.49 ± 1.11**	**0.66 ± 0.11**	**0.20 ± 0.02**	Time * LR * DR	Time	2, 15	**173.63 (<0.001)**
CONDENSED TANNINS									
21. MeOH-soluble	62.53 ± 0.69	47.20 ± 2.16	6.13 ± 0.37	2.33 ± 0.29	1.07 ± 0.22	Time * LR * DR	Time	2, 15	**93.08 (<0.001)**
22. MeOH-insoluble	10.23 ± 1.43	28.19 ± 0.49	17.84 ± 0.54	14.31 ± 0.74	7.42 ± 0.59	Time * LR * DR	Time	2, 15	**187.43 (<0.001)**
SUM PHENOLIC COMPOUNDS	**98.93 ± 1.92**	**94.78 ± 1.90**	**28.46 ± 1.46**	**17.30 ± 0.78**	**8.63 ± 0.69**	Time * LR * DR	Time	2, 15	**136.67 (<0.001)**



Supporting Information Figure 1. Numerical prefixes that identify all OTUs that are presented in the GNMDS ordination plots of fungal community composition in Figure 4, for **(a)** all litter types, **(b)** bilberry, **(c)** pine, and **(d)** spruce litter after 14, 61, 250, and 370 days after incubation in the soil. Numerical prefixes identify all OTUs sorted by abundance for all litter types **(a)**: *Phacidium pseudophacidioides* (1), Helotiales spp. (2, 18, 32, 36, 37), *Muriformistrickeria rosae* (3), *Chalara* spp. (4, 10, 21, 22, 26), *Rhizosphaera oudemansii* (5), *Endoconidioma populi* (6), *Hormonema macrosporum* (7), Agaricales sp. (8), *Hydnum* sp. (9), *Didymella urticicola* (11), *Phlogicylindrium* sp. (12), *Phialocephala* sp. (13), *Penicillium penicillioides* (14), Leotiomycetes sp. (15), *Sistotrema* spp. (16, 17, 23), *Infundichalara minuta* (19), Tremellales sp. (20), *Cladosporium delicatulum* (24), Agaricomycetes sp. (25), *Lachnum rhytismatis* (27), Hyaloscyphaceae sp. (28), *Gyoerffyella entomobryoides* (29), *Mycena arcangeliana* (30), Xylariales sp. (31), Tremellomycetes sp. (33), unknown fungi (34), *Cistella acuum* (35), *Pezoloma ericae* (38), *Dermea viburni* (39), Ceratobasidiaceae sp. (40), *Mollisia cinerea* (41), Herpotrichiellaceae sp. (42), unknown ascomycota (43), Serendipitaceae sp. (44), *Trichoderma parapiluliferum* (45), Cantharellales sp. (46), *Vishniacozyma victoriae* (47), *Ramularia rhabdospora* (48), *Arachnopeziza* sp. (49), *Vestigium* sp. (50). Numerical prefixes identify all OTUs sorted by abundance for bilberry litter **(b)**: Helotiales spp. (1, 15,

17, 20, 23, 24, 32, 35, 46), *Endoconidioma populi* (2), *Phacidium pseudophacidioides* (3), *Phlogicylindrium* sp. (4), *Lachnum rhytismatis* (5), *Gyoerffyella* spp. (6, 21, 36), *Cladosporium delicatulum* (7), Agaricales sp. (8), Unknown fungi (9), *Dermea viburni* (10), *Phialocephala* sp. (11), *Pezoloma ericae* (12), *Hormonema macrosporum* (13), *Ramularia rhabdospora* (14), Sympoventuriaceae sp. (16), *Cadophora* sp. (18), Hyaloscyphaceae sp. (19), *Sistotrema sp.* (22), Tremellales sp. (25), *Vishniacozyma victoriae* (26), *Mollisia cinerea* (27), Rhytismatales sp. (28), Tremellomycetes sp. (29), *Didymella gardeniae* (30), Pezizales sp. (31), *Fontanospora fusiramosa* (33), *Hydnum* sp. (34), *Muriformistrickeria rosae* (37), *Alpinaria* sp. (38), *Hyaloscypha* sp. (39), *Arachnopeziza* sp. (40), *Didymella urticicola* (41), *Polyscytalum sp.* (42), *Exobasidium japonicum* (43), Auriculariales sp. (44), *Mycena arcangeliana* (45), *Leptodontidium* sp.(47), Herpotrichiellaceae sp. (48), Chaetothyriales sp. (49), *Curvibasidium cygneicollum* (50). Numerical prefixes identify all OTUs sorted by abundance for pine litter **(c)**: *Phacidium pseudophacidioides* (1), *Hormonema macrosporum* (2), Leotiomycetes sp. (3), *Didymella urticicola* (4), *Hydnum* sp. (5), *Sistotrema* spp. (6, 11), Agaricales sp. (7), *Infundichalara minuta* (8), *Phialocephala* sp. (9), Agaricomycetes sp. (10), *Chalara piceae-abietis* (12), Tremellales sp. (13), Hyaloscyphaceae sp. (14), *Chalara holubovae* (15), Tremellomycetes sp. (16), *Trichoderma parapiluliferum* (17), Cantharellales spp. (18, 30), Herpotrichiellaceae sp. (19), Helotiales spp. (20, 26), *Lophodermium pinastri* (21), *Cryptosporiopsis* sp. (22), *Mollisia cinerea* (23), Dermateaceae sp. (24), *Vestigium* sp. (25), *Scleropezicula* sp. (27), Unknown fungi (28), Dothideomycetes sp. (29), *Cladophialophora* spp. (31, 46), *Truncatella spadicea* (32), *Cladosporium delicatulum* (33), *Infundichalara* sp. (34), *Muriformistrickeria rosae* (35), *Lophodermium conigenum* (36), Chaetothyriales sp. (37), *Penicillium penicillioides* (38), *Xenochalara* sp. (39), *Genolevuria* sp. (40), Ascomycota spp. (41,48), Chaetosphaeriaceae sp. (42), *Chlorencoelia torta* (43), *Arachnopeziza* sp. (44), *Athelia* sp. (45), Serendipitaceae sp. (47), *Pyrenopeziza revincta* (49), *Trichoderma spirale* (50). Numerical prefixes identify all OTUs sorted by abundance for spruce litter **(d)**: *Muriformistrickeria rosae* (1), *Chalara* spp. (2, 10, 13, 19, 40, 50), *Rhizosphaera oudemansii* (3), *Penicillium penicillioides* (4), *Chalara piceae-abietis* (5), *Sistotrema* spp. (6, 28), Helotiales spp. (7, 31, 49), *Chalara pseudoaffinis* (8), *Phacidium pseudophacidioides* (9), Tremellales sp. (11), Xylariales sp. (12), *Mycena arcangeliana* (14), Agaricales sp. (15), *Hydnum sp.* (16), *Cistella acuum* (17), Ceratobasidiaceae sp. (18), *Cladosporium delicatulum* (20), *Phialocephala* spp. (21, 47), Ascomycota sp. (22), *Didymella urticicola* (23), Chaetothyriales sp. (24), Serendipitaceae sp. (25), unknown fungi (26, 27), Hyaloscyphaceae sp. (29), *Phacidium lacerum* (30), *Alatospora acuminata* (32), *Tomentella terrestris* (33), *Chlorencoelia torta* (34), *Mollisia sp.* (35), Microbotryomycetes sp. (36), *Polyscytalum* sp. (37), Cantharellales sp. (38), *Lachnum pulverulentum* (39), Tricholomataceae sp. (41), *Cystofilobasidium capitatum* (42), *Curvibasidium cygneicollum* (43), *Mycena sanguinolenta* (44), *Vishniacozyma victoriae* (45), *Endoconidioma populi* (46), *Phialocephala sphaeroides* (47), *Piskurozyma* sp. (48).

Paper II

Faster initial litter decomposition under native birch than planted spruce

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Manuscript

Summary

Litter decomposition rates are highly influenced by plant species. Shifts in the dominant tree species may therefore have a significant impact on the early stage of litter decomposition process. Plant litter is predicted to have a home-field advantage (HFA hypothesis) with faster decay rates underneath the plant from which it originates. We tested this hypothesis in a one-year reciprocal litterbag experiment by placing litter material of birch and spruce in parallel stands of native birch and planted spruce. Birch litter mass loss was generally more rapid compared to spruce litter, while both litter types had a higher mass loss in the birch stands. Condensed tannin loss and fungal biomass was highest in birch litter. Initial C:N ratio and condensed tannin concentration was correlated with mass loss. The greater negative HFA for spruce litter offset the positive HFA for birch litter, which resulted in a net negative home-field advantage. We found no significant effect of microclimatic conditions on early-stage litter decay. Our results indicate that a shift from birch-dominated forest to planted spruce will result in slower decomposition rates in the early stage of decomposition.

Keywords: *Betula pubescens*, boreal forest, fungal biomass, litter decomposition, mass loss, *Picea abies*, soil carbon, tree influence, litter quality

Introduction

Trees are major producers of litter in forest ecosystems. The species-specific rate of litter decomposition has been proposed to explain the effects that tree species appears to have on soil C stocks (Hansen et al. 2009, Vesterdal et al. 2008), a process in which litter quality, decomposer communities, and microclimatic conditions play key functional roles (Coûteaux et al. 1995, Hobbie et al. 2006, Prescott 2002). Shifts in tree species composition will thus have an impact on the decomposition process, which in turn will have implications for both carbon (C) dynamics and nutrient cycling. However, the effects of tree species change on soil C is complex and far from fully understood (Jandl et al. 2007, Vesterdal et al. 2013).

Litter quality is an important driver in the early stages of litter decay (Djukic et al. 2018). Tree species produce leaf litter with species-specific concentration and composition of chemical components, such as nitrogen (N), lignin, and secondary metabolites, that decompose at different rates. When plant litter enters the soil, a flush of nutrients is rapidly released through leaching of soluble compounds and by microbial decomposer activity (Nykvist 1963, van der Heijden et al. 2007). Generally, initial N concentrations are positively related to early decomposition rates, while lignin:N ratio are negatively correlated due to recalcitrant lignin compounds (Talbot et al. 2012, Melillo et al. 1982, Aerts 1997). In line with this, initial decomposition of broadleaved litter tends to be faster than conifer litter, which is largely attributed to greater labile compound concentration, higher N concentration, and lower lignin concentration in broadleaved leaf litter (Cornwell et al. 2008, Prescott et al. 2000). Moreover, the importance of secondary metabolites such as condensed tannins on decay rates has often been overlooked (Chomel et al. 2016). Condensed tannins are polymeric compounds that may hamper the decay process in several ways, which include being toxic to microorganisms, inhibit enzyme activity, and form insoluble complexes with biological polymers (Hättenschwiler & Vitousek 2000, Kraus et al. 2003). Thus, labile compounds including cellulose and low molecular weight secondary metabolites are expected to be released in the early decomposition phase, while condensed tannins will be released together with other recalcitrant compounds at later stages (Chomel et al. 2016). To get a better understanding of the effect that tree species has on litter decay rates, it is necessary to know how species-specific secondary metabolites affect C and nutrient cycling.

Plant litter is predicted to decompose faster in the soil under the plant from which it originates, a mechanism commonly known as the home-field advantage (HFA) hypothesis (Ayres et al.

2009, Gholz et al. 2000). This HFA is attributed to the adaption of decomposer communities to species-specific litter types and qualities. Fungi are the main decomposers of litter in boreal forests, through their ability to produce a wide range of extracellular enzymes (Lindahl et al. 2021). The decomposing activity of fungal communities and decomposition rates have been found to correlate with fungal biomass (Albright et al. 2020, Lodato et al. 2021), and accumulation of fungal biomass can thus be used as proxy for decomposing activity. However, decomposing activity and soil dynamics are also impacted by the local microclimate, which is known to differ significantly under the canopy of different tree species (Frenne et al. 2021). Microclimates within forests of different tree species are influenced in various ways, for example by ground temperature and moisture conditions being modified by species-specific canopy characteristics (Prescott 2002). In the growing season, conifer-dominated forests are normally cooler than those dominated by broadleaved species, as the coniferous canopy cover is denser, and the penetration of light and wind is generally low and reduces lateral transfer of humidity and heat (Greiser et al. 2018).

Forests of native pine or broadleaved birch-dominated forests have been extensively replaced by planted spruce, predominantly Norway spruce (*Picea abies* L. (Karst); hereafter spruce), across Western Norway since the middle of the 20th century (Granhus et al. 2012, Hylen 2019). This forestry practice has historically been applied to increase timber production, and more recently to increase C sequestration. Kjønaas et al. (2021) examined the effect of a tree species change from natural birch to planted Norway spruce on the carbon stocks at stand level and found a significantly higher C stock in the forest floor under planted spruce compared to native birch. Still, the overall soil C stock for the first meter of depth was unaffected by the tree species change.

In this study, we investigate the differences in early-stage decomposition rates and chemical characteristics of litter material from planted Norway spruce and native birch-dominated stands and test the HFA hypothesis in the same study system as in Kjønaas et al. (2021). We conducted a one-year reciprocal litterbag experiment by placing site-specific litter material of birch and spruce in both stands. We hypnotized that spruce litter would decompose more slowly than birch litter based on litter quality. Further, we expected lower mass loss in spruce relative to birch stands, due to lower temperatures in the spruce stands. By transplanting litter between the stands, we further expected that the litter would decompose more rapidly in the stand it originates from, according to the HFA hypothesis.

Materials and methods

Study locations

The study was conducted at four locations (Stranda, Ørsta, Jølster I, and Jølster II) in Vestland county and Møre and Romsdal county in western Norway (Fig. 1, Table 1) in stands of adjacent mature native downy birch (*Betula pubescens* Ehrh.) and planted spruce (Kjønaas et al. 2021). At each location, three paired plots (144m^2) in parallel birch and spruce stands were established, with six randomly positioned 0.5 × 0.5 m subplots within each plot. While the spruce stands were planted 45–60 years ago, uneven-aged stands of naturally occurring downy birch dominate the landscape with scattered common juniper (*Juniperus communis* L.). The birch stands were characterized by diverse and abundant understories of bryophytes, forbs, graminoids, ferns and ligneous species, while mainly a few dominant bryophyte species were present in the spruce stands understories (Kjønaas et al. 2021). Both stand types have been subjected to some rough grazing by sheep and wild deer. All locations were located on hillsides, where varying slopes, elevations, and aspects contributed to some variation in the local climate (Table 1). For a complete description of environmental conditions, vegetation, soil characteristics, and experimental design, see Kjønaas et al. (2021).

Microclimatic measurements

Surface and soil temperature and moisture (at 6 cm depth) were recorded at each subplot using TMS-4 soil probe sensors (TOMST, Praha, Czech Republic; ± 0.5 °C accuracy) set to 15 min measurement frequency (Kjønaas et al. 2021). The measurements took place throughout the whole study period, and gap filling was performed when missing data occurred. For a detailed

Table 1. Location description and environmental conditions (data from Kjønaas et al. 2021).

	Stranda	Ørsta	Jølster I	Jølster II
Location	62°16'N, 6°52'E	62°9'N, 6°12'E	61°30'N, 6°18'E	61°30'N, 6°12'E
MAT (C°)	3.41	6.17	4.99	3.95
MAP (mm)	1584	1951	2614	2394
Aspect	S–SE	E–NE	N	N
Elevation (m asl)	430	210	225–250	335–345
Stand age (years birch/spruce)	87/45	104/60	101/45	103/60

Notes: Meteorological data are average values for the period 1986–2015, based on gridded climate data adjusted according to elevation, whereas stand age for birch is based on tree ring analysis in increment cores from dominant trees (see Kjønaas et al. 2021).

description on the gap filling of procedure, see Supplementary Information (Details on gap filling methodology). The sum of growing degree days (GDD) was calculated for temperature measured at the soil surface and at 6 cm soil depth. The calculations were based on 24-hour periods (96 records per day) with an average temperature above 0 °C and 5 °C, which were summed up for the one-year study period to assess potential differences in temperature sums related to microbial- and plant activity, respectively.

Litterbag experiment

Senescent leaves from living birch and spruce twigs were collected in birch and spruce stands at all four locations in the beginning of October 2018 and stored separately for each location. The plant material was oven dried (30 °C), before 1 g of each litter type was placed individually inside approximately 5 × 5 cm sachets constructed of 50-micron masked nylon. The initial dry mass was determined by oven dried (70 °C, 48h) plant material and the ratio between air and

Figure 1. Map of the locations (Stranda, Ørsta, Jølster I, and Jølster II) of the paired stands of native birch and planted spruce in Western Norway. The location Molde was excluded from assessments of a species change due to the spruce stand being subject to nutrient rich water from a well. (Retrieved from Kjønaas et al. 2021, Appendix, Supplementary Figures, Figure S1).

oven dried weights were calculated. Six subsamples of the litter from each species were used to determine the initial concentration of measured variables prior to decomposition. At the end of October 2018, one litter bag of each litter type was placed on top of the forest floor in subplots where the understory vegetation biomass previously had been harvested (Jøster I and II in 2016; Stranda and Ørsta in 2017) (Kjønaas et al. 2021), and thus allowing similar conditions in ground position between the stand types. Litter material was distributed at the location it originated, and the litterbags were attached on top of the forest floor with a stick. This gave a total of 288 litterbags, 144 bags of each litter type, which was deployed in a total of 72 birch and 72 spruce subplots. Litterbags were collected after 358, 359, 358, and 358 days at Stranda, Ørsta, Jølster I, and Jølster II, respectively. Mass remaining after the experiment period was determined after freeze-drying (48 h) litterbags and mass loss was calculated as the proportion of initial oven dry mass. The material was then homogenized by grinding it to powder with a ball mill and subsampled into smaller samples for various assays. Concentrations of C and N were measured using a vario MICRO cube elemental analyser (Elementar, Hanau, Germany). To calculate the amount of released N, the total mass × N concentration after incubation were subtracted from the initial mass × N concentration and expressed as the proportion of the initial mass × N concentration before incubation (Wardle 2002).

Condensed tannins analyses

For analysis of condensed tannins concentrations, we identified both MeOH-soluble and MeOH-insoluble fractions using the acid butanol assay for proanthocyanidins described in Hagerman (2002). Approximately 100 mg of ground litter sample was extracted with methanol (MeOH) as described by Nybakken et al. (2018). Dried MeOH extraction residues were re-dissolved in 100–200 µl MeOH and diluted with 100–200 µl of ultra-pure water (USF ELGA Maxima HPLC; Veolia Water Technologies, Saint-Maurice, France), depending on predicted condensed tannins concentration (start material or partly decomposed material). From this, 25–100 µl was used to determine the quantities of MeOH-soluble condensed tannins. Approximately 5 mg of the residues left in the vials after the extraction process described above were used to examine the amount of MeOH-insoluble condensed tannins. Both liquid and dried extractions were added the adequate amount of MeOH for the total volume to be 0.5 ml. After adding further 3 ml of acid butanol (95% butanol, 5% HCI) and 100 µl of iron reagent (2% ferric ammonium sulphate in 2N HCI), duplicate samples were placed in boiling water for 1 h. Absorbance (550 nm) of cooled samples was detected by a UV spectrophotometer

(Shimadzu, Kyoto, Japan). Concentrations were calculated by averaging measurements in duplicate samples, using purified extracts of spruce needles as a standard. The loss of condensed tannins was calculated by the method used for released N.

Ergosterol analysis

As a proxy for fungal biomass, ergosterol was analysed using a modified version of the protocol of Ransedokken et al. (2019). Approximately 100 mg of prepared litter sample was mixed with 7 ml 3M KOH in MeOH, vortexed and sonicated in a 70 °C ultrasonic water bath in darkness for 90 min. After being vortexed and centrifuged (c. 16 400 rpm, 15 min), the supernatant was mixed with 2 ml purified water in new tubes. Ergosterol was extracted twice by adding 5 ml hexane, vigorous vortexed (approx. 1 min), and the hexane phase was collected after the liquid separated into two phases. Both extractions were collected in the same vial and evaporated using an Eppendorf Concentrator Plus 5301 (Eppendorf, Hamburg, Germany). Dried extracts were re-dissolved in 500 µl MeOH and analysed for ergosterol concentration using a 1200 Series HPLC (Agilent Technologies, Waldbronn, Germany). Ergosterol was separated using a reversed phase ODS ultra sphere column (250 mm × 4.6 mm; particle size 5 µm) with MeOH as the mobile phase (flow rate 1.5 ml min^{-1}, total analysis time 12 min). Absorption of ergosterol was detected at 280 nm and identified by comparing retention time and online UV-spectra with that of a commercial standard of ergosterol (Sigma, St. Louis, USA). A response curve of the commercial standard was used to quantify the sample concentrations of ergosterol.

Statistical analyses

Linear mixed-effects models were used to test for the effect of stand type, litter type, location, and climatic factors on mass loss, C concentration, N release, C:N ratio, ergosterol concentration and loss of MeOH-soluble and MeOH-insoluble condensed tannins after one year of decomposition with subplot nested within plot nested within location as random factor. Climatic variables were standardized by subtracting the mean and dividing by the standard deviation. To test for multicollinearity of climatic factors we ran Pearson correlations. Because temperature parameters were highly correlated (Supplementary Information, Figure S1), we ran separate models for each climatic parameter and compared their AIC values to determine which variables to include in the final models (Supplementary Information, Table S1). Similar linear mixed effects models were used to test for the effect of stand type and location on climatic factors, and simple linear regression models were used to test for the effect for stand

type and location on initial litter properties. When location effects were significant, the emmeans function from the R package emmeans (Length et al. 2021) using Tukey adjusted p-values was used to test which locations that were significantly different.

The home-field advantage index (HFAI) was calculated for pairs of subplots from paired plots in adjacent birch and spruce stands following Ayres et al. (2009) as presented in Asplund et al. (2018):

$$\text{HFAI} = \left[\left(\frac{Ff}{Ff + Pf} + \frac{Pp}{Pp + Fp}\right) / \left(\frac{Pf}{Ff + Pf} + \frac{Fp}{Pp + Fp}\right)\right] \times 100 - 100$$

Here, *Ff* represents the mass loss of litter from species *F* in the forest *f*. The HFAI is the net value for both species and represents the percent faster mass loss of litter when it decomposes at home vs away. The function emmeans was used to test whether the HFAI differed from 0.

All statistical analyses were performed in R 4.1.2 (R Core Team 2021), and graphical illustrations were generated in Veusz 3.4 (Sanders 2020).

Results

Concentrations of N and both fractions of condensed tannins were higher in birch than in spruce litter prior to decomposition, while there was no significant difference in initial C concentration (Table 2). Initial C:N ratio and ergosterol concentration were significantly higher in spruce litter compared to birch litter (Table 2). Both litter types varied in initial quality among locations (Table 2). Birch litter from Jølster II had higher C and N concentrations compared to birch litter from the other three locations, Jølster I had the highest ergosterol and MeOH-soluble condensed tannins concentration, and Stranda had the highest MeOH-insoluble condensed tannins. Spruce litter from Stranda had the lowest C and highest N and ergosterol concentration compared to the other three locations and lower MeOH-insoluble condensed tannins concentration than Jølster II.

Spruce stands had lower average soil temperature compared to birch stands during the one-year study period (Supplementary Information, Table S2), although the difference in mean soil temperature was only 0.17 °C. The GDD above 5 °C in soil surface, and above 0 °C and 5 °C in the soil were significantly higher in birch stands. All climatic factors varied significantly between locations (Supplementary Information, Table S2).

Table 2. Initial concentrations or ratios (mean ± SE) of carbon (%), nitrogen (%), C:N ratio, ergosterol (mg g^{-1}), MeOH-soluble and MeOH-insoluble condensed tannins (mg g^{-1}) in litter of birch and spruce. n=48. For each variable, values marked with contrasting letters indicate significant differences among locations (Tukey, $P < 0.05$). *F* and *P* values derived from one-way ANOVAs to test for the effect of stand type on litter properties. n = 12. DF = 1, 40; 3, 40; 3,40 for litter type (LT), location (L), and LT × L, respectively. Statistically significant results ($P < 0.05$) are printed in bold.

		Stranda	Ørsta	Jølster I	Jølster II	All locations		*F* (*P*)
Carbon	Birch	47.01 ± 0.06^{b}	47.08 ± 0.14^{b}	47.60 ± 0.23^{b}	48.29 ± 0.09^{a}	47.49 ± 0.13	Litter type (LT)	3.43 (0.071)
	Spruce	46.43 ± 0.04^{d}	47.31 ± 0.06^{c}	48.62 ± 0.03^{a}	48.27 ± 0.12^{b}	47.66 ± 0.18	Location (L)	**70.72 (<0.001)**
							LT × L	**13.97 (<0.001)**
Nitrogen	Birch	0.88 ± 0.03^{b}	0.71 ± 0.01^{c}	0.92 ± 0.03^{b}	1.31 ± 0.06^{a}	0.96 ± 0.05	Litter type (LT)	**227.22 (<0.001)**
	Spruce	0.75 ± 0.01^{a}	0.51 ± 0.01^{d}	0.66 ± 0.01^{c}	0.70 ± 0.01^{b}	0.65 ± 0.02	Location (L)	**66.58 (<0.001)**
							LT × L	**27.91 (<0.001)**
C:N ratio	Birch	53.81 ± 1.72^{b}	66.47 ± 1.09^{a}	51.95 ± 1.50^{b}	37.34 ± 1.70^{c}	52.39 ± 2.24	Litter type (LT)	**463.20 (<0.001)**
	Spruce	61.98 ± 0.98^{d}	93.17 ± 1.56^{a}	74.17 ± 0.75^{b}	68.70 ± 0.90^{c}	74.50 ± 2.43	Location (L)	**128.77 (<0.001)**
							LT × L	**23.76 (<0.001)**
Ergosterol	Birch	0.05 ± 0.00^{b}	0.04 ± 0.00^{b}	0.09 ± 0.00^{a}	0.05 ± 0.00^{b}	0.06 ± 0.00	Litter type (LT)	**129.01 (<0.001)**
	Spruce	0.13 ± 0.00^{a}	0.07 ± 0.00^{b}	0.09 ± 0.00^{b}	0.09 ± 0.01^{b}	0.09 ± 0.01	Location (L)	**32.78 (<0.001)**
							LT × L	**32.98 (<0.001)**
MeOH-soluble	Birch	20.68 ± 0.86^{b}	13.88 ± 3.54^{b}	37.56 ± 4.63^{a}	24.19 ± 1.34^{b}	24.22 ± 2.41	Litter type (LT)	**100.80 (<0.001)**
CT	Spruce	2.30 ± 0.08^{a}	10.94 ± 0.86^{a}	7.98 ± 0.83^{a}	6.50 ± 0.30^{a}	6.93 ± 0.71	Location (L)	**8.89 (<0.001)**
							LT × L	**10.28 (<0.001)**
MeOH-	Birch	35.26 ± 1.85^{a}	24.67 ± 1.41^{bc}	28.82 ± 0.69^{b}	20.29 ± 0.51^{c}	27.26 ± 1.29	Litter type (LT)	**313.74 (<0.001)**
insoluble CT	Spruce	10.31 ± 0.24^{b}	14.87 ± 0.92^{ab}	13.67 ± 0.31^{ab}	17.32 ± 0.52^{a}	14.04 ± 0.59	Location (L)	**5.44 (0.003)**
							LT × L	**38.62 (<0.001)**

Table 3. Linear mixed effects model, with subplot nested within plot nested within location as random factor, testing for the effect of litter type (birch, spruce), stand type (birch, spruce), location (Stranda, Ørsta, Jølster I, Jølster II), and either mean moisture (%), soil temperature (°C), soil growing degree days (GDD) above 5°C, or none, on mass loss (%), carbon (%), nitrogen release, C:N ratio, ergosterol concentration (mg g^{-1}) and loss of MeOH-soluble and MeOH-insoluble condensed tannins (%) after one year of decomposition. Significant *F* and *P* values are printed in bold, only significant effects are shown.

Response variable	Selected model	Significant variables	DF	*F* (*P*)
Mass loss	Litter type * Stand type * Location	Litter type (LT)	1, 1	**238.29 (<0.001)**
		Stand type (S)	1, 1	**47.05 (<0.001)**
		Location (L)	3, 1	**14.51 (0.002)**
		LT × S	1, 2	**9.32 (0.002)**
		LT × L	3, 2	**10.17 (0.017)**
		S × L	3, 2	**8.68 (0.034)**
Carbon	Litter type * Stand type * Location * Soil temperature	Litter type (LT)	1, 1	**181.13 (<0.001)**
		Location (L)	3, 1	**59.98 (<0.001)**
		LT × L	3, 2	**27.24 (<0.001)**
		Stand type × L × Soil temperature	3, 2	**8.94 (0.030)**
Nitrogen release	Litter type * Stand type * Location * Moisture	Litter type (LT)	1, 1	**40.67 (<0.001)**
		Stand type (S)	1, 1	**11.30 (<0.001)**
		Location (L)	3, 1	**15.35 (0.002)**
		Moisture (M)	1, 1	**5.85 (0.016)**
		LT × S	1, 2	**14.75 (<0.001)**
		LT × L	1, 2	**74.24 (<0.001)**
		S × L	3, 2	**8.90 (0.031)**
		S × L × M	3, 3	**10.63 (0.014)**
		LT × S × L × M	3, 4	**11.36 (0.010)**
C:N ratio	Litter type * Stand type * Location * Soil temperature	Litter type (LT)	1, 1	**3499.86 (<0.001)**
		Stand type (S)	1, 1	**4.78 (0.029)**
		Location (L)	3, 1	**620.45 (<0.001)**
		Soil Temperature (ST)	1, 1	**5.36 (0.021)**
		LT × S	1, 2	**8.83 (0.003)**
		LT × L	3, 2	**70.72 (<0.001)**
		L × S	1, 2	**8.13 (0.043)**
Ergosterol	Litter type * Stand type * Location * Soil GDD above 5°C	Litter type (LT)	1, 1	**2250.60 (<0.001)**
		Stand type (S)	1, 1	**5.96 (0.015)**
		Location (L)	3, 1	**67.07 (<0.001)**
		LT × L	3, 2	**24.45 (<0.001)**
		S × L	3, 2	**11.41 (0.010)**
		LT × S × L	3, 3	**23.74 (<0.001)**

Table 3. continued

Response variable	Selected model	Significant variables	DF	*F* (*P*)
MeOH-soluble CT loss	Litter type * Stand type * Location * Soil GDD above 5°C	Litter type (LT)	1, 1	**1416.89 (<0.001)**
		Stand type (S)	1, 1	**24.46 (<0.001)**
		Location (L)	3, 1	**331.22 (<0.001)**
		LT × S	1, 1	**20.77 (<0.001)**
		LT × L	3, 2	**347.49 (<0.001)**
		S × L	3, 2	**21.11 (<0.001)**
		S × GDD > 5 soil	1, 1	**9.33 (0.002)**
		L × GDD > 5 soil	3, 2	**9.98 (0.019)**
		LT × S × L	3, 3	**18.91 (<0.001)**
		LT × S × GDD > 5 soil	1, 2	**8.21 (0.004)**
		LT × L × GDD > 5 soil	3, 3	**8.74 (0.033)**
MeOH-insoluble CT loss	Litter type * Stand type * Location * Moisture	Litter type (LT)	1, 1	**249.80 (<0.001)**
		Stand type (S)	1, 1	**9.09 (0.003)**
		Location (L)	3, 1	**102.34 (<0.001)**
		Moisture (M)	1, 1	**4.88 (0.027)**
		LT × L	3, 2	**241.12 (0.032)**

Birch litter decomposed faster than spruce litter, and both litter types decomposed more rapidly in the birch stands (Fig. 2a). Overall, mass loss after one year of decomposition was 9.4 % higher for birch litter compared with spruce litter, and 4.3 % higher in birch stands compared with spruce stands (Fig. 2a, Tukey, $P < 0.05$). The overall mass loss (%) for both litter types combined differed significantly among the locations (Table 3, Supplementary Information, Fig. S2), where highest mass loss was found in Ørsta (35.7 ± 1.06; mean ± 1 SE), followed by Stranda (34.1 ± 0,63), Jølster I (33.2 ± 0.84), and Jølster II (32.68 ± 0.98). None of the measured climatic variables significantly affected mass loss rates nor improved the model (Supplementary Information, Table S1). The pattern of C concentration during litter decomposition resembled that of mass loss. We found an overall significant negative net home-field advantage of on average –6.63 ± 1.56 % (t = –4.26, $P < 0.001$). This show that both litter types decomposed faster in the birch stands, and that the decomposition of spruce litter was greater in the birch stand environment compared to the decomposition of native birch litter. All locations, except Ørsta, had a significant negative mean HFA effect (Fig. 3).

The highest N release (%) was found in spruce litter in the birch stands (13.1 ± 1.15; mean ± 1 SE), while minor changes were observed for spruce litter in spruce stands and birch litter in general (Fig. 2b). Even though both birch and spruce litter immobilised N during the

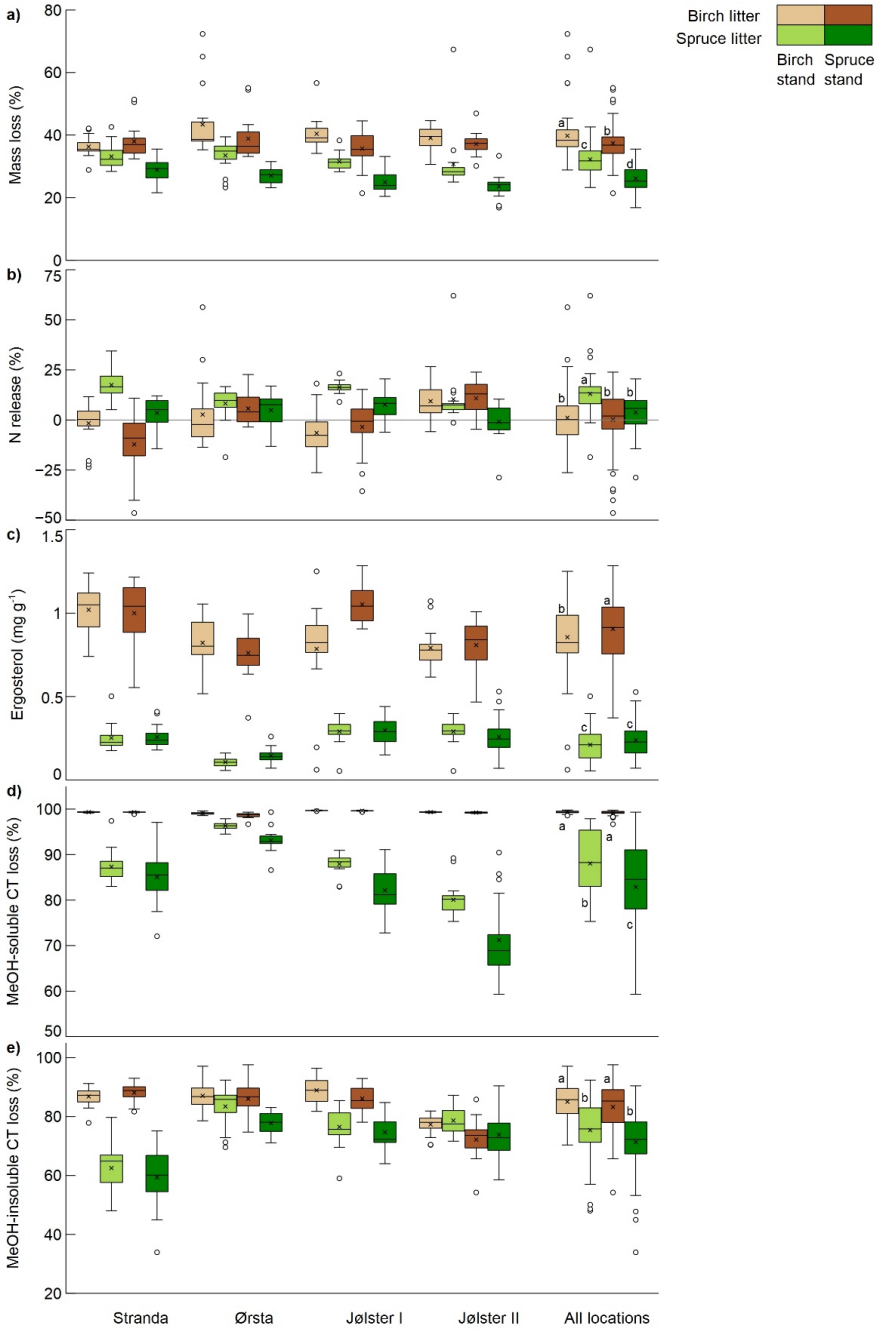

Figure 2. Boxplots of (**a**) mass loss (%), (**b**) nitrogen release (%), (**c**) ergosterol concentration (mg g^{-1}), (**d**) MeOH-soluble condensed tannins (CT) loss (%), and (**e**) MeOH-insoluble CT loss (%) in litterbags of birch and spruce litter after one year of decomposition in birch and spruce stands at all locations (Stranda, Ørsta, Jølster I, Jølster II), as well as for all locations combined. Boxplots marked with contrasting letters indicate significant differences (Tukey, $P < 0.05$) for all locations combined.

decomposition period at some locations, there was an overall net N release (Fig. 2b). Overall, there were no significant differences in N release among locations although the birch litter in spruce stands at Stranda released less N compared to similar stands at Ørsta and Jølster II (Supplementary Information, Fig. S3, Tukey, $P < 0.05$). The release of N was positively correlated to mean moisture (Table 3, Supplementary Information, Table S1). The N release after one year of decomposition resulted in lower C:N ratios compared to initial values. Spruce litter still had significantly higher C:N ratios after one year of decomposition compared to birch litter, regardless of stand type (Tukey, $P < 0.05$). Overall, the mean C:N ratio in litter at Ørsta was higher compared to Stranda and Jølster II (Tukey, $P < 0.05$), reflected in the differences in litter type × stand type among the locations (Supplementary Information, Fig. S4). The C:N ratio was positively correlated to mean soil temperature (Table 3, Supplementary Information, Table S1).

Birch litter contained roughly four times higher ergosterol concentration than spruce litter after one year of decomposition, and the concentration was highest for the birch litter incubated in the spruce stands (Fig. 2c). While the ergosterol concentration was up to fifteen times higher in birch litter after one year of decomposition, it was only two times higher for spruce litter (Table 2, Fig. 2c). Stranda had the highest initial concentration of ergosterol followed by Jølster I, Jølster II, and Ørsta (Tukey, $P < 0.05$), there were also variances in litter type × stand type among locations (Supplementary Information, Fig. S5). Ergosterol was positively correlated to GDD above 5 °C in the soil (Table 3, Supplementary Information, Table S1).

Spruce litter had lower loss of MeOH-soluble condensed tannins compared to birch litter after the soil incubation, and this loss was significantly lower in the spruce stands (Table 3, Fig. 2d). However, both litter types had a mean MeOH-soluble condensed tannin loss > 83 % regardless of stand type, indicating a rapid loss of MeOH-soluble condensed tannins during the first year of decomposition. The MeOH-soluble condensed tannin loss in Ørsta was significantly higher than that in Jølster II (Tukey, $P < 0.05$), mainly driven by the low loss of spruce litter in spruce stands at Jølster II (Fig. 2d, Supplementary Information, Fig. S6). MeOH-soluble condensed tannin loss was positively correlated to GDD above 5 °C in the soil (Table 3, Supplementary Information, Table S1). The loss of MeOH-insoluble condensed tannins was highest in birch litter after one year of decomposition, with no difference in average loss between forest types (Fig. 2e). Overall, the loss of MeOH-soluble compared to MeOH-insoluble condensed tannins were 10-16 % lower (depending on litter and stand type) for the insoluble fraction. Although

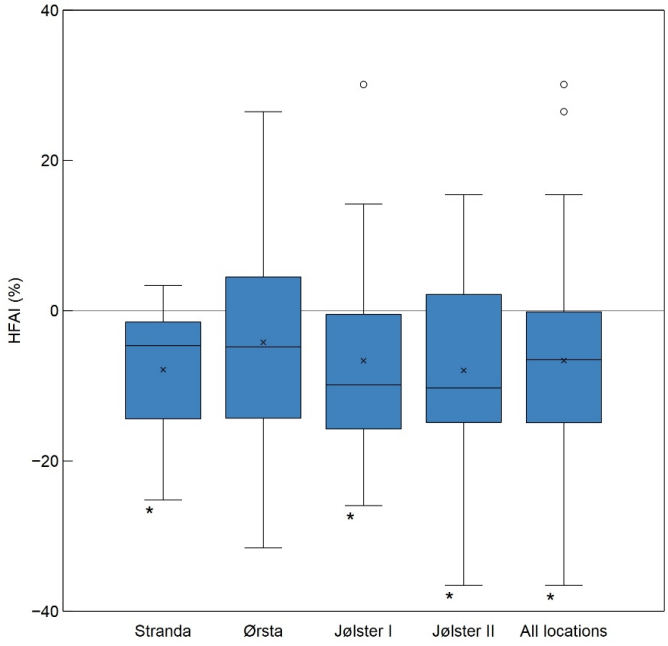

Figure 3. Home-field advantage index (HFAI) calculated for all locations (Stranda, Ørsta, Jølster I, Jølster II, and all locations combined)**,** significant values are indicated (*).

differences were found among locations in litter type × stand type (Fig. 2e, Supplementary Information, Fig. S7), no significant differences among locations in MeOH-insoluble condensed tannin loss were found. Loss of MeOH-insoluble condensed tannins were positively correlated to mean moisture (Table 3, Supplementary Information, Table S1).

Discussion

We found spruce litter to decompose at a slower pace compared to birch litter, as predicted in our first hypothesis. The decomposition rates were also slower in spruce stands compared to birch stands, partly supporting our second hypothesis. The mass loss of spruce litter increased on average by 6 % when placed in birch stands compared to spruce stands. Birch litter, on the other hand, decomposed slightly slower in the spruce stands compared to birch stands. Interestingly, we found a net negative home-field advantage as spruce litter decomposed faster in the birch stands, which contrasts with our third hypothesis that litter will decompose faster in the stands of litter origin. We did not find support for our hypothesis that microclimatic conditions, such as temperature and moisture, would affect mass loss rates. These findings and

their implications will be explored below. The more rapid mass loss of birch litter relative to spruce litter supports the findings in previous studies where broadleaf litter have been found to decompose faster than conifer needle litter in the early decay phase (Cornwell et al. 2008, Prescott et al. 2000). Litter type was more important than stand type in determining litter mass loss, which is supported by previous studies that underline the importance of litter quality (Makkonen et al. 2012, Fanin et al. 2016). The different rates of decay reflect the differences in initial litter quality in our study. Before decay, birch litter had significantly higher N and MeOH-soluble condensed tannin concentrations compared to the spruce litter. This may reflect the placement of birch and spruce along the leaf economic spectrum, where the plant C and nutrient investments return species-specific traits (e.g., N, lignin, pH, phenolics) that define them along the spectrum as nutrient conservative or acquisitive species, which has important afterlife effects on the litter decomposition rate (Freschet et al. 2012, Wright et al. 2004). However, the early stage of decomposition is characterized by leaching of soluble compounds, where leaf toughness constitutes an important aspect of litter quality (Gallardo & Merino 1993). Further, a higher concentration of water-soluble compounds in birch leaves compared to spruce needles may have contributed strongly to the mass loss in the early decay stage since water-soluble compounds are released faster from leaf litter compared to needle litter (Johansson 1995, Nykvist 1963).

After the one-year decomposition, almost all MeOH-soluble condensed tannins were lost from the birch litter. Meanwhile the loss was higher for spruce litter when placed in birch stands compared to spruce stands, thus following the same pattern as litter mass loss. This suggests that the loss of MeOH-soluble condensed tannins explain a large part of the difference in mass loss in the early decomposition of spruce vs birch. In comparison, a larger part of MeOH-insoluble condensed tannins were left after one year of decomposition in both litter types, although significantly less in birch litter compared to spruce litter. This reflects the general assumption of condensed tannins resistance to degradation (Haase & Wantzen 2008) and importance at later stages of the decomposition process (Chomel et al 2016, Prescott & Vesterdal 2021). Condensed tannins are a diverse group of compounds, with high degree of variation in polymerization and other chemical characteristics. They are difficult to characterize down to single compounds and are therefore commonly analysed in bulk assays like in this study. An extract from one tissue type from one species may contain a mixture of different condensed tannins of different structures and complexities (Shay et al. 2017) and the two fractions separated here tells us that some are easily released from litter, while others are

more recalcitrant. Further, the mixture of condensed tannins may be affected by the species genotype and environmental conditions (Lindroth et al. 2002, Preston & Trofymov 2015, Liu et al. 2005), indicating that the compound composition in the insoluble fraction from spruce may be different from that of birch.

The fungal biomass (concentration of ergosterol) was significantly higher in birch than spruce litter after one year of decomposition. The higher fungal biomass concentration in birch compared to spruce litter after the one-year decay, corresponds with the lower net mass remaining of birch litter. This may be attributed to fungal decomposers being more abundant in N-rich birch litter, compared to N-poor spruce litter at early decay stages.

The differences in overall mass loss after one year of decomposition among the four locations may be related to site-specific litter being used which is likely prone to genetic variation, together with variance of chemical responses to environmental stresses such as leaf senescence (Gallet & Lebreton 1995, Lindroth et al. 2002). The initial N concentration varied significantly among locations for both litter types. Spruce litter at Stranda had the highest initial N concentration, which reflects the higher N stock in the LFH soil layer at Stranda compared to the other locations (Kjønaas et al. 2021). The lack of similar response for birch litter at Stranda compared to the other locations may be due to differences in timing of senescence which is negatively correlated to N concentration. Another important factor determining senescence of tree leaves may be sunlight. The senescence was likely earliest at Stranda, which is positioned towards the south, at a higher elevation, and located further north than the other locations. Based on this, Jølster I is likely to be covered by shadow before Jølster II since it is positioned at lower elevation in a north facing hillslope, which is potentially reflected in the lower N concentration in the litter compared to Jølster II. However, other site-specific factors may cause the initial litter differences. The early stage of litter decomposition is often N limited (Berg 2000, Parton et al. 2007). Still, we found a general trend of N release after one year of decomposition, with a significantly higher release of N of spruce litter placed in birch stands compared to the other litter and stand types.

Our results oppose the dominant trend of HFA in similar studies, but HFA effects are far from universal (Fanin et al. 2021). The effect is usually quite small (4-8 %) (Ayres et a. 2009, Wang et al. 2013), although higher effects have also been found (24 %) (Asplund et al. 2018). Moreover, Wang et al. (2013) found a lower litter mass loss HFA for conifer species compared

to broadleaves, and tendencies of spruce decomposing faster in broadleaf forest have also been found in previous studies (Prescott et al. 2000). Indeed, in our study the birch litter mass loss was highest in the birch stand, suggesting a positive HFA, but decomposition of spruce litter was even more favored by the birch stand conditions. Generally, higher temperature, access of oxygen, higher nutrients levels, higher pH, and richer understory vegetation leads to more favourable conditions for decomposer communities (Prescott & Greyson 2013). Altogether, this suggests that birch forests harbour a more favourable environment for decomposer organisms compared to spruce forest. The shift from native birch to planted spruce in the current study system has previously been found to cause changes in fungal community composition and a reduction of fungal diversity in the forest floor layer (Mundra et al. in review), which also concurs with similar studies (Danielsen et al. 2021). Our pattern of slower decomposition rates in spruce stands is further consistent with the findings of Kjønaas et al. (2021) from the same study system, where higher C stocks were found in the forest floor in spruce stands compared to the birch stands. Thus, such tree species change induced shifts in fungal decomposer communities are likely to impact soil C accumulation, and part of the explanation may be differences in litter quality, as shown here.

In our study, litter type and stand type overshadowed small scale variation in climatic variables. Tree species richness and composition have been found to impact the microclimatic conditions in forests, with the consequence of microclimatic conditions having a greater control on early-stage decomposition rates than climate at global scale (Joly et al. 2017). The proportion of sunlight that penetrates the tree canopy and reaches the forest floor are likely to influence the soil temperature. Although the soil temperature was slightly higher in the birch stands, this did not affect the mass loss rates in the early stage of decomposition. The soil temperature was more responsive than surface temperature, which may be the results of opposing seasonal temperature trends in birch and spruce stands that may be neutralized by the annual estimates. The removal of understory vegetation alters the original microclimate and may have influenced the results as understory vegetation generally tend to be thicker in conifer forests compared to broadleaved forests. The thick bryophyte cover may insulate both the climate sensors as well as incoming tree litter, creating more favourable decomposition conditions during cold winter periods, and less favourable during warm summer days. The moss layer has been found by Jackson et al. (2013) to have a strong positive effect on decomposition rates, with faster litter decay at greater depths in the moss layer. However, moss layer removal does not always influence decomposition rates (Fanin et al. 2019).

In summary, our study showed that litter type had a stronger influence on mass loss in the early stage of decomposition, compared to stand type and microclimatic conditions. The faster decomposition of birch litter compared to spruce litter in the early decay phase reflected the initial litter quality, the loss of MeOH-soluble condensed tannins and accumulation of fungal biomass. In contrast, the MeOH-insoluble condensed tannins fraction may have regulatory effects in the later decomposition process. Our results give insights into the nutrient release at the early stage of decomposition when a substantial fraction of the C is lost from the plant litter. The net negative HFA was caused by a higher benefit of spruce litter when the litter decomposed in birch stands compared to when birch litter decomposed in birch stands. Mass loss from the early stage of litter decomposition cannot be used to predict long term storage of soil C (Prescott et al. 2004, Prescott & Vesterdal 2021). However, in the current study, the slower decomposition rates in the spruce stands compared to birch stands along with higher C stocks in the forest floor of the spruce stands reflect short term processes which mirror the long-term effects of the tree species change. Transplanting experiments conducted for a longer period will be useful to uncover additional long-term litter decomposition dynamics.

Acknowledgement

This study was funded by NIBIO and the Research Council of Norway [grant number 255307]. Thanks are due to Annie Aasen and Claus D. Kreibich for assistance with chemical analyses. The authors declare no conflict of interest.

References

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* 79: 439–449. https://doi.org/3546886

Albright, M.B.N., Johansen, R., Thompson, J., Lopez, D., Gallegos-Graves, L.V., Kroeger, M.E., Runde, A., Mueller, R.C., Washburne, A., Munsky, B., Yoshida, T. & Dunbar, J. 2020. Soil bacterial and fungal richness forecast patterns of early pine litter decomposition. *Frontiers in Microbiology* 11: 542220. https://doi.org/10.3389/fmicb.2020.542220

Asplund, J., Kauserud, H., Bokhorst, S., Lie, M.H., Ohlson, M. & Nybakken, L. 2018. Fungal communities influence decomposition rates of plant litter from two dominant tree species. *Fungal Ecology* 32: 1–8. https://doi.org/10.1016/j.funeco.2017.11.003

Ayres, E., Steltzer, H., Simmons, B. L., Simpson, R. T., Steinweg, J. M., Wallenstein, M. D., Mellor, N., Parton, W. J., Moore, J. C. & Wall, D. H. 2009. Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biology & Biochemistry* 41: 606–610. https://doi.org/10.1016/j.soilbio.2008.12.022

Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. *Forest Ecology and Management 133*: 13–22. https://doi.org/10.1016/S0378-1127(99)00294-7

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., Jackson, B. G. & Baldy, V. 2016. Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. *Journal of Ecology*. https://doi.org/10.1111/1365-2745.12644

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Bodegom, P.V., Brovkin, V., Chatain, A., Callaghan, T.W., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V. & Westoby, M. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11: 1065–1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

Coûteaux, M.-M., Bottner, P. & Berg, B. 1995. Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution* 10: 63–66. https://doi.org/10.1016/S0169-5347(00)88978-8

Danielsen, J.S., Morgado, L., Mundra, S., Nybakken, L., Davey, M. & Kauserud, H. 2021. Establishment of spruce plantations in native birch forests reduces soil fungal diversity. *FEMS Microbiology Ecology* 97: fiab074. https://doi.org/10.1093/femsec/fiab074

Djukic, I., Kepfer-Rojas, S., Schmidt, I. K., Larsen, K. S., Beier, C., Berg, B. & Verheyen, K. 2018. Early stage litter decomposition across biomes. *Science of The Total Environment* 628–629: 1369–1394. https://doi.org/10.1016/j.scitotenv.2018.01.012

Fanin, N., Fromin, N. & Bertrand, I. 2016. Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. *Ecology* 97: 1023–1037. https://doi.org/10.1890/15-1263.1

Fanin, N., Kardol, P., Farrell, M., Kempel, A., Ciobanu, M., Nilsson, M.-C., Gundale, M.J. & Wardle, D.A. 2019. Effects of plant functional group removal on structure and function of soil communities across contrasting ecosystems. *Ecology Letters* 22: 1095–1103. https://doi.org/10.1111/ele.13266

Fanin, N., Lin, D., Freschet, G.T., Keiser, A., Augusto, L., Wardle, D.A. & Veen, G.F. (Ciska). 2021. Home-field advantage of litter decomposition: from the phyllosphere to the soil. *New Phytologist 231*: 1353–1358. https://doi.org/10.1111/nph.17475

Frenne, P.D., Lenoir, J., Luoto, M., Scheffers, B.R., Zellweger, F., Aalto, J., Ashcroft, M.B., Christiansen, D.M., Decocq, G., Pauw, K.D., Govaert, S., Greiser, C., Gril, E., Hampe, A., Jucker, T., Klinges, D.H., Koelemeijer, I.A., Lembrechts, J.J., Marrec, R., Meeussen, C., Ogée, J., Tyystjärvi, V., Vangansbeke, P. & Hylander, K. 2021. Forest microclimates and climate change: Importance, drivers and future research agenda. *Global change biology* 27: 2279–2297. https://doi.org/10.1111/gcb.15569

Freschet, G.T., Aerts, R., & Cornelissen, J.H.C. 2012. A plant economics spectrum of litter decomposability. *Functional Ecology* 26: 56–65. https://doi.org/10.1111/j.1365-2435.2011.01913.x

Gallardo, A. & Merino, J. 1993. Leaf decomposition in two Mediterranean ecosystems of southwest Spain: influence of substrate quality. *Ecology* 74: 152–161. https://doi.org/10.2307/1939510

Gallet, C. & Lebreton, P. 1995. Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. *Soil Biology and Biochemistry* 27: 157–165. https://doi.org/10.1016/0038-0717(94)00167-Y

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E. & Parton, W.J. 2000. Longterm dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology* 6, 751–765. https://doi.org/10.1046/j.1365-2486.2000.00349.x

Granhus, A., Hylen, G., & Ørnelund Nilsen, J.-E. 2012. Statistics of forest conditions and resources in Norway. Report 03/2012, Skog og Landskap, Ås, Norway.

Greiser, C., Meineri, E., Luoto, M., Ehrlén, J. & Hylander, K. 2018. Monthly microclimate models in a managed boreal forest landscape. *Agricultural and Forest Meteorology* 250-251: 147–158. https://doi.org/10.1016/j.agrformet.2017.12.252

Haase, K. & Wantzen, K. M. 2008. Analysis and decomposition of condensed tannins in tree leaves. *Environmental Chemistry Letters* 6: 71-75. https://doi.org/10.1007/s10311-008-0140-7

Hagerman, A.E. 2002. The Tannin Handbook. Oxford: Miami University.

Hansen, K., Vesterdal, L., Schmidt, I.K., Gundersen, P., Sevel, L., Bastrup-Birk, A., Pedersen, L.B. & Bille-Hansen, J. 2009. Litterfall and nutrient return in five tree species in a common garden experiment. *Forest Ecology and Management* 257: 2133–2144. https://doi.org/10.1016/j.foreco.2009.02.021

Hättenschwiler S. & Vitousek, P. M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution* 15: 238–243. https://doi.org/10.1016/S0169-5347(00)01861-9

van der Heijden, M. G. A., Bardgett, R. D. & van Straalen, N. M. 2007. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11: 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Hobbie, S.E., Reich, P.B., Oleksyn, J., Ogdahl, M., Zytkowiak, R., Hale, C. & Karolewski, P. 2006. Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology* 87: 2288–2297. https://doi.org/10.1890/0012-9658(2006)87[2288:TSEODA]2.0.CO;2

Hylen, G. 2019. Norges skoger gjennom 100 år, in: S. M. Tomter (Ed.), Landsskogtakseringen 1165 1919-2019, NIBIO, NIBIO. pp. 64–79. (*In Norwegian*)

Jackson, B.G., Nilsson, M.-C. Wardle, D.A. 2013. The effects of the moss layer on the decomposition of intercepted vascular plant litter across a post-fire boreal forest chronosequence. *Plant and Soil* 367: 199–214. https://doi.org/10.1007/s11104-012-1549-0

Jandl, R., Lindner, M., Bauwens, B., Vesterdal, L., Baritz, R., Hagedorn, F., Johnson, D.W., Minkkinen, K., Byrne, K.A. 2007. How strongly can forest management influence soil carbon sequestration? *Geoderma* 137: 253–268. https://doi.org/10.1016/j.geoderma.2006.09.003

Johansson, M.-B. 1995. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. *Forestry* 68: 49–62. https://doi.org/10.1093/forestry/68.1.49

Joly, F.-X., Milcu, A., Scherer-Lorenzen, M., Jean, L.-K., Bussotti, F., Dawud, S.M., Müller, S., Pollastrini, M., Raulund-Rasmussen, K., Vesterdal, L. & Hättenschwiler, S. 2017. Tree species diversity affects decomposition through modified micro-environmental conditions across European forests. *New Phytologist* 214: 1281–1293. https://doi.org/10.1111/nph.14452

Kjønaas, O. J., Bárcena, T. G., Hylen, G., Nordbakken, J.-F. & Økland, T. 2021. Boreal tree species change as a climate mitigation strategy: impact on ecosystem C and N stocks and soil nutrient levels. *Ecosphere* 12: e03826. https://doi.org/10.1002/ecs2.3826

Kraus, T. E. C., Dahlgren, R. A. & Zasoski, R. J. 2003. Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil* 256: 41–66. https://doi.org/10.1023/A:1026206511084

Lenth, R. V. 2021. emmeans: estimated marginal means, aka least-squares means. R package version 1.6.3. https://CRAN.R-project.org/package=emmeans

Lindahl, B. D., Kyaschenko, J., Varenius, K., Clemmensen, K. E., Dahlberg, A., Karltun, E. & Stendahl, J. 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. *Ecology Letters* 47: 1341–1351. https://doi.org/10.1111/ele.13746

Lindroth, R.L., Osier, T.L. & Barnhill, H.R.H., Wood, S.A. 2002. Effects of genotype and nutrient availability on phytochemistry of trembling aspen (*Populus tremuloides* Michx.) during leaf senescence. *Biochemical Systematics and Ecology* 30: 297–307. https://doi.org/10.1016/S0305-1978(01)00088-6

Liu, L.L., King, J.S. & Giardina, C.P. 2005. Effects of elevated concentrations of atmospheric CO2 and tropospheric O3 on leaf litter production and chemistry in trembling aspen and paper birch communities. *Tree Physiology* 25:1511–1522. https://doi.org/10.1093/treephys/25.12.1511

Lodato, M.B., Boyette, J.S., Smilo, R.A., Jackson, C.R., Halvorson, H.M. & Kuehn, K.A. 2021. Functional importance and diversity of fungi during standing grass litter decomposition. *Oecologia* 195: 499–512. https://doi.org/10.1007/s00442-020-04838-y

Makkonen, M., Berg, M.P., Handa, I.T., Hättenschwiler, S., van Ruijven, J., van Bodegom, P.M. & Aerts, R. 2012. Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecology Letters* 15: 1033–1041. https://doi.org/10.1111/j.1461-0248.2012.01826.x

Melillo, J.M., Aber, J.D. & Muratore, J.F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63: 621–626. https://doi.org/1936780

Mundra, S., Kauserud, H., Økland, T., Nordbakken, J.-F., Ransedokken, Y. & Kjønaas, O.J. Shift in tree species leads to dramatic changes in the belowground biota in boreal forests. *In review.*

Nybakken, L., Lie, M.H., Julkunen-Tiitto, R., Asplund, J. & Ohlson, M. 2018. Fertilization Changes Chemical Defense in Needles of Mature Norway Spruce (*Picea abies*). *Frontiers in Plant Science 9*: 770. https://doi.org/10.3389/fpls.2018.00770

Nykvist, N. 1963. Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. *Studia forestalia Suecica* 3: 33.

Parton, W., Silver, W. L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C. & Fasth, B. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* 315: 361–364. https://doi.org/10.1126/science.1134853

Prescott, C. E. 2002. The influence of the forest canopy on nutrient cycling. *Tree Physiology* 22: 1193–1200. https://doi.org/10.1093/treephys/22.15-16.1193

Prescott, C.E. & Grayston, S.J. 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. *Forest Ecology and Management* 309: 19–27. https://doi.org/10.1016/j.foreco.2013.02.034

Prescott, C.E. & Vesterdal, L. 2021. Decomposition and transformations along the continuum from litter to soil organic matter in forest soils. *Forest Ecology and Management* 498:119522. https://doi.org/10.1016/j.foreco.2021.119522

Prescott, C.E., Vesterdal, L., Preston, C.M. & Simard, S.W. 2004. Influence of initial chemistry on decomposition of foliar litter in contrasting forest types in British Columbia. *Canadian Journal of Forest Research* 34: 1714–1729. https://doi.org/10.1139/x04-040

Prescott, C.E., Zabek, L.M., Staley, C.L. & Kabzems, R. 2000. Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures. *Canadian Journal of Forest Research* 30: 1742–1750. https://doi.org/10.1139/x00-097

Preston, C.M. & Trofymow, JA. 2015. The chemistry of some foliar litters and their sequential proximate analysis fractions. *Biogeochemistry* 126:197–209. https://doi.org/10.1007/s10533-015-0152-x

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ransedokken, Y. R., Asplund, J., Ohlson, M. & Nybakken, L. 2019. Vertical distribution of soil carbon in boreal forest under European beech and Norway spruce. *European Journal of Forest Research* 138: 353–361. https://doi.org/10.1007/s10342-019-01176-4

Sanders, J. 2020. Veusz – a scientific plotting package. https://veusz.github.io/

Shay, P.E., Trofymow, J.A. & Constabel, C.P. 2017. An improved butanol-HCl assay for quantification of water-soluble, acetone:methanol-soluble, and insoluble proanthocyanidins (condensed tannins). *Plant Methods* 13: 63. https://doi.org/10.1186/s13007-017-0213-3

Talbot, J.M., Yelle, D.J., Nowick, J. & Treseder, K.K. 2012. Litter decay rates are determined by lignin chemistry. *Biogeochemistry* 108: 279–295. https://doi.org/10.1007/s10533-011-9599-6

Vesterdal, L., Clarke, N., Sigurdsson, B.D. & Gundersen, P. 2013. Do tree species influence soil carbon stocks in temperate and boreal forests? *Forest Ecology and Management* 309: 4–18. https://doi.org/10.1016/j.foreco.2013.01.017

Vesterdal, L., Schmidt, I.K., Callesen, I., Nilsson, L.O. & Gundersen, P. 2008. Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management* 255: 35–48. https://doi.org/10.1016/j.foreco.2007.08.015

Wang, Q., Zhong, M. & He, T. 2013. Home-field advantage of litter decomposition and nitrogen release in forest ecosystems. *Biology and Fertility of Soils* 49, 427–434. https://doi.org/10.1007/s00374-012-0741-y

Wardle, D.A., 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., & Villar, R. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827. https://doi.org/10.1038/nature02403

Supplementary Information

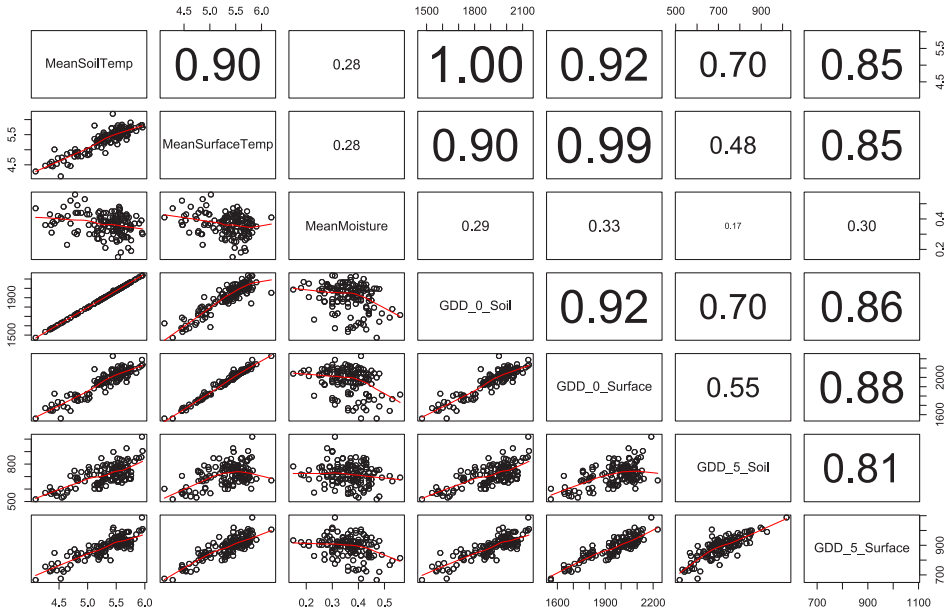

Figure S1. Correlation coefficients (where size of values reflect p-values) of all climatic variables, with correlation plots.

Table S1. Coefficient estimates (±SE), t-values, and model AIC of climatic factors added separately as a fixed effect in the linear mixed-effects models testing for the effect of litter type, stand type, and location as on mass loss after one year of decomposition with subplot nested within plot nested within location as random factor. Significant effects found for climate related variables are indicated (*). The lowest AIC for each response variable is marked with bold values.

Response variables	Climatic factors	Estimate ± SE	t-value	AIC
Mass loss	Mean soil temperature	0.104811 ± 0.101412	1.034	-794.1
	Mean surface temperature	-0.009403 ± 0.125647	-0.075	-782.2
	Mean moisture	-0.12137 ± 0.14407	-0.842	-801.4
	GDD > 0 soil	2.891e-04 ± 2.823e-04	1.024	-794.0
	GDD > 0 surface	1.289e-04 ± 3.565e-04	0.362	-783.3
	GDD > 5 soil	3.453e-04 ± 2.647e-04	1.305	-798.1
	GDD > 5 surface	2.507e-04 ± 4.014e-04	0.625	-787.3
	Without climatic factors			**-832.8**
C	Mean soil temperature	0.2982 ± 2.9332	0.102	**1053.7**
	Mean surface temperature	1.40494 ± 3.60906	0.389	1060.9
	Mean moisture	2.15566 ± 4.33709	0.497	1056.0
	GDD > 0 soil	8.373e-04 ± 8.167e-03	0.103	1054.0
	GDD > 0 surface	3.286e-03 ± 1.023e-02	0.321	1059.3
	GDD > 5 soil	8.532e-04 ± 7.909e-03	0.108	1067.2
	GDD > 5 surface	0.003308 ± 0.011551	0.286	1056.9
	Without climatic factors			1069.2
N release	Mean soil temperature	-11.92 ± 19.88	-0.599	2105.9
	Mean surface temperature	3.2156 ± 24.5528	0.131	2110.9
	Mean moisture	7.2878 ± 27.9567	0.261	**2085.1***
	GDD > 0 soil	-0.03441 ± 0.05530	-0.622	2105.8
	GDD > 0 surface	3.596e-03 ± 6.940e-02	0.052	2108.3
	GDD > 5 soil	-0.02568 ± 0.05297	-0.485	2108.7

Table S1. continued

Response variables	Climatic factors	Estimate ± SE	t-value	AIC
	GDD > 5 surface	-7.463e-03 ± 7.834e-02	-0.095	2108.4
	Without climatic factors			2154.1
C:N ratio	Mean soil temperature	2.1814 ± 6.9806	0.312	**1526.8***
	Mean surface temperature	-0.26894 ± 8.48143	-0.032	1528.5
	Mean moisture	-3.5337 ± 10.4376	-0.339	1534.9
	GDD > 0 soil	0.005636 ± 0.019433	0.290	1527.0*
	GDD > 0 surface	5.916e-03 ± 2.418e-02	0.245	1530.0
	GDD > 5 soil	0.010439 ± 0.018603	0.561	1534.6
	GDD > 5 surface	9.014e-03 ± 2.752e-02	0.328	1532.0
	Without climatic factors			1566.2
Ergosterol	Mean soil temperature	0.4196 ± 0.2417	1.736	-317.8
	Mean surface temperature	-0.09596 ± 0.29769	-0.322	-309.7
	Mean moisture	-0.40878 ± 0.35481	-1.152	-306.8*
	GDD > 0 soil	0.0011607 ± 0.0006726	1.726	-317.9
	GDD > 0 surface	-1.100e-04 ± 8.384e-04	-0.131	-314.8*
	GDD > 5 soil	0.0012082 ± 0.0006502	1.858	**-306.4**
	GDD > 5 surface	0.0005118 ± 0.0009482	0.540	-316.2*
	Without climatic factors			-335.9
MeOH-soluble CT	Mean soil temperature	-0.1488 ± 6.4429	-0.023	1478.9
	Mean surface temperature	-0.03699 ± 7.92886	-0.005	1487.0
	Mean moisture	0.17805 ± 9.23979	0.019	1476.9
	GDD > 0 soil	-4.070e-04 ± 1.789e-02	-0.023	1477.9
	GDD > 0 surface	-2.337e-04 ± 2.259e-02	-0.010	1488.3
	GDD > 5 soil	-5.150e-04 ± 1.614e-02	-0.032	**1452.6**

Table S1. continued

Response variables	Climatic factors	Estimate ± SE	t-value	AIC
	GDD > 5 surface	--3.890e-04 ± 2.547e-02	-0.015	1485.1
	Without climatic factors			1499.7
MeOH-insoluble CT	Mean soil temperature	-0.1604 ± 11.0245	-0.015	1778.9
	Mean surface temperature	12.6863 ± 13.6320	0.931	1783.1
	Mean moisture	4.8560 ± 17.0700	0.284	**1126.5***
	GDD > 0 soil	-1.168e-03 ± 3.065e-02	-0.038	1778.3
	GDD > 0 surface	3.202e-02 ± 3.881e-02	0.825	1786.4
	GDD > 5 soil	-8.259e-04 ± 2.897e-02	-0.029	1774.1
	GDD > 5 surface	0.02656 ± 0.04375	0.607	1787.7
	Without climatic factors			1835.9

Table S2. Climatic factors for birch and spruce stands at each location and results of linear mixed-effects models, with subplot nested within plot nested within location, to test for the effect of stand type and location. Significant *F* and *P* values are printed in bold, only significant effects are shown.

		Stranda	Ørsta	Jølster I	Jølster II	All locations		DF	*F* (*P*)
Mean soil temperature	Birch	4.90 ± 0.03^{d}	5.61 ± 0.04^{a}	5.60 ± 0.03^{b}	5.38 ± 0.05^{c}	5.40 ± 0.04	Stand type (S)	1, 11	**19.02 (<0.001)**
	Spruce	4.49 ± 0.04^{d}	5.47 ± 0.02^{b}	5.67 ± 0.02^{a}	5.23 ± 0.02^{c}	5.23 ± 0.05	Location (L)	3, 11	**397.07 (<0.001)**
							S × L	3, 11	**21.02 (<0.001)**
Mean surface temperature	Birch	4.89 ± 0.02^{a}	5.48 ± 0.04^{a}	5.53 ± 0.02^{a}	5.33 ± 0.05^{a}	5.33 ± 0.03	Stand type (S)	1, 11	0.33 (0.566)
	Spruce	4.60 ± 0.06^{a}	5.64 ± 0.04^{a}	5.64 ± 0.06^{a}	5.39 ± 0.03^{a}	5.34 ± 0.05	Location (L)	3, 11	**277.11 (<0.001)**
							S × L	3, 11	**19.49 (<0.001)**
Mean soil moisture	Birch	0.42 ± 0.02^{a}	0.39 ± 0.01^{b}	0.36 ± 0.02^{c}	0.34 ± 0.01^{d}	0.37 ± 0.01	Stand type (S)	1, 11	2.50 (0.114)
	Spruce	0.40 ± 0.02^{a}	0.35 ± 0.02^{b}	0.32 ± 0.01^{d}	0.35 ± 0.02^{c}	0.35 ± 0.01	Location (L)	3, 11	**12.49 (0.006)**
							S × L	3, 11	1.18 (0.757)
GDD >0 soil	Birch	1758 ± 9.17^{b}	2013 ± 14.62^{a}	2011 ± 9.87^{a}	1933 ± 17.28^{a}	1939 ± 13.67	Stand type (S)	1, 11	**19.35 (<0.001)**
	Spruce	1612 ± 14.99^{b}	1965 ± 8.35^{a}	2035 ±. 6.66^{a}	1878 ± 6.01^{a}	1876 ± 19.41	Location (L)	3, 11	**388.01 (<0.001)**
							S × L	3, 11	**20.40 (<0.001)**
GDD >0 surface	Birch	1786 ± 7.25^{b}	2010 ± 13.47^{a}	2043 ± 8.30^{a}	1978 ± 19.44^{ab}	1964 ± 13.22	Stand type (S)	1, 11	0.10 (0.748)
	Spruce	1695 ± 19.15^{b}	2061 ± 14.00^{a}	2082 ± 6.21^{a}	1966 ± 10.19^{a}	1955 ± 19.22	Location (L)	3, 11	**271.65 (<0.001)**
							S × L	3, 11	**12.99 (0.005)**
GDD >5 soil	Birch	708 ± 9.59^{b}	803 ± 9.30^{a}	768 ± 10.45^{ab}	807 ± 17.46^{a}	775 ± 7.74	Stand type (S)	1, 11	**150.89 (<0.001)**
	Spruce	580 ± 9.97^{b}	720 ± 7.52^{a}	694 ± 6.57^{a}	645 ± 6.86^{ab}	661 ± 7.36	Location (L)	3, 11	**75.73 (<0.001)**
							S × L	3, 11	**14.31 (0.003)**
GDD >5 surface	Birch	839 ± 7.50^{a}	949 ± 8.62^{a}	925 ± 7.29^{a}	923 ± 14.74^{a}	913 ± 7.03	Stand type (S)	1, 11	**22.30 (<0.001)**
	Spruce	758 ± 12.88^{b}	929 ± 7.78^{a}	911 ± 4.85^{ab}	850 ± 7.28^{ab}	864 ± 8.94	Location (L)	3, 11	**107.12 (<0.001)**
							S × L	3, 11	**8.39 (0.039)**

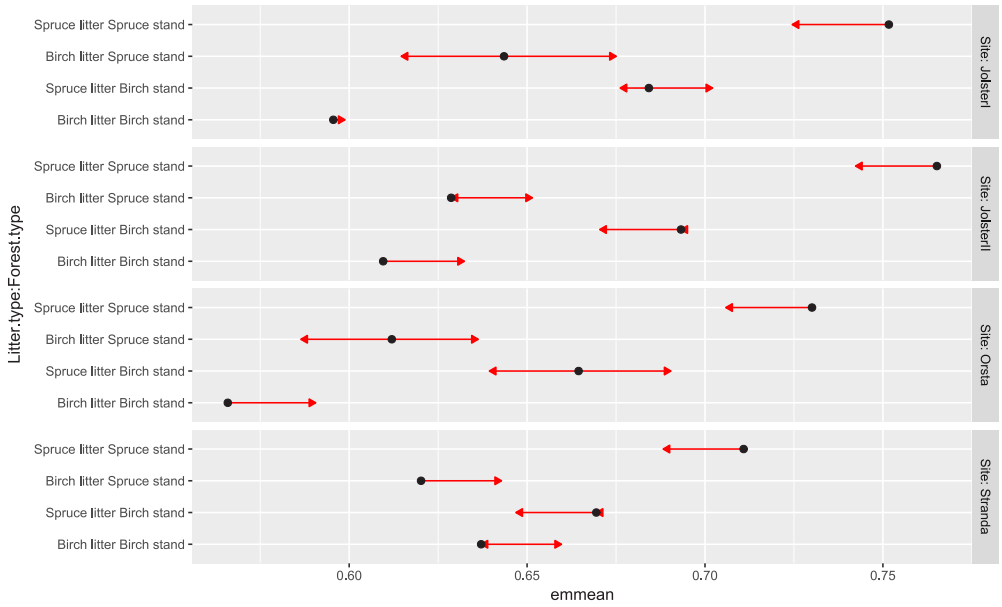

Figure S2. Graphical comparison of estimated marginal means for mass loss. Red arrows indicate the comparison among EMMs. Arrows that do not overlap are significantly different, based on Tukey ($P < 0.05$).

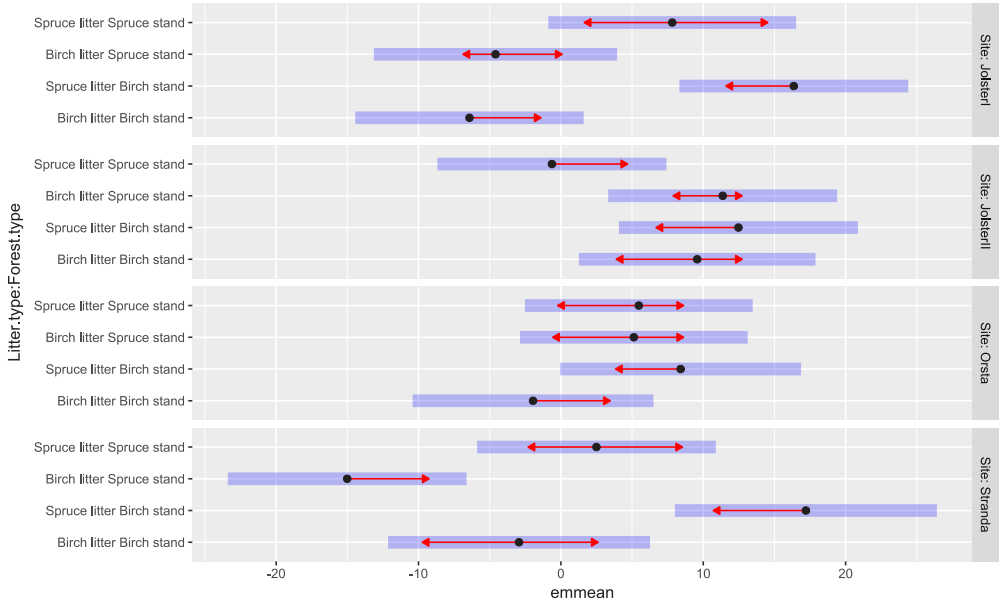

Figure S3. Graphical comparison of estimated marginal means (EMMs) for N release. Blue bars show confidence intervals, and red arrows indicate the comparison among EMMs. Arrows that do not overlap are significantly different, based on Tukey ($P < 0.05$).

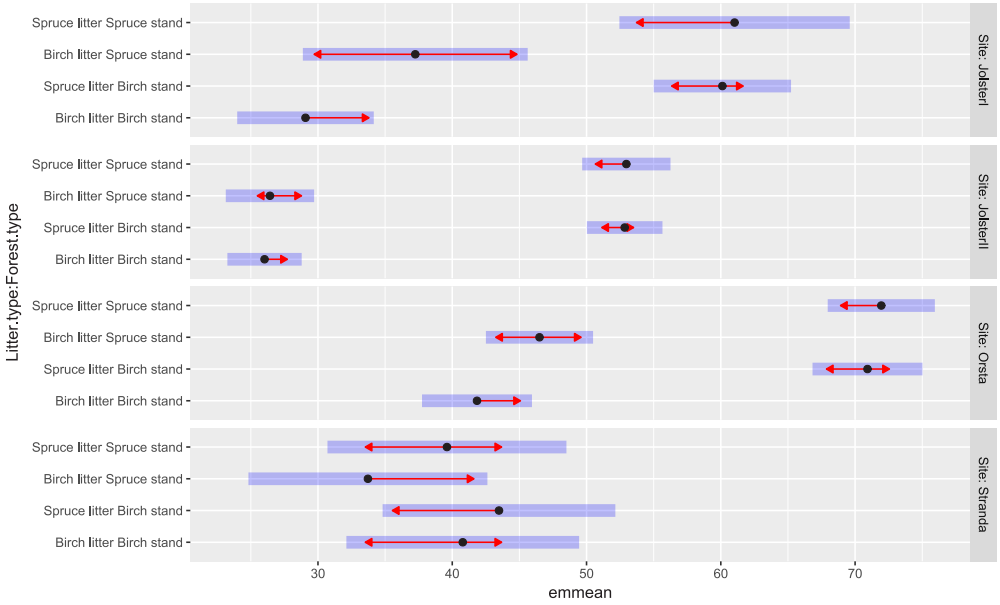

Figure S4. Graphical comparison of estimated marginal means (EMMs) for C:N ratio. Blue bars show confidence intervals, and red arrows indicate the comparison among EMMs. Arrows that do not overlap are significantly different, based on Tukey ($P < 0.05$).

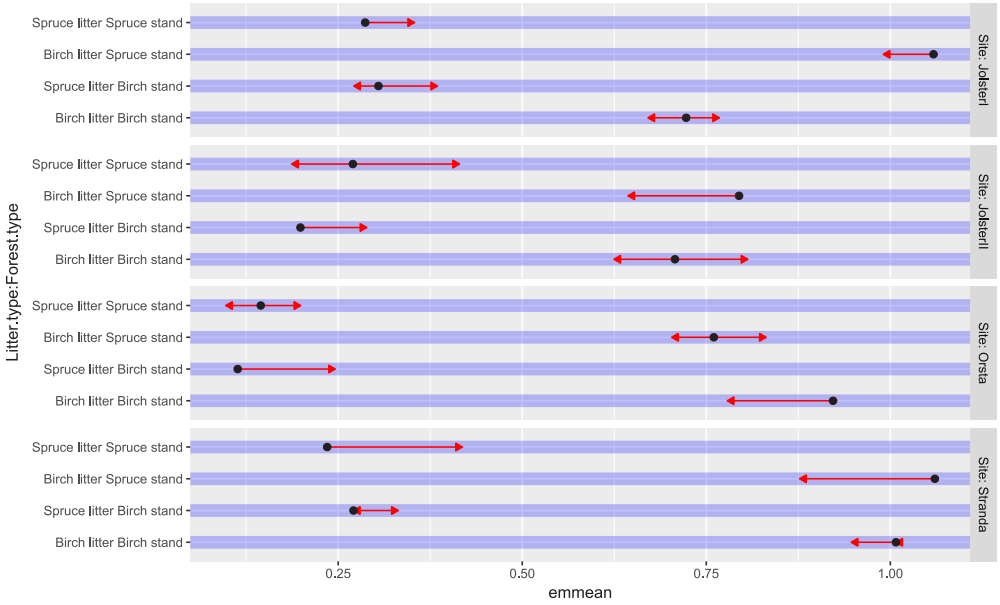

Figure S5. Graphical comparison of estimated marginal means for ergosterol. Blue bars show confidence intervals, and red arrows indicate the comparison among EMMs. Arrows that do not overlap are significantly different, based on Tukey ($P < 0.05$).

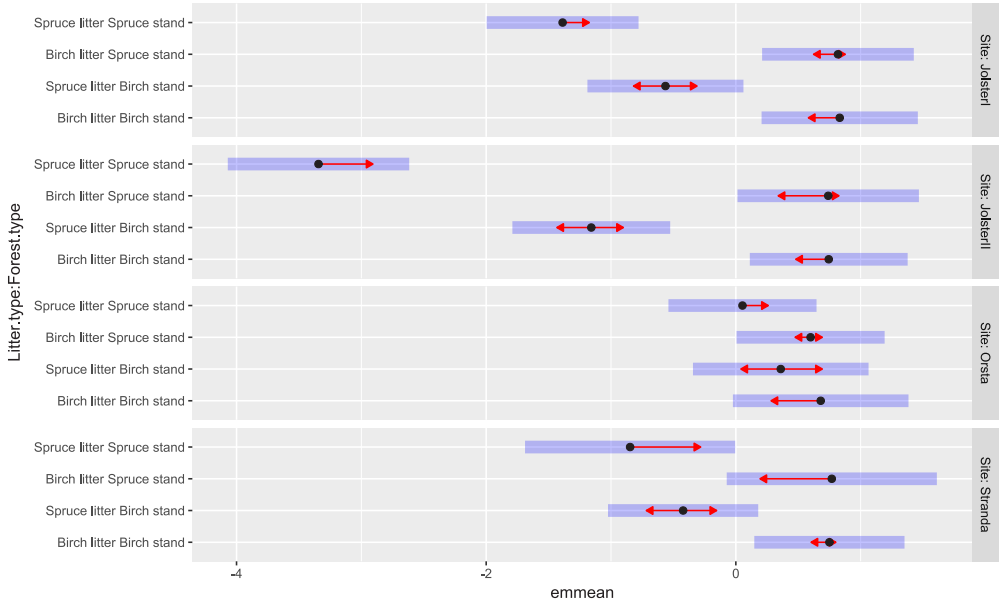

Figure S6. Graphical comparison of estimated marginal means (EMMs) for MeOH-soluble condensed tannin loss. Blue bars show confidence intervals, and red arrows indicate the comparison among EMMs. Arrows that do not overlap are significantly different, based on Tukey ($P < 0.05$).

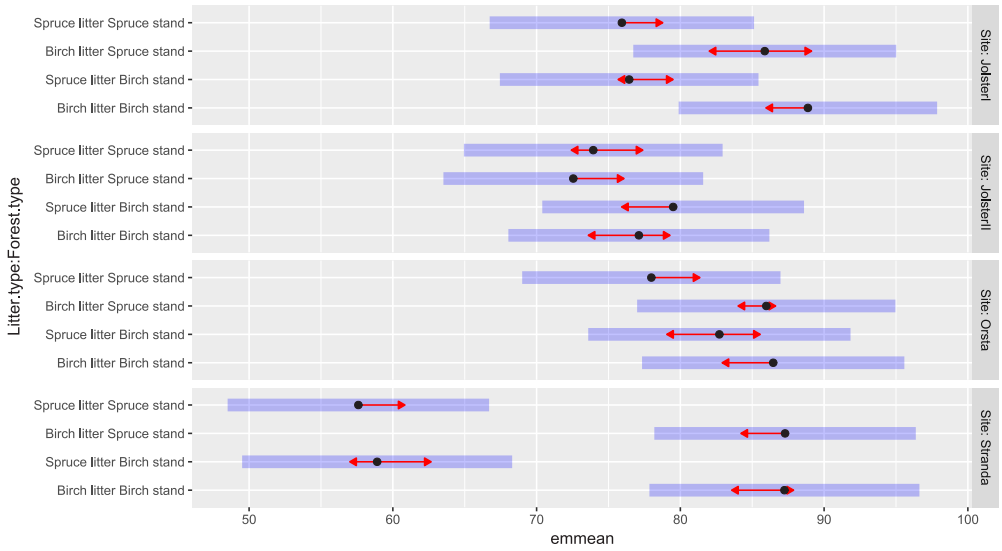

Figure S7. Graphical comparison of estimated marginal means (EMMs) for MeOH-insoluble condensed tannin loss. Blue bars show confidence intervals, and red arrows indicate the comparison among EMMs. Arrows that do not overlap are significantly different, based on Tukey ($P < 0.05$).

Details on gap filling methodology

Surface temperature and soil moisture and temperature at 6 cm soil depth were recorded at each subplot by use of TMS-4 soil probes sensors (TOMST, Praha, Czech Republic; ± 0.5°C accuracy) with a 15 min measurement frequency. The measurements took place throughout the whole study period, and gap filling was done when missing data occurred. Different approaches were used depending on the length of the period with missing data. Of the total of TOMST 144 loggers deployed at the 4 locations, 5 of the loggers had stopped recording data over the entire experiment period, thus these data were excluded (4 loggers in Stranda birch, 1 in Stranda spruce). Missing temperature and soil moisture data over a short-term period (3-5 days) were replaced by data of another TOMST logger in the given stand and location based on the best fit average value for a period of 4 days prior to and after the missing 24- hour data. (1 logger in Jølster I birch, 1 logger in Ørsta birch, and 1 logger in Ørsta spruce). One additional TOMST sensor had a short-term (5 days) gap only in the soil moisture data (Ørsta birch). Four sensors were found to lack temperature data over a longer period (2 in Stranda birch (133 and 146 days) and 2 in Stranda spruce (51 and 75 days)), where the gap filling was based on the best fit average value of another TOMST in the stand for a period of 2 months after and/or prior to the period with missing data. Most gaps in soil moisture data, or obvious erratic values most probably due to disturbance from animals, were typically extending over longer time periods. Missing /deviating soil moisture data for extended periods were found for a total of 13 loggers: 5 in Jølster I birch (11days, 73days, and 220 days for 3 loggers), 2 in Jøster II spruce (145 days), 5 in Stranda birch (133, 134 in 2 loggers), 146 and 170 days), and 1 in Stranda spruce (51 days). The relative soil moisture content tended to vary between loggers with patterns differing between years. Gap filling of missing soil moisture data were estimated based on the best correlation of soil moisture between a given sensor and other loggers in a given stand and location over a period of time that was similar to the missing data period tested for the two preceding years. The soil moisture from the best fit correlation was multiplied with the average soil moisture of the given logger for the incubation period based on the period after and/or prior to the period with missing data. The first factor reflects the best fit for the variability in soil moisture over a given period, and the latter correct for difference in the actual soil moisture content between the best fit logger and the logger with the missing data.

Paper III

European Journal of Forest Research (2019) 138:353–361
https://doi.org/10.1007/s10342-019-01176-4

ORIGINAL PAPER

Vertical distribution of soil carbon in boreal forest under European beech and Norway spruce

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Received: 2 August 2018 / Revised: 26 January 2019 / Accepted: 1 February 2019 / Published online: 15 February 2019

Abstract
Past forest management decisions have resulted in European beech being replaced with Norway spruce across Europe. Previous studies have revealed variances in soil carbon (C) under different dominating tree species. Yet, there is a scarcity of knowledge about how beech and spruce differ in impact on forest soil C in boreal regions, where beech has its northern distribution limit. We have therefore compared soil C in a natural beech forest (Be) with that of two spruce forests: one planted on former beech forest (SpBe) and the other on former spruce forest (Sp), in South-East Norway. Analyses of biochemical parameters and fungal biomass were performed along fine-scaled soil profiles, covering both the organic and mineral layers. We found no significant difference between the forests when comparing estimates of total C stocks per area. However, throughout the soil profile, the distribution of soil C in Be varied significantly from SpBe, while Sp was intermediate. The distribution of fungal biomass along the soil profile in Be varied significantly from the two other forests. Hence, fungal biomass may drive the observed differences. Soil C, nitrogen (N), and C/N ratios were forest type and soil depth dependent, whereas forest type had an effect on the vertical distribution of condensed tannins and fungal biomass. Our results suggest that the presence of beech or spruce as the dominant tree species in the studied area has an effect on the vertical distribution of soil properties, while there is no major difference when comparing the whole soil profile.

Keywords Boreal forest · Soil carbon · *Fagus sylvatica* · *Picea abies* · Fungal biomass · Condensed tannins

Introduction

Boreal forest soils store the largest amount of carbon (C) in the terrestrial biosphere and play an important role in mitigating climate change (Scharlemann et al. 2014). Soil C storage is ultimately determined by the balance of C and nitrogen (N) input from plant production and output from decomposition. Low temperatures and relatively short growing seasons hamper decomposition rates in boreal forest soils, thus facilitating C sequestration. Forestry and choice of tree species have an impact on the soil C stock and distribution by many mechanisms, including the quantity and chemical quality of litter, the depth and distribution of roots, as well as the associated community of decomposers.

A typical example of a forestry-driven choice of tree species is the replacement of European beech (*Fagus sylvatica* L.) with Norway spruce (*Picea abies* (L.) Karst) in Europe. Here, beech forests have been substituted with spruce for decades, and this is the case from their northern distribution limit in Norway to their heart of distribution in Germany (Albers et al. 2004). The reason for this change in tree species is that spruce is considered economically more favourable than beech (Hanewinkel et al. 2013), due to its suitability in construction work, biomass energy, and production of pulp and paper. Interestingly, forest soils under beech have been reported to store less C compared to spruce (Cremer et al. 2016), as adverse environmental conditions in spruce forest soils retard decomposition (Berger and Berger 2012).

Communicated by Agustín Merino.

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Plant secondary compounds, particularly large tannin molecules, may affect ecosystem processes by several mechanisms including changes in soil C and N cycling (Smolander et al. 2012). Condensed tannins represent a major portion of the stable soil C in boreal forests, and the concentration and chemical composition of these recalcitrant compounds vary between tree species, microbial community composition, and environmental conditions (Kraus et al. 2003). Moreover, high content of condensed tannins in plants that grow on soil with low pH and soil fertility, typical for boreal forest ecosystems, are known to retard the decomposition process by affecting the composition and activity of decomposers (Adamczyk et al. 2018).

Aboveground plant litter from trees and understory plants have traditionally been assumed the main source of forest soil C, but recent studies have found that belowground roots and root-associated fungi are major contributors to the soil C pool in boreal forest ecosystems (Clemmensen et al. 2013). Here, mycorrhizal fungi are a key component of the soil microbial biomass, and the heavily decomposable mycelial necromass contributes to the stable soil C (Treseder and Holden 2013). Tree species influence the soil microbial community by their associated mycorrhizal community (Prescott and Grayston 2013), which may contribute to differences in forest C stock and stability. Fungal communities are vertically separated in the soil, where mycorrhizal fungi occur in the rhizosphere. Beech and spruce forests differ in belowground fungal community composition, with larger differences in the litter layer than in the mineral soil (Asplund et al. 2018, 2019).

Previous research has compared differences between tree species in soil C over coarse spatial scales on forest floors or has used relatively large depth intervals. However, research along more fine-scaled soil depth profiles with high spatial resolution is needed to explain soil C dynamics. The effect of tree species on soil C and N is most pronounced in organic soil layers (Hansson et al. 2011), although the distribution pattern in organic soil does not necessarily reflect that of mineral soil (Vesterdal et al. 2008). As C in organic soil is more vulnerable to external disturbance than C in the underlying mineral soil, the mineral soil C is thus a potentially stable reservoir.

To our knowledge, no studies have compared fine spatial patterns in soil C distributions in beech and spruce forests at the northern limit of the beech forest distribution. More knowledge about the underlying mechanisms and factors that contribute to the effect of tree species on soil C, such as fungal biomass and plant litter chemistry, is essential to understand the differences between forest types. In this study, we compared the vertical distribution of soil C in a natural beech forest (Be) with that of two spruce forests; one planted on former beech forest (SpBe) and the other on former spruce forest (Sp). Analyses of soil C properties were done on fine spatial scales across soil profiles, and we tested the following hypotheses: (1) the spruce forests have an overall higher accumulation of soil C than the beech forest, and that (2) differences in soil properties between forest types are depth dependent.

Materials and methods

Study sites

The study sites consisted of three distinct forest stands in the same forest landscape in Vestfold, South-East Norway, selected due to their similar environmental conditions. The present northern margin of the beech forest range is overlapping the southern margin of the boreal spruce forest in this area. Both species are native to the region. The natural beech forest (Be) is located within Brånakollane nature reserve (59°11′N, 10°02′E, 188 m a.s.l.), with no significant forestry activity since 1837. The nature reserve has a clear boundary to the surrounding spruce forest (SpBe), which was planted after a clear-cut of the previous beech forest in 1956. The other spruce forest (Sp) is located about 1.5 km south of the reserve (59°10′N, 10°02′E, 68 m a.s.l.). The preceding spruce forest was clear-cut and re-planted in 1981. Understory vegetation at each site is generally sparse. For detailed information of understory species composition and the sites forest history, see Ohlson et al. (2017).

Soil depths at the study sites are relatively shallow, and there were analogous underlying bedrock in the Brånakollane area and the separate spruce forest (monzonite/quartz monzonite, and syenite/quart syenite, respectively). The latter forest is situated below the marine limit (marine sediment deposits). The two spruce forests have no sign of podsolization, and the thickness of the organic soil was similar for beech and spruce forests (~ 6 cm). The annual average precipitation was 1029 mm, with a mean February temperature of − 3.8 °C and a mean July temperature of 16.3 °C, for the period 1961–1990.

Soil sampling

In September 2015, soil samples were collected at four randomly located plots (with soil depths of approximately 50 cm) within each site. At each plot, a soil corer (10 cm in diameter) was used to collect soil from the organic soil layer (including LFH horizons altogether). The weight of this sample was used for estimation of total C and N stocks, while a subsample of this soil was stored in a 20-ml vial (avoiding compression) for soil chemical analyses. Underneath, a hole of approximately 50 cm in diameter was dug. Here, samples were collected along the mineral soil profile and stored separately in 20-ml vials (avoiding compression).

The samples were taken horizontally using a steel cylinder with a 2.5 cm diameter, from the top of the mineral soil profile (0 cm depth) and at every other 2.5 cm of depth, leaving a 2.5 cm interval of soil between each sample taken. This was done down to 47.5 cm of depth (or down to 42.5 cm in plots with shallower soil depths). Depth ranges of the mineral soil are therefore in following intervals: 0–2.5 cm, 5–7.5 cm, 10–12.5 cm, etc. Sealed vials were placed on dry ice in a Styrofoam cooler in the field. The data set consisted of 129 soil samples (9 plots × 11 samples, 3 plots × 10 samples). Prior to subsequent analyses, all samples were freeze-dried (48 h), sieved (2.5 mm, organic fragments were added), weighted, milled in a ball mill (MM 400, Retsch, Haag, Germany) to powder, and weighted into smaller subsamples for the various assays. All samples were stored at − 80 °C.

Soil chemical analyses

Concentrations of C and N were quantified by a vario MICRO cube elemental analyser (Elementar, Hanau, Germany), and C/N ratios were determined. C and N content were converted to mg cm^{-3} by using the volumetric mass of each sample (20 ml). Estimates of total C and N stocks were calculated separately for the organic soil layer, the mineral soil, and the whole soil profile, without including larger roots or stones, by using the concentration of C and N of the measured samples in each plot. Weight of the initially sampled organic soil layer was used to calculate the estimate at this soil depth. For the mineral soil and the whole soil profile, the content of measured depth intervals along the soil profile was summarized with the means of the two adjacent values for intervals that were not initially measured. Estimates of total C and N at each forest are the means of, respectively, plots converted to kg m^{-2}.

For pH analyses, 3 ml prepared soil sample was placed in a 15-ml glass tube with 8 ml purified water, vortexed, and left overnight. The following day, samples were vortexed and pH values were measured with an intoLab 720 precision pH meter (WTW GmbH, Weilheim, Germany).

The applied extraction method for analysing condensed tannins (CT) is described in Kanerva et al. (2008). In short, approximately 200 mg prepared soil sample was mixed with 4 ml 70% acetone and placed on a planar shaker (200 rpm) for 1 h. After being vortexed and centrifuged (c. 16,400 rpm, 10 min), the supernatant was collected in a 15-ml glass tube. This extraction procedure was repeated three times. All three extractions were collected in the same vial and evaporated using an Eppendorf Concentrator Plus 5301 (Eppendorf, Hamburg, Germany). The extractions were analysed for condensed tannins by the butanol–HCl–iron assay according to Hagerman (2002). Dried extractions were re-dissolved in 0.5 ml MeOH and vigorously vortexed. After adding 3 ml of acid butanol (95% butanol, 5% HCl) and 0.1 ml of iron reagent (2% ferric ammonium sulphate in 2N HCl), samples were placed in boiling water for 1 h. Absorbance (550 nm) of cooled samples was detected by a UV spectrophotometer (Shimadzu, Kyoto, Japan), and purified extracts of *P. abies* were used as a standard. Soil CT content was converted to mg cm^{-3} by using the volumetric mass of each sample (20 ml), and CT/C ratios were determined.

Ergosterol was used as a proxy to estimate fungal biomass, and total ergosterol was measured by a modified version of the protocol of Davey et al. (2009). Briefly, approximately 200 mg prepared soil sample was mixed with 7 ml 3 M KOH in MeOH, vortexed, and sonicated in a 70 °C ultrasonic water bath in darkness for 90 min. After being vortexed and centrifuged (c. 16,400 rpm, 15 min), the supernatant was mixed with 2 ml purified water in new tubes. Ergosterol was extracted by adding 5 ml hexane, vortexed vigorously (approx. 1 min), and the hexane phase was collected after the two phases divided. This extraction was repeated twice. Both extractions were collected in the same vial and evaporated using an Eppendorf Concentrator Plus 5301 (Eppendorf, Hamburg, Germany). Dried extractions were re-dissolved in 500 µl MeOH, and the supernatant was analysed for ergosterol content using high-performance liquid chromatography (HPLC). The extractions were analysed on an 1100 Series HPLC (Agilent Technologies, Waldbronn, Germany). Ergosterol was separated using a reversed phase ODS ultrasphere column (250 mm × 4.6 mm; particle size 5 µm). MeOH was used as the mobile phase (flow rate 1.5 ml min^{-1}, total analysis time 12 min). Absorption of ergosterol was detected at 280 nm and identified by comparing retention time, online UV spectra and co-chromatography of a commercial standard of ergosterol (Sigma, St. Louis, USA). Ergosterol content was converted to mg cm^{-3} by using the volumetric mass of each sample (20 ml), and ergosterol/C ratios were determined.

Statistical analyses

Linear mixed-effects models, followed by two-way ANOVA, were used to test for the effect of forest type and soil depth on soil properties. Plot was used as random factor. All soil data were log-transformed to improve model fit. When forest type effects were significant, we performed separate Tukey's post hoc tests. Kendall's tau correlation test and covariance matrix were used to examine the relationship between the soil property data. Linear regression models, followed by one-way ANOVA, were used to determine whether there were any significant differences between the sites in estimates of total C and N. All statistical analyses were performed in R Studio (version 3.2.4) and graphic illustrations were generated in Veusz (version 1.23.2).

Results

Estimates of total C and N stocks at the three forests were not significantly different for the organic soil layer, the mineral soil, or the whole soil profile (Table 1; Fig. 1). When comparing the measured depth intervals, soil C and N in all three forests were lower in the organic soil layer compared to the mineral soil above 20 cm and the mineral soil below 20 cm (Table 2). While soil C and N in Be and Sp generally constantly decreased along the mineral soil profile, the pattern in SpBe was a quick decline in the first 10 cm and then a slight increase throughout the rest of the soil profile (Fig. 2a, b). This resulted in a significant site × depth interaction (Table 3). Furthermore, there was a strong correlation between C and N ($r = 0.821$; $P < 0.001$). Overall, throughout the soil profile, the content of C and N in Be differed significantly from SpBe (Tukey, $P < 0.05$). C/N ratios generally declined in Sp, while contrasting directions between Be and SpBe to depth in the upper 15 cm resulted in a significant site × depth interaction (Table 3; Fig. 2c).

Concentration of CT and ergosterol roughly declined with increasing soil depth in all three forests (Table 2; Fig. 2d, e). We found a moderate correlation between CT and ergosterol ($r = 0.653$; $P < 0.001$), and both showed a positive, but poor, correlation with concentrations of C ($r = 0.391$ $P < 0.001$, $r = 0.483$ $P < 0.001$, respectively) and N ($r = 0.336$ $P < 0.001$, $r = 0.451$ $P < 0.001$, respectively). The amount of ergosterol in Be differed significantly from both SpBe and Sp along the soil profile (Tukey, $P < 0.05$). Ratios of CT/C and ergosterol/C strongly decreased with depth, implying that both CT and ergosterol concentrations decreased more rapidly than C (Table 2). In contrast, soil pH was negatively correlated with all variables above. Soil pH generally increased with increasing depth in all three forests (Table 2; Fig. 2f). Forest type had an effect on all variables, except for pH values, resulting in different distribution patterns along the soil profile (Table 3).

Table 1 Mean (± 1 SE) for estimates of total carbon (C) and nitrogen (N) stocks at beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp) in the organic soil layer, the mineral soil, and the whole soil profile. Statistics (F and P values) are derived from one-way ANOVAs testing for the effect of forest type

	C (kg m^{-2})	N (kg m^{-2})
Be		
Organic soil layer	2.39 ± 0.19	0.12 ± 0.01
Mineral soil	26.05 ± 2.48	1.36 ± 0.15
Whole soil profile	28.44 ± 2.52	1.48 ± 0.16
SpBe		
Organic soil layer	1.65 ± 0.32	0.07 ± 0.02
Mineral soil	21.70 ± 1.55	0.99 ± 0.09
Whole soil profile	23.35 ± 1.78	1.06 ± 0.10
Sp		
Organic soil layer	1.56 ± 0.18	0.07 ± 0.01
Mineral soil	17.22 ± 2.66	0.90 ± 0.13
Whole soil profile	18.78 ± 2.73	0.98 ± 0.13
Statistics[a]		
Organic soil layer	1.941 (0.199)	2.577 (0.130)
Mineral soil	3.327 (0.083)	2.914 (0.106)
Whole soil profile	3.502 (0.075)	3.149 (0.092)

[a]Degrees of freedom: C = 2, N = 2

Discussion

In contrast to our first hypothesis, there were no significant differences between estimated total C stocks of the three forests. However, the distribution of soil C in Be differed significantly from SpBe throughout the whole soil profile. This was the case even though Be and SpBe are located only about 100 m apart, and SpBe was a part of the then larger beech forest until 1956. Sp, however, was intermediate (not significantly different from the two other forests). Our results imply that the C stock in the studied area is not affected by the presence of beech or spruce as the dominant tree species, but that the forest types have an effect on the vertical distribution of C. This indicates that the soil C stock under beech and spruce varies between forests and regions; the outcome is climate and context dependent.

We found the distribution of fungal biomass in the Be to differ significantly from both SpBe and Sp along the soil profile, suggesting that fungal biomass drives the significant difference in C distribution between Be and SpBe. In addition, Be is the oldest of the three forests in this study, which may partly explain the differences. Previous studies have found that mycelial turnover declines with increasing forest age (Hagenbo et al. 2017), and ectomycorrhizal fungi increase in relative abundance with increasing forest age (Kyaschenko et al. 2017). A fungal community rich in ectomycorrhizal species may suppress decomposition rates and favour accumulation of C in the beech forest soil (Bödeker et al. 2016). However, it has also been found that ectomycorrhizal fungi stimulate decomposition rates and nutrient cycling (Fernandez and Kennedy 2016).

Soil C, N, and C/N ratios in the three forests were dependent on soil depth, providing mixed support for our second hypothesis that differences in soil properties between forest types are depth dependent. However, forest type had an impact on the vertical distribution of condensed tannins and fungal biomass. The cause of this variation in vertical distribution of soil properties is unclear, but may be a result of differences in fungal community composition between the forests along the soil profile. Clear-cutting has a major and long-lasting impact on fungal diversity in forest soils

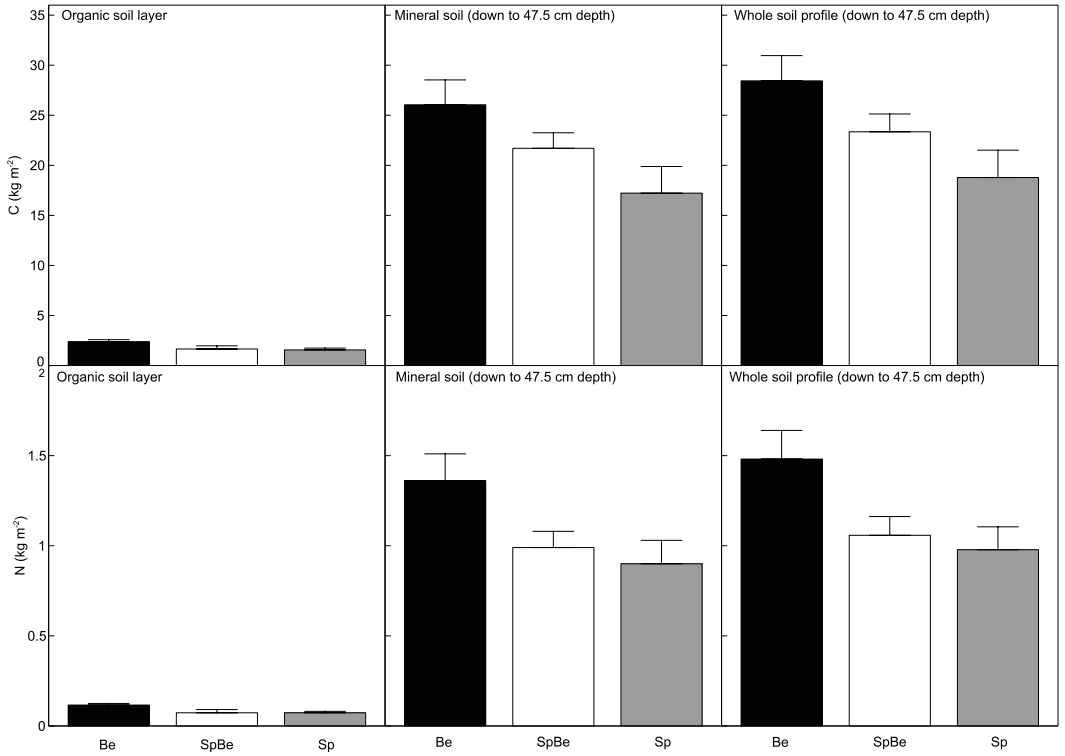

Fig. 1 Mean (±1 SE) for estimates of total carbon (C) and nitrogen (N) stocks at beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp) in the organic soil layer, the mineral soil, and the whole soil profile

resulting in compositional differences of fungal communities between forests of dissimilar ages (Kyaschenko et al. 2017). Hence, each forest has distinct functional groups of fungi that are abundant at different levels of soil depth and vary in regulation of decomposition and accumulation of organic matter. Sp also differs from the others in that it is situated below the marine limit, and therefore has a finer soil texture towards the bedrock. This finer soil texture might imply lower levels of soil respiration and lower input of roots and organic matter that is hampering decomposition, together with lower nutrient content and less active decomposer system in the mineral soil (Baritz et al. 2010).

Fungal community composition can drive the variations in the distribution of condensed tannins as local decomposer communities are specialized in breaking down the litter of dominant tree species (Freschet et al. 2012). However, the biochemical characteristics of condensed tannins potentially regulate decomposition and affect microbial communities. Although little is known of these effects, condensed tannins have been found to potentially inhibit enzyme activity, form recalcitrant complexes with proteins and chitin, and influence N availability (Adamczyk et al. 2018; Chomel et al. 2016). Persistence and vertical distribution of condensed tannins in soil have been shown to vary between tree species (Kanerva et al. 2008). Thus, the amount and chemical structure of litter input from beech and spruce forests may cause variance in soil C. However, since all three forests displayed similar C/N ratios and amounts of condensed tannins, there are likely similar quantities of complex C compounds in the soil.

Although pH correlated negatively with the other variables, this is unlikely to involve causation because higher pH generally provides more favourable soil conditions stimulating higher rates of plant productivity and decomposition, and thus nutrient cycling (Berger et al. 2002). Sterkenburg et al. (2015) suggested that the optimum pH for fungal biomass is around 5, since fertile soils with higher pH generally enhance the environment for soil fauna, which prevents the establishment of large and long-lived mycelial networks. Fungal biomass content declined with increasing soil depth

Table 2 Mean (±1 SE) for carbon (C), nitrogen (N), C/N ratio, condensed tannins (CT), ergosterol, pH values, CT/C ratio, and ergosterol/C ratio at beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp) in the organic soil layer, the mineral soil above 20 cm depth, the mineral soil below 20 cm depth, and the whole mineral soil profile. $n=4$, except for SpBe for which $n=2$ and Sp for which $n=3$ at the deepest soil depth (45–47.5 cm)

	C (mg g^{-1})	C (mg cm^{-3})	N (mg g^{-1})	N (mg cm^{-3})	C/N ratio	CT (mg cm^{-3})	Ergosterol (mg cm^{-3})	pH	CT/C ratio (‰)	Ergosterol/C ratio (‰)
Be										
Organic soil layer	430.02±1.51	56.75±3.53	21.09±1.39	2.82±0.34	20.74±1.32	0.366±0.086	0.082±0.005	4.40±0.10	1.46±0.08	6.79±1.82
Mineral soil (above 20 cm)	1096.40±60.90	315.95±9.44	57.27±3.09	16.83±0.47	19.39±0.49	1.235±0.026	0.204±0.009	4.75±0.14	4.55±0.72	0.62±0.06
Mineral soil (below 20 cm)	369.44±7.74	237.73±2.87	18.94±0.29	12.30±0.08	19.48±0.80	0.812±0.026	0.061±0.001	5.36±0.05	4.12±0.59	0.26±0.02
Mineral soil	1465.84±41.22	553.68±7.38	76.22±2.13	29.13±0.39	19.44±0.52	2.046±0.033	0.265±0.007	5.12±0.11	4.29±0.46	0.41±0.06
SpBe										
Organic soil layer	332.19±57.11	65.26±5.69	14.11±2.37	2.79±0.23	23.72±2.13	0.700±0.227	0.091±0.016	4.24±0.11	12.23±5.27	1.52±0.42
Mineral soil (above 20 cm)	451.69±48.87	205.21±13.59	20.73±2.15	9.52±0.58	21.30±0.74	0.831±0.046	0.075±0.007	4.77±0.22	4.69±0.64	0.36±0.04
Mineral soil (below 20 cm)	480.55±2.59	291.22±2.44	21.52±0.16	13.11±0.14	22.50±0.36	0.591±0.011	0.035±0.000	5.05±0.02	2.19±0.24	0.13±0.01
Mineral soil	932.24±20.26	496.43±5.64	42.25±0.90	22.63±0.25	22.02±0.41	1.422±0.026	0.110±0.004	4.94±0.10	3.19±0.49	0.22±0.04
Sp										
Organic soil layer	207.76±46.41	73.02±3.26	9.55±1.83	3.42±0.12	21.34±0.57	0.687±0.145	0.069±0.006	4.05±0.14	9.79±2.41	0.96±0.12
Mineral soil (above 20 cm)	257.34±13.97	242.99±12.78	12.62±0.61	11.86±0.54	20.01±0.32	0.456±0.039	0.038±0.003	4.73±0.15	2.56±0.66	0.21±0.04
Mineral soil (below 20 cm)	116.26±3.20	133.36±3.65	6.77±0.15	7.84±0.17	16.94±0.51	0.243±0.008	0.026±0.001	5.16±0.03	1.91±0.29	0.22±0.05
Mineral soil	373.61±9.13	376.35±8.16	19.39±0.41	19.70±0.35	18.17±0.58	0.698±0.020	0.064±0.001	4.99±0.09	2.17±0.33	0.22±0.03

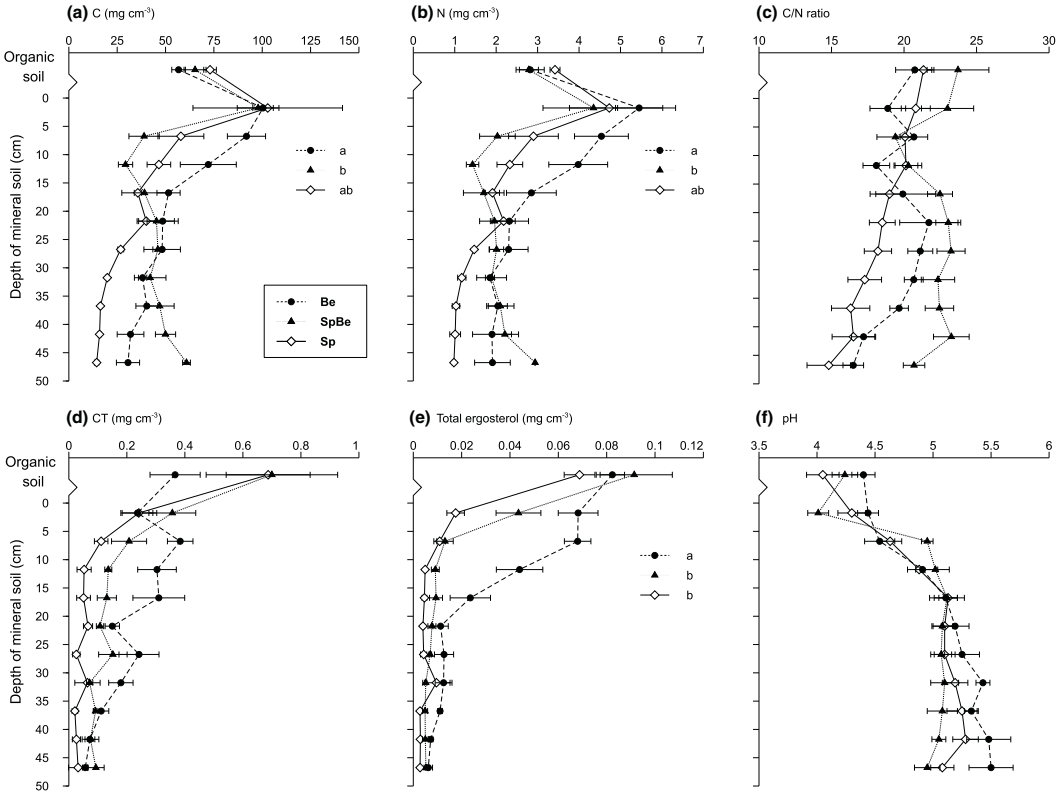

Fig. 2 Mean (±1 SE) for **a** carbon (C), **b** nitrogen (N), **c** C/N ratio, **d** condensed tannins (CT), **e** total ergosterol, and **f** pH values displayed at different soil depths (organic soil, mineral soil 0–47.5 cm) at beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp). $n=4$, except for SpBe for which $n=2$ and Sp for which $n=3$ at the deepest soil depth (45–47.5 cm). For each graph, lines marked with contrasting letters indicate significant differences between forests (Tukey, $P<0.05$)

in all three forests, indicating a larger proportion of mycelium production in the upper levels of the soil profile. This corresponds with the decrease in ergosterol/C ratio with soil depth, which is expected as saprotrophic fungi feed on dead organic matter that is more abundant at the upper soil profile, and mycorrhizal fungi are concentrated in the rhizosphere. Additionally, higher turnover rates in the beech forest could cause greater mycelial production to compensate for the loss, and thereby increase the fungal biomass (Clemmensen et al. 2013).

Besides differences in fungal biomass and litter chemistry, differing rooting patterns between beech and spruce might have contributed to the observed differences in soil properties. From our results it appears that beech maintain the mycorrhizal activity at deeper soil depths than spruce by recycling nutrients from deeper soil depths through its deeper root system, thus potentially increasing the soil C accumulation. However, the effect of tree species may only be attributed to the upper soil layers. Ahmed et al. (2016) found that C storage to a depth of 1 m was unaffected by tree species identity; it only affected the C concentration of soil in the top 20 cm. In our study, visual inspection of C distribution in all three forests shows the same tendency of highest variation roughly in the upper 20 cm of the soil profile. This coincides with a study by Schmid and Kazda (2001) where they found spruce roots to be predominately concentrated in the upper soil layers (with maximum root density in top 10 cm) compared to beech roots (with maximum root density in 10–20 cm soil depth). However, they found no differences in total rooting depth down to 1 m. In accordance with these findings, a review by Vesterdal et al. (2013) highlighted that the trend among forest types in soil C accumulation seems to be similar when combining organic and mineral soil. Thus, tree species influence the soil

Table 3 Two-way split-plot ANOVAs (F and P values) testing for the effect of site [beech forest (Be), previous beech forest (SpBe), and spruce forest (Sp)] and soil depth (organic soil, mineral soil 0–47.5 cm) as the main plot factors, and holes (1–12) as the random plot factor, on carbon (C), nitrogen (N), C/N ratio, condensed tannins (CT), ergosterol, pH values, CT/C ratio, and ergosterol/C ratio

	Site (S)	Depth (D)	$S \times D$
C	**10.22 (0.006)**	**114.52 (< 0.001)**	**44.84 (< 0.001)**
N	**6.17 (0.046)**	**83.80 (< 0.001)**	**26.00 (< 0.001)**
C/N ratio	**7.75 (0.021)**	**19.21 (< 0.001)**	**24.74 (< 0.001)**
CT	**23.29 (< 0.001)**	**92.62 (< 0.001)**	4.87 (0.087)
Ergosterol	**31.44 (< 0.001)**	**169.63 (< 0.001)**	0.90 (0.637)
pH	1.78 (0.412)	**142.79 (< 0.001)**	2.38 (0.304)
CT/C ratio	**6.35 (0.042)**	**15.49 (< 0.001)**	2.09 (0.352)
Ergosterol/C ratio	**15.52 (< 0.001)**	**74.81 (< 0.001)**	**10.137 (0.006)**

Degrees of freedom: $S = 2$, $D = 1$, $S \times D = 2$

Bold values indicate significant effects at $P = 0.05$

C distribution rather than the soil C stock. Regardless, there has not been reported any tree species effect on the quantity and vertical distribution of labile and stable soil C fractions (Jandl et al. 2007).

Conclusion

The beech forest did not store less soil C than spruce forest in the present study, suggesting that beech forest soils at its northern distribution limit are different from areas where beech has its main distribution. Moreover, our results show that a transformation from beech to spruce forest is likely to change the vertical distribution of C in boreal forest soils. We are aware of the risks associated with few replications for each forest type and that imprecise separation of the organic and mineral soil layer may have caused offsets along the soil profile, as the separation is hard to standardize. However, the small amounts of soil subjected to analysis in this study undoubtedly contributed to much stochastic variation, which makes the significant results more noteworthy. The significant differences among all three forests indicate considerable variation within a single forest landscape. The dynamics of C storage in beech forests soils in boreal ecosystems need to be further explored to better understand how a shift from beech to spruce may influence the soil C reservoir of boreal forests.

Acknowledgements We are grateful to Eivind Thomassen for help in the field, to Annie Aasen for laboratory assistance, and for technical support from The Norwegian Institute of Bioeconomy Research (NIBIO). Permit to conduct research in Brånakollane Nature Reserve was issued by the County Governor of Vestfold (2013/3878). We thank Kjell Lie, on behalf of the forest owner, for kind help with access to the forest area. The Research Council of Norway funded this study (Grant No. 225018).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adamczyk B, Adamczyk S, Smolander A, Kitunen V, Simon J (2018) Plant secondary metabolites—missing pieces in the soil organic matter puzzle of boreal forests. Soil Syst 2:2. https://doi.org/10.3390/soils2010002

Ahmed IU, Smith AR, Jones DL, Godbold DL (2016) Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. For Ecol Manag 359:352–360. https://doi.org/10.1016/j.foreco.2015.07.018

Albers D, Migge S, Schaefer M, Scheu S (2004) Decomposition of beech leaves (*Fagus sylvatica*) and spruce needles (*Picea abies*) in pure and mixed stands of beech and spruce. Soil Biol Biochem 36:155–164. https://doi.org/10.1016/j.soilbio.2003.09.002

Asplund J, Kauserud H, Bokhorst S, Lie MH, Ohlson M, Nybakken L (2018) Fungal communities influence decomposition rates of plant litter from two dominant tree species. Fungal Ecol 32:1–8. https://doi.org/10.1016/j.funeco.2017.11.003

Asplund J, Kauserud H, Ohlson M, Nybakken L (2019) Spruce and beech as local determinants of forest fungal community structure in litter, humus and mineral soil. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiy232

Baritz R, Seufert G, Montanarella L, Van Ranst E (2010) Carbon concentrations and stocks in forest soils of Europe. For Ecol Manag 260:262–277. https://doi.org/10.1016/j.foreco.2010.03.025

Berger TW, Berger P (2012) Greater accumulation of litter in spruce (*Picea abies*) compared to beech (*Fagus sylvatica*) stands is not a consequence of the inherent recalcitrance of needles. Plant Soil 358:349–369. https://doi.org/10.1007/s11104-012-1165-z

Berger TW, Neubauer C, Glatzel G (2002) Factors controlling soil carbon and nitrogen stores in pure stands of Norway spruce (*Picea abies*) and mixed species stands in Austria. For Ecol Manag 159:3–14. https://doi.org/10.1016/S0378-1127(01)00705-8

Bödeker ITM, Lindahl BD, Olson Å, Clemmensen KE (2016) Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Funct Ecol 30:1967–1978. https://doi.org/10.1111/1365-2435.12677

Chomel M, Guittonny-Larcheveque M, Fernandez C, Gallet C, DesRochers A, Pare D, Jackson BG, Baldy V (2016) Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. J Ecol 104:1527–1541. https://doi.org/10.1111/1365-2745.12644

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD, Wardle DA, Lindahl BD (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339:1615–1618. https://doi.org/10.1126/science.1231923

Cremer M, Kern NV, Prietzel J (2016) Soil organic carbon and nitrogen stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. For Ecol Manag 367:30–40. https://doi.org/10.1016/j.foreco.2016.02.020

Davey ML, Nybakken L, Kauserud H, Ohlson M (2009) Fungal biomass associated with the phyllosphere of bryophytes and vascular plants. Mycol Res 113:1254–1260. https://doi.org/10.1016/j.mycres.2009.08.001

Fernandez CW, Kennedy PG (2016) Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? New Phytol 209:1382–1394. https://doi.org/10.1111/nph.13648

Freschet GT, Aerts R, Cornelissen JHC (2012) Multiple mechanisms for trait effects on litter decomposition: moving beyond home-field advantage with a new hypothesis. J Ecol 100:619–630. https://doi.org/10.1111/j.1365-2745.2011.01943.x

Hagenbo A, Clemmensen KE, Finlay RD, Kyaschenko J, Lindahl BD, Fransson P, Ekblad A (2017) Changes in turnover rather than production regulate biomass of ectomycorrhizal fungal mycelium across a *Pinus sylvestris* chronosequence. New Phytol 214:424–431. https://doi.org/10.1111/nph.14379

Hagerman AE (2002) Tannin handbook. Miami University, Oxford

Hanewinkel M, Cullmann DA, Schelhaas M-J, Nabuurs G-J, Zimmermann NE (2013) Climate change may cause severe loss in the economic value of European forest land. Nat Clim Change 3:203–207. https://doi.org/10.1038/nclimate1687

Hansson K, Olsson BA, Olsson M, Johansson U, Kleja DB (2011) Differences in soil properties in adjacent stands of Scots pine, Norway spruce and silver birch in SW Sweden. For Ecol Manag 262:522–530. https://doi.org/10.1016/j.foreco.2011.04.021

Jandl R, Lindner M, Vesterdal L, Bauwens B, Baritz R, Hagedorn F, Johnson DW, Minkkinen K, Byrne KA (2007) How strongly can forest management influence soil carbon sequestration? Geoderma 137:253–268. https://doi.org/10.1016/j.geoderma.2006.09.003

Kanerva S, Kitunen V, Loponen J, Smolander A (2008) Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. Biol Fertil Soils 44:547–556. https://doi.org/10.1007/s00374-007-0234-6

Kraus TEC, Dahlgren RA, Zasoski RJ (2003) Tannins in nutrient dynamics of forest ecosystems—a review. Plant Soil 256:41–66. https://doi.org/10.1023/A:1026206511084

Kyaschenko J, Clemmensen KE, Hagenbo A, Karltun E, Lindahl BD (2017) Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. ISME J 11:863–874. https://doi.org/10.1038/ismej.2016.184

Ohlson M, Ellingsen VM, del Olmo MV, Lie MH, Nybakken L, Asplund J (2017) Late-Holocene fire history as revealed by size, age and composition of the soil charcoal pool in neighbouring beech and spruce forest landscapes in SE Norway. Holocene 27:397–403. https://doi.org/10.1177/0959683616660174

Prescott CE, Grayston SJ (2013) Tree species influence on microbial communities in litter and soil: current knowledge and research needs. For Ecol Manag 309:19–27. https://doi.org/10.1016/j.foreco.2013.02.034

Scharlemann JPW, Tanner EVJ, Hiederer R, Kapos V (2014) Global soil carbon: understanding and managing the largest terrestrial carbon pool. Carbon Manag 5:81–91. https://doi.org/10.4155/cmt.13.77

Schmid I, Kazda M (2001) Vertical distribution and radial growth of coarse roots in pure and mixed stands of *Fagus sylvatica* and *Picea abies*. Can J For Res 31:539–548. https://doi.org/10.1139/x00-195

Smolander A, Kanerva S, Adamczyk B, Kitunen V (2012) Nitrogen transformations in boreal forest soils—does composition of plant secondary compounds give any explanations? Plant Soil 350:1–26. https://doi.org/10.1007/s11104-011-0895-7

Sterkenburg E, Bahr A, Durling MB, Clemmensen KE, Lindahl BD (2015) Changes in fungal communities along a boreal soil fertility gradient. New Phytol 207:1145–1158. https://doi.org/10.1111/nph.13426

Treseder KK, Holden SR (2013) Fungal carbon sequestration. Science 339:1528–1529. https://doi.org/10.1126/science.1236338

Vesterdal L, Schmidt IK, Callesen I, Nilsson LO, Gundersen P (2008) Carbon and nitrogen in forest floor and mineral soil under six common European tree species. For Ecol Manag 255:35–48. https://doi.org/10.1016/j.foreco.2007.08.015

Vesterdal L, Clarke N, Sigurdsson BD, Gundersen P (2013) Do tree species influence soil carbon stocks in temperate and boreal forests? For Ecol Manag 309:4–18. https://doi.org/10.1016/j.foreco.2013.01.017

ISBN: 978-82-575-1893-6
ISSN: 1894-6402

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