Short Communication

Interaction between ectomycorrhizal and ericoid mycorrhizal plants decelerates stable soil organic matter decomposition

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A B S T R A C T

Boreal forests are N-limited ecosystems storing globally significant amounts of carbon (C) belowground as soil organic matter (SOM). The significant role of mycorrhizal fungi, both ericoid (ERM) and ectomycorrhizal (ECM) in decomposition and building up SOM has been proposed, but it is still largely unknown how these two types of mycorrhiza interact in terms of SOM changes. Here we try to elucidate the combined effect of these mycorrhizal types on the SOM decomposition and accumulation in a pot study with ECM-pine (*Pinus sylvestris* L.) and ERM-heather (*Calluna vulgaris* L.). Experimental design included plants growing alone or in a mixture, and a non-planted control. In contrast to the mixture, heather growing alone exerted higher biomass, higher uptake of N from stable SOM pool, slightly higher microbial biomass C and fungal biomass. Moreover, heather soil had the highest dissolved organic carbon and nitrogen. On the contrary, mixed cultures showed lower growth of heather and stable SOM decomposition. Our results suggest that interaction between ERM and ECM plants may lead to stable SOM accumulation in boreal forest soils.

1. Introduction

Boreal forests store significant amounts of the global carbon (C) pool, of which a major fraction is stored belowground mainly as soil organic matter (SOM) (Lehmann and Kleber, 2015). SOM is built up from persistent and labile organic compounds forming supramolecular aggregates stabilized by chemical stability and interactions, mineral adsorption, and physical inaccessibility (Adamczyk et al., 2019a; Clarholm et al., 2015; Schmidt et al., 2011). Decisive factors for the direction of changes in SOM pool, i.e. its decomposition or stabilization are not yet well understood, though urgently needed to predict and mitigate climate change (Cotrufo et al., 2015; Dijkstra et al., 2020; Liang et al., 2017). Work done up to date underlines the role of roots and root-associated microbes in enhanced decomposition via rhizosphere priming and effective SOM decomposition by mycorrhizal fungi (Lindahl et al., 2021; Meyer et al., 2022) but also in the stabilization of SOM (Clemmensen et al., 2013; Liang et al., 2017; Meyer et al., 2023). It has recently been shown that the interaction between root-derived tannins with fungal necromass leads to SOM stabilization (Adamczyk, 2021; Adamczyk et al., 2019b).

Nitrogen (N) in boreal forest soil is mainly present in organic forms, i. e. as peptide-like compounds and amino sugars (Clarholm et al., 2015; Liang et al., 2017), which are bound in supramolecular structures, leading to very low N availability (Clarholm et al., 2015). To improve N uptake from these persistent N pools boreal plants evolved symbioses with effective decomposers, i.e. fungi. In boreal forest, two types of mycorrhizal fungi dominate: ectomycorrhizal fungi (ECM), symbionts of trees, and ericoid mycorrhiza fungi (ERM) living in symbioses with shrubs from Ericaceae family, like *Calluna vulgaris* (heather). ERM fungi have previously been hypothesized to be more efficient than ECM fungi in accessing organic N (Read and Perez-Moreno, 2003), but recent evidence suggests that some ECM fungi have a similar capacity as ERM to decompose persistent SOM, including tannin-protein complexes (Adamczyk et al., 2016; Lindahl and Tunlid, 2015; Shah et al., 2016). On the other hand, as ERM plants and especially heather are known to restrict the growth of competitors, they may build up stable SOM (Fanin

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et al., 2022; Hawkins et al., 2023). More precisely, ERM-shrubs may contribute to SOM accumulation through the production of recalcitrant, melanin-rich necromass (Clemmensen et al., 2021, 2015; Kennedy and Maillard, 2023). However, an understanding of how the interaction of ERM-shrubs with ECM-trees affects SOM decomposition vs stabilization in the boreal forest remains still scarce.

Here we studied the interactive effect of ERM-heather and ECM-pine seedlings on decomposition of SOM and a representative of hard-to-decompose SOM fungal necromass-condensed tannin (FNM-CT) complexes. We have chosen FNM-tannin complexes as they may be abundant representative of stable SOM in boreal forests (Adamczyk et al., 2019a). We hypothesize that 1) ERM-heather and ECM-pine facilitate stable SOM decomposition as they are both effective decomposers for organic N, which could be seen as enhanced uptake of ^{15}N from labelled FNM-CT complexes. An increase in a small molecular weight DOC/DON in soil extracts of planted microcosms would provide support for increased SOM decomposition, as these compounds are the main products of decomposition. In particular, an increase in the concentration of free glucosamine as a fungal marker in DOC would indicate more intensive decomposition of FNM in plant mixtures than alone. In addition, we hypothesize that 2) growing together, ERM-heather and ECM-pine interact, which can lead to decelerated SOM decomposition compared to the treatments where pine and heather are grown alone, seen as decreased ^{15}N uptake from labelled FNM-CT and lower concentration of free glucosamine. Our study discovers how the interaction between ERM-shrubs and ECM-pine affects SOM decomposition.

2. Materials and methods

2.1. Experimental design

The soil used in this study was collected from the organic layer of a forest in proximity to the SMEAR II station of the University of Helsinki at Hyytiälä (61°84′N, 24°26′E) in Finland (Ilvesniemi et al., 2000). The soil of this forest is haplic podzol with Scots pine (*Pinus sylvestris* L.) and *Vaccinium myrtillus*, *V. vitis-idaea* and *Calluna vulgaris* as dominant tree and shrub species, respectively. Carbon and N stocks in the humus layer and the uppermost 0.5 m of mineral soil were 6.4 kg m^{-2} and 0.22 kg m^{-2}, respectively. The humus type at this site is mor, which is typical for boreal coniferous acidic soil with fungi as dominating organisms. Most abundant fungal genera include (in order of relative abundance) *Lactarius* (ECM), *Mortierella*, *Mycena* (saprotrophs, SAP), *Piloderma*, *Suillus*, *Cortinarius* (ECM), *Umbelopsis* (SAP), *Boletus*, *Russula* (ECM), *Resinicium*, *Marasmius* (SAP), *Tylospora*, *Inocybe* (ECM), *Rhizoscypus*, *Meliniomyces* (ERM) (Santalahti et al., 2016). The soil was taken from the organic layer (OF, OH), visible roots were removed, and the soil was homogenized and sieved through a 4-mm mesh.

Heather seedlings were germinated using soil from the organic layer. Due to very slow growth, heather plants were approximately 6 months old before transfer to microcosms. The aim was to have similar size seedlings rather than similar age seedlings as both cannot be achieved simultaneously (Adamczyk et al., 2016). Pine seeds were obtained from tree breeding seed collection (lot M29-92-0059 sv. Ullanristi), sterilized with 30 % H$_2$O$_2$ and germinated on glucose agar and grown aseptically on Brown-Wilkins medium for 2.5 months (Heinonsalo et al., 2015) before being transplanted into the pots with soil. These plants were cultivated in pots for 6 months, with the addition of mesh bags containing fungal necromass bound to condensed tannins, a representative of stable SOM (Adamczyk et al., 2019b, 2019a) (see below for preparation). Seedlings were cultivated one per pot (for monocultures) or two per pot (for treatment with two species). Each treatment was replicated 8 times. The pots were of size 15 × 15 × 15 cm. The seedling shoots were exposed to 160–220 μmol s^{-1} m^{-2} light intensity, 18 °C temperature during the 18 h day and 14 °C during the 6 h night. Soil was regularly watered in order to maintain soil moisture. Soil samples were collected from the pots at the end of the experiment. Soil and plant samples were

vacuum-dried and milled for further analysis, for DOC and DON pools fresh soil was used.

2.2. Fungal necromass-condensed tannin complexes and measurements of ^{15}N uptake

Fungal necromass-tannin complexes were prepared as before (Adamczyk et al., 2019b). Briefly, the fungal biomass (basidiomycete fungi *Dichomitus squalens*, strain code FBCC 312) was cultivated on Hagem's liquid medium where 10 % of the required NH$_4$Cl was replaced with 99 % ^{15}NH$_4$Cl (Larodan, Solna, Sweden). After cultivation, the fungal biomass was autoclaved, lyophilized, and homogenized. The condensed tannins (CT) were extracted and purified from Norway spruce needles (*Picea abies* (L.) Karst) and precisely characterized as in Adamczyk et al. (2019b). Briefly, FNM was mixed with 0.1 % CT, and after centrifugation supernatant was removed and the precipitate was washed twice with water to remove unbound compounds. Wet FNM-CT complexes were placed in the mesh bags (50 μm mesh size, 1 × 1 cm) and transferred to the pots (one mesh bag per pot). These mesh bags allow fungal, but not root ingrowth. Each bag contained FNM (corresponding to 10 mg dry weight per mesh bag) and bound CT (0.45 mg per bag). The ^{15}N content of plant leaves was analyzed by isotope-ratio mass spectrometry coupled to an elemental analyzer, with an analytical precision better than 0.2 ‰ for the δ^{15}N based on five replicates of the standards. The nitrogen isotope values were calculated as atom% ^{15}N excess (at.% ^{15}N sample –at.% ^{15}N natural abundance) and for the treatments with seedlings given as ^{15}N content (μg ^{15}N) in stems, needles and roots by taking into account the mass, nitrogen content and atom% ^{15}N excess values of each plant pool.

2.3. Amino sugars in soil samples

We determined four amino sugars (glucosamine, galactosamine, mannosamine and muramic acid) using gas chromatography (GC) as described in (Zhang and Amelung, 1996), and modified by (Liang et al., 2012). Briefly, 1 g of freeze-dried soil was hydrolyzed in 6 M HCl at 105 °C for 8 h. The resulting solution was then filtered, neutralized and dried. Next, the amino sugars were washed from the residue with methanol and evaporated to dryness. Residues were dissolved in 1 mL of water, then lyophilized and subjected to derivatization with aldononitrile acetate. After removing the excess derivatization agent the liquid was dried at 45 °C and re-suspended in a 300 μL of ethylacetate-hexane mixture (1:1). Separation of amino sugar derivatives was conducted on a GC system (Agilent 6890) with a J&W ScientificUltra-2 column (25 m × 0.2 mm × 0.33 μm) and a flame ionization detector. Samples were injected onto the column with hydrogen as the carrier gas. The GC inlet was set to 250 °C and operated in split mode with a 30:1 ratio. The individual amino sugars were identified by comparing their retention time with those of known standards, with myo-inositol serving as the internal standard. Glucosamine can be seen as fungal marker, and muramic acid derives from bacterial peptidoglycan; galactosamine and mannosamine are unspecific microbial markers (Joergensen, 2018).

2.4. Fungal biomass and microbial biomass C

Fungal biomass was measured through its biomarker, ergosterol, with the high-performance liquid chromatography (HPLC) method (Frostegård and Bååth, 1996). Briefly, ergosterol was extracted from 0.25 g soil samples by adding 1 mL cyclohexane and 4 mL 10 % KOH in methanol, 15 min of sonication and liquid-liquid extraction with water and cyclohexane. Cyclohexane phase was evaporated and samples redissolved in methanol. The concentration of ergosterol was measured with HPLC (Arc HPLC Waters) using a C18 100 A reverse-phase column (Phenomenex).

Microbial biomass C (MBC) was determined by the chloroform fumigation-extraction method (Vance et al., 1987). Fumigated soil

samples were prepared by fumigating 2.5 g of soil with ethanol-free chloroform overnight and extracting with 40 mL of 0.05 M K_2SO_4. The extracts were analyzed for total organic C using a TOC-VCPH Shimadzu (Japan). MBC was calculated as a difference between TOC of fumigated and non-fumigated counterpart. A correction factor was not used (Leckie et al., 2004).

2.5. Dissolved organic C (DOC) and total dissolved N (TDN)

For the analyses, 5 g of fresh soil was taken from each pot and shaken in 50 mL ultra-pure water for 2 h at 200 rev min^{-1} and then filtered through a paper filter (Schleicher & Schuell no. 5893, blue ribbon, ashless). The filtered DOC and TDN extracts were then divided into 3 subsamples. The first subsample (10 mL) was used to analyze the total organic C and N using TOC-VCPH Shimadzu (Japan). The second sub-sample (2 mL) was used to measure glucosamine released from FNM. The third subsample of extracts was filtered through Ultracel regener-ated cellulose membrane filters with a nominal molecular weight limit of 1 kDa (Amicon Stirred Cell model 8000). The <1 kDa DOC and TDN concentrations were measured by TOC-VCPH Shimadzu (Japan) (Kiikkilä et al., 2006). DOC and TDN are presented as µg g^{-1} soil DW and as µg g^{-1} root DW, for which we took into account root dry mass, as root masses differed significantly between treatments.

Amount of glucosamine released from FNM (thus from chitin) was measured after derivatization with FMOC according to protocol by (Adamczyk et al., 2020). Derivatized glucosamine was injected into Agilent 1010 HPLC with fluorescence detector.

2.6. Statistics

The effect of treatments was compared with one-way ANOVA fol-lowed by Tukey test. The difference was reported as significant when $P < 0.05$. The assumption of normality was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests and homogeneity of variances using Levene's test. All statistical analyses were made using SPSS 24.0 software.

3. Results

Heather growing alone obtained higher shoot mass than pine alone or when heather and pine grew in a mixture ($p < 0.01$). However, the total shoot biomass in the mixed treatment (heather and pine together) did not differ from the shoot biomass of heather growing alone (Fig. 1A). Similarly, the root biomass of individual plants was decreased in mixed treatment, as their sum were at a similar level as the root biomass of each species growing alone.

Seedlings growing alone obtained more ^{15}N from mesh bag than seedlings in a mixture; however, a significant difference was observed only for heather ($p < 0.05$) (Fig. 1B). Similarly, potentially more intensive decomposition of FNM, as indicated by more free glucosamine in DOC, was detected under pine and heather growing alone than in a mixture (p < 0.01) (Fig. 1C). The lowest glucosamine (GlcN) concen-tration in DOC was detected in control treatment (no plants).

The soil under seedlings growing alone had slightly higher living fungal biomass (higher concentration of ergosterol) than under seed-lings growing in a mixture. The lowest results were found in control soil, though not statistically different from mixed treatment (Fig. 1D).

The concentration of microbial biomass C was the lowest in control pots (Fig. 1E).

When DOC and total dissolved N (TDN) concentrations were given per root biomass, differences between treatments with plants were observed (p < 0.05, Table 1). The presence of heather increased DOC and TDN most, treatment with pine was at the same level as mixed treatment.

Both total DOC and TDN were somewhat higher in pots with plants compared to control treatment, but the trend was not statistically sig-nificant. The DOC fraction passing 1 kDa molecular sieve corresponded to approximately 12 to 18 % of the total DOC, but no treatment-related differences were observed (Table 1). However, in the <1 kDa fraction, there were 15 to 17 times more dissolved N in planted treatments compared to the non-planted control (Table 1).

Though differences between amino sugars were not statistically significant due to high variation, some trends could be observed (Table 2). Treatment with pine exerted slightly higher GlcN
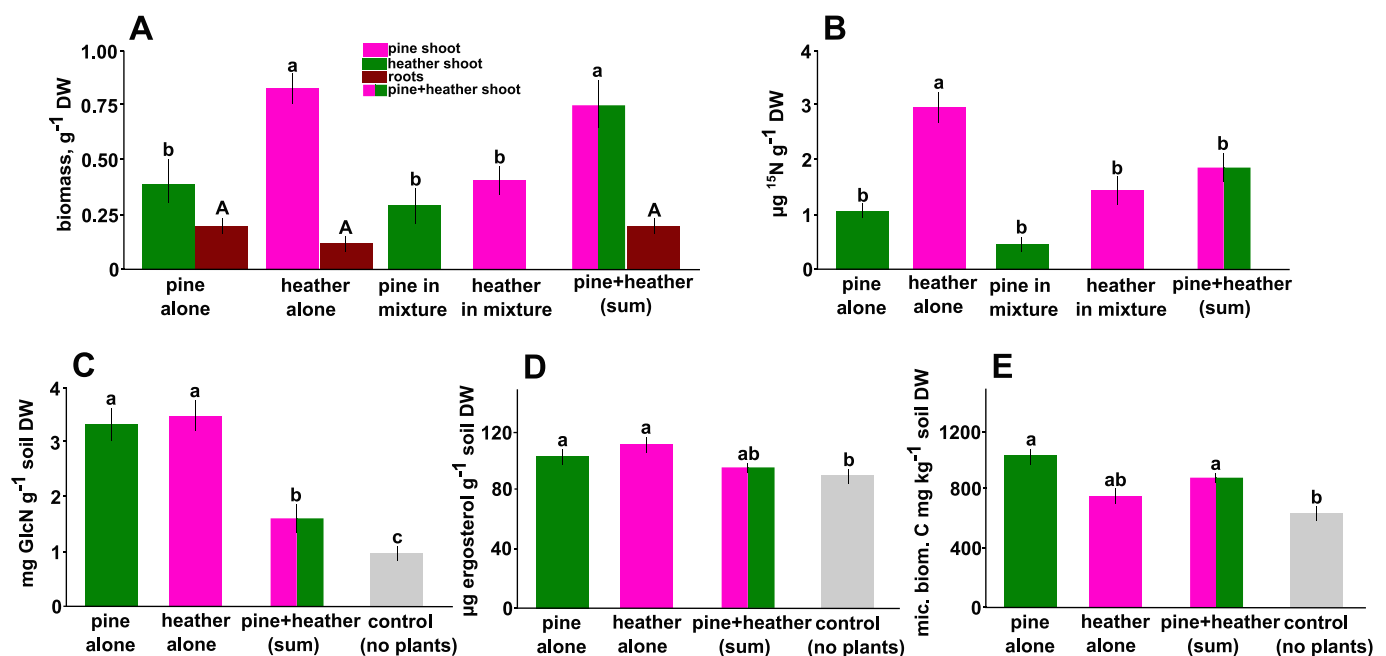

Fig. 1. Biomass of seedlings, their uptake of ^{15}N and soil characteristics: A) plant biomass, B) Content of ^{15}N in leaves/needles of seedlings, C) amount of glucosamine in DOC pool, D) ergosterol concentration in the soil, E) microbial biomass C in the soil. Significant differences ($P < 0.05$) are indicated by different letters. For plant biomass significant differences (P < 0.05) between shoot growth are indicated by different letters, and the differences between root growth are indicated by capital letters. All values are presented as mean of 8 replicates ± SE.

Table 1
Dissolved organic C (DOC) and total N (TDN), and <1 kDa fractions. Mean values ±SE. Significant differences (P<0.05) are indicated by different letters.

	DOC μg g^{1} DW	TDN μg g^{-1} DW	<1 kDa DOC μg g^{1} DW	<1 kDa TDN μg g^{1} DW	<1 kDa TDN μg g^{-1} root	DOC μg g^{-1} root	TDN μg g^{-1} root
Pine	252(17)	4.8(0.28)	31.4(4.6)	3.68(0.2)[a]	26.2(5.4)[a]	1676(145)[b]	33.6(3.2)[b]
heater	216(23)	4.0(0.28)	39.1(9.6)	4.04(0.2)[a]	90.2(19.5)[b]	4280(676)[a]	90.4(17.2)[a]
Pine-heater	204(16)	4.0(0.32)	29.2(6.2)	4.08(0.4)[a]	35.6(7.8)[a]	1788(416)[b]	33.6(7.2)[b]
No plant	160(8)	3.2(0.32)	25.6(4.6)	0.24(0.1)[b]			

Table 2
Concentrations of amino sugars in soil samples (mg kg^{-1}). GlcN – glucosamine, GalN – galactosamine, MurA- muramic acid. Mean values ±SE.

	GlcN	GalN	MurA	GlcN/ MurA	GlcN/ GalN	Total amino sugars
Pine	3320 (159)	865 (45.7)	69.8 (2.9)	47.9 (2.5)	3.9(0.1)	4256(200)
heather	3294 (81)	967 (32.5)	78.3 (2.8)	42.2 (1.0)	3.4(0.1)	4340(127)
No plant	3391 (131)	998 (55.3)	78.3 (3.2)	43.4 (0.3)	3.4(0.1)	4467(182)

concentrations than heather and lower muramic acid concentration. This difference is underlined by the ratio of GlcN to muramic acid. Galactosamine was slightly higher in controls, and lowest in pots with pine.

4. Discussion

The potential role of ERM and ECM fungi in both, decomposition and building up stable SOM has been recognized (Adamczyk et al., 2016; Clemmensen et al., 2015; Fanin et al., 2022), however, how these two mycorrhizal plant types interact in term of the SOM changes remains unclear. Here we compared the access of ERM-heather and ECM-pine seedlings to FNM-CT complexes, with special attention to their mixed effect.

In our short-term pot experiment (6 months), we observed symptoms of plant competition leading to pine outcompeting heather resulting in a decreased growth of heather and changes in the decomposition of FNM-CT complexes. The phenomena of interaction between pine and heather have been observed before with contradictory results. Heather may act negatively on pine growth via allelopathy (Kerley and Read, 1997; Norberg et al., 2001) but positively on pine seedling establishment in forest regeneration (Hyppönen et al., 2013). However, these studies were done mainly in field experiments with mature heather and small pine seedlings. In our experiment, we used seedlings of similar biomass, and we observed that pine outcompeted heather. The mechanism underneath such an effect of interaction needs more studies. Our results suggest that both mycorrhizal plants access FNM-CT complexes, however, faster decomposition was observed for plants growing alone compared to a species mixture, supporting our hypothesis 2. This difference is especially visible for heather, which is even more effective in accessing hard-to-decompose organic N than pine; this is in line with a recent view stating that ERM are more capable degrading C compounds than ECM ones (Tedersoo et al., 2020; Ward et al., 2022), with exception of some ECM showing similar abilities to decompose SOM as ERM (Lindahl et al., 2021). These differences in the ability to decompose SOM are well reflected also in a free glucosamine (chitin monomer) concentration in soil solution, as free GlcN was the highest for plants growing alone than in a mixture, further supporting hypothesis 2. However, as this free glucosamine was not studied for ^{15}N, we cannot say that it represents only glucosamine uptake from added FNM-CT complexes. Smaller molecular size DOC did not differ between planted and control pots but there was 15 to 17 times more dissolved N in the pots with plants compared to controls. This observation means that plant-related activity has significantly increased the release of small molecular

weight organic N compounds from soil. As there were no differences in small molecular weight DOC, it seems that plant-associated microbes preferentially target N-containing compounds in SOM, maybe including FNM-CT complexes. As the root biomass differed in the pots, the DOC and dissolved TDN data were also given per g root weight. These calculations revealed the highest values for heather, for both DOC and TDN. This is in line with the study on peatlands, where shrubification (expansion of shrubs) increased the concentration of dissolved organic compounds in the peat beneath heather (Bragazza et al., 2012).

Fungal and bacterial necromass did not differ much between pine and heather, as revealed by amino sugar analysis with muramic acid (a marker of bacteria) and glucosamine (a marker of fungi). Soil amino sugars not only reflect the microbial community at the time point of sampling but can also be used to monitor medium-to-long-term changes in the microbial community (Glaser et al., 2004). However, slight dominance of fungal residues under pine compared to heather was detected, which could be explained by the difference in ECM vs ERM hyphal abundance in soil; ECM forms widely spreading mycelium in the soil and ERM as endomycorrhizal type does not (Chodak et al., 2015; Pearson and Read, 1973). Amino sugars are also markers for microbial contribution to soil organic matter formation (Liang et al., 2019), as studies have shown that microbial residues and especially those associated with roots build up most of the stable SOM (Clemmensen et al., 2015; Liang et al., 2017). However, in our study, we did not observe differences in amino sugars comparing planted to control soil, which potentially emerged from the fact that our experiment was a short-term one with not enough time to build up a significant amount of new SOM. Also, amino sugar concentrations in organic layers are high and variable (Liang et al., 2019), and small changes would likely be difficult to detect against this large background.

Future studies should take into account also abiotic factors of interaction between ERM-shrubs and ECM-trees. Abiotic factors, like topographic factors, climatic factors and physico-chemical characteristics of soil affect plant diversity and thus their interaction (Arellano et al., 2021; Zhang et al., 2021). In addition, pH differences between sites and microsites may significantly shape fungal community structure and level of root mycorrhization (Canini et al., 2019). However, the influence of abiotic factors is not present in pot studies conducted in controlled conditions of the laboratory, like in this pot-experiment. Another limitation of our experimental design is the number of seedlings per pot (one in monocultures and two in a mixed treatment), size of the pot and the demand for nutrients of monocultures vs mixed treatment. However, taking into account that seedlings were small, size of pots was relatively big and N content of soil was relatively high, we believe that our results are representative for the interaction of pine vs shrub at the pot experiment level and not driven by nutrient and space limitations.

5. Conclusions

All in all, the interplay of pine and heather leading to decreased heather growth and changes in the decomposition of more stable FNM-CT complexes suggest that interaction between ERM and ECM plants may lead in the long term to SOM accumulation in boreal forest soils. Our study highlights also that plants partially degrade SOM during N mining: although plants could access SOM-bound N compounds and

take it up, they increased the concentrations of dissolved low molecular weight N compounds manifold but without altering low molecular weight DOC concentrations. Future studies should extrapolate ERM vs ECM interaction and its impact on soil chemistry to field scale.

CRediT authorship contribution statement

Sylwia Adamczyk: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Rashmi Shrestha:** Writing – review & editing, Formal analysis, Data curation. **Bartosz Adamczyk:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Chao Liang:** Writing – review & editing, Methodology, Data curation. **Christina Biasi:** Writing – review & editing, Investigation, Data curation. **Jussi Heinonsalo:** Writing – review & editing, Funding acquisition, Conceptualization. **Kristiina Karhu:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Bartosz Adamczyk reports financial support was provided by the Academy of Finland. Kristiina Karhu reports financial support was provided by the Academy of Finland. Jussi Heinonsalo reports financial support was provided by the Academy of Finland.

Data availability

Data will be made available on request.

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