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Soil carbon and nitrogen near alder, birch and spruce trees in four mixed Norway spruce-dominated stands

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ABSTRACT

To increase understanding of soil processes in mixed forests, we examined soil nitrogen (N) and carbon (C) near alder, birch and spruce trees in Norway spruce (*Picea abies*)-dominated stands in southern Finland. Two sites were middle-fertile stands aged 20—40 years, and the other two were fertile stands aged 60 years. Grey alder (*Alnus incana*) was present at all sites and birch (*Betula pendula* and *Betula pubescens*) was present at three sites. At each site, we determined C and N stocks of forest floor and mineral soil (0—10 cm depth) close to (max distance 1.5 m) alder, birch, and spruce trees. We found forest floor C stocks to be 0.8 kg m^{-2} higher under alder than under birch trees. Forest floor N stocks were higher near alder than near birch or spruce. Alder decreased C:N-ratio of both forest floor and mineral soil by around 5 units compared to spruce; birch decreased C:N-ratio of mineral soil. Diffusive fluxes of soil N, as determined by microdialysis method, showed no significant differences between species. At the two fertile 60-year-old stands, microbial biomass N was lower near alder than near birch, birch decreased microbial biomass C:N-ratio compared to spruce, and alder decreased C mineralisation rate and dissolved organic C concentration compared to spruce. Several other properties, such as the concentrations of monoterpenes and condensed tannins, were unexpectedly similar under alder, birch and spruce trees. This suggests the presence of complex interactions between trees in mixed forests, driven by above- and belowground processes involving litter dispersal.

1. Introduction

Boreal forest soils store a significant amount of carbon (C) on a global scale. Soil organic carbon (SOC) stocks can be influenced by forest management practices, such as selection of tree species (Vesterdal et al., 2013). Replacing monocultures with mixed-species stands can have several positive effects, including increased biodiversity and the resilience of the forest ecosystems towards pest and pathogen outbreaks leading to improved risk management in changing climate (Huuskonen et al., 2021). Tree species composition determines the quality and quantity of litter inputs and influences microclimate, soil biota, and soil organic matter (SOM) chemistry (Saetre, 1999; Vancampenhout et al., 2009). The area of young Norway spruce (*Picea abies* (L.) Karst) stands has increased in southern Finland in recent decades as spruce has been favoured for forest regeneration (Korhonen et al., 2021). It has been observed that spruce reduces soil fertility, as evidenced by a decrease in soil pH, microbial biomass C and nitrogen (N), and nutrient availability compared to broadleaved trees such as birch (Priha, 1999; Smolander and Kitunen, 2011; Augusto et al., 2015). The presence of high concentrations of certain groups of plant secondary compounds, condensed tannins and monoterpenes, in spruce litter has been shown to inhibit N cycling processes (Smolander et al., 2012). Condensed tannins have also been found to form complexes with several organic compounds, protecting them from microbial decomposition and stabilising C and N in the soil (Adamczyk et al., 2011). The recalcitrance of spruce litter, combined with microclimatic conditions and other factors, reduces the rate of organic matter decomposition, resulting in the formation of a thick C-rich forest floor under spruce. Conversely, broadleaved trees have been observed to increase the allocation of C to mineral soil (Frouz et al., 2009; Vesterdal et al., 2013).

One solution to improve soil fertility and increase C sequestration in mineral soil of spruce stands could be to introduce broadleaved tree species, especially those capable of N-fixation symbiosis, as an admixture. On one hand, C allocation to mineral soil is more favourable for

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SOC stability, as it enables the associations between SOC and reactive mineral surfaces, which is together with environmental factors considered more important for SOC persistence than the inherent chemical recalcitrance of organic matter (Schmidt et al., 2011). On the other hand, the accumulation of mineral-associated organic matter depends on soil texture and can only increase to the point of saturation of reactive mineral surfaces, whereas particulate organic matter can, in theory, accumulate indefinitely (Cotrufo et al., 2019). Studies reporting C stocks of mineral soil under different tree species in boreal forests are scarce (Vesterdal et al., 2013). On two tree species experiments on forest soils in Fennoscandia, higher SOC stocks were found under spruce monocultures compared to birch monocultures, attributed to higher forest floor C stocks, with no difference in mineral soil up to 30 cm depth (Hansson et al., 2011; Olsson et al., 2012). When it comes to mixed stands, situation is more complex; Dawud et al. (2017) found that C stocks (forest floor + 0–20 cm mineral soil) decreased with increasing conifer proportion in Finland, while the opposite was observed in the temperate zone. The development of SOC stocks under mixed conifer-broadleaved stands on diverse soil and site types remains to be fully elucidated. Birch has been shown to produce high-quality litter that promotes the biomass and activity of soil micro- and macrofauna (Priha, 1999; Saetre, 1999; Saetre et al., 1999; Smolander and Kitunen, 2011) and has a deeper rooting system compared to spruce (Laitakari, 1935). Consequently, the incorporation of birch admixture into spruce stands has the potential to enhance the allocation of C to the mineral soil. However, the effectiveness of this process depends on factors such as site type, stand age, and soil texture (Smolander and Kitunen, 2011; Olsson et al., 2012).

Grey alder (*Alnus incana* (L.) Moench) lives in root nodule symbiosis with actinomycetes of the genus *Frankia*, which are able to fix atmospheric nitrogen (N_2) into a plant-available form, ammonia (NH_3). The N content of grey alder litter is notably high, approximately 3 % of dry mass (Mikola, 1959, Morozov et al., 2019). Consequently, the annual litterfall of a pure grey alder stand has the potential to add approximately 50–100 kg ha^{-1} of N to the soil, based on studies conducted in Finland and Estonia (Mikola, 1966; Saarsalmi and Mälkönen, 1989; Aosaar., 2012). The following case studies reporting differences between alder stands, conifer stands, and alder-conifer mixtures have been conducted in the temperate zone. Red alder (*Alnus rubra* Bong.) litter migrated 8–18 m from the alder-conifer stand boundary into the conifer stand at 10–45-year-old stands, increasing total C, N, ammonium-N (NH_4-N) and nitrate-N (NO_3-N) concentrations of the topsoil (10 cm depth) of the conifer stand several metres beyond the stand boundary at the oldest site (Lavery et al., 2004). The presence of red alder admixture has been shown to increase conifer growth, soil N content, and dissolved N leaching on a low fertility site (Binkley et al., 1992). Conversely, on a high fertility site, red alder admixture decreased conifer growth and increased NO_3-N leaching in the same study. In an afforested post-mining site, spruce (a mixture of *Picea omorica* and *Picea pungens*) produced a higher C stock in the forest floor than alder (*Alnus glutinosa*), whereas alder stored more C in the organo-mineral A layer, with higher total C stocks found in alder monocultures than in spruce monocultures (Frouz et al., 2009). Additional N from fertilisation can increase soil C sequestration by increasing litter input while decreasing decomposition and C mineralisation rates (Mayer et al., 2020). Analogous mechanisms may be involved in the context of N-fixing vegetation. A greater amount of C was sequestered under N-fixers compared to non-N-fixers, due to enhanced retention of old C and the accumulation of recently fixed C (Resh et al., 2002). In Finland, grey alder was a prevalent tree species in forests following slash-and-burn cultivation in the early 20th century. However, the proportion of forests with alder admixture has decreased considerably since then (Henttonen et al., 2024). To our knowledge, there are only a couple of studies examining the effects of grey alder as an admixture in Norway spruce stands in the boreal zone, dated back to the 1930–60 s (Kalela, 1937; Mikola, 1966).

Previous studies have focused mainly on comparing soil properties of single-species stands (monocultures) of different tree species; more information is needed especially regarding C stocks under boreal tree species and their mixtures. The objective of this study was to increase understanding of soil processes within mixed Norway spruce-dominated stands, focusing on the tree species -induced spatial variation. We examined whether the alder, birch or spruce trees in spruce-dominated stands differ with regard to their effects on soil C and N, microbial biomass and activities, and organic matter quality. We analysed the diffusive fluxes of soil N using an *in situ* microdialysis sampling method, acquiring fine-scale information about plant-available N pool (Inselsbacher et al., 2011). Many applications of microdialysis in soil environments have emerged (Buckley et al., 2020), but to our knowledge, no study has investigated the effect of tree species on diffusive N fluxes. To discover the potential differences between tree species in C allocation patterns into forest floor and topmost mineral soil (0–10 cm layer) and in diffusive N fluxes, we collected samples near grey alders, birches (silver birch and downy birch) and Norway spruces in four 21—63-year-old mixed spruce-dominated stands representing two different fertility classes (middle-fertile and fertile). In addition, we analysed organic matter quality and microbial biomass and activities at the two fertile 62—63-year-old stands. We hypothesised that: (I) alder and birch increase inorganic N (NH_4-N and NO_3-N) and N mineralisation in the forest floor, whereas under spruce, N remains more in organic form; (II) additional N originating from N fixation symbiosis under alder enhances C accumulation; and (III) more C is allocated to the forest floor under spruce, whereas birch allocates more C to the mineral soil through its deeper root system.

2. Material and methods

2.1. Study sites

Our study sites were located in the boreal zone of southern Finland, two in Karkkila (MT-21 and MT-40 sites) and two in Lapinjärvi (OMT-62 and OMT-63 sites; Table 1). Sites represented two different fertility classes according to Finnish forest site type classification (Cajander, 1949); Karkkila sites represented a middle-fertile *Vaccinium myrtillus* type (MT) and Lapinjärvi sites represented a more fertile *Oxalis acetosella* – *Vaccinium myrtillus* type (OMT). All soils were podzols or developing towards podzols, according to the WRB soil classification (IUSS Working Group WRB, 2015a). Humus type was mor in MT sites and mor-moder in OMT sites. According to statistics from 1991 to 2020, the mean annual temperature was + 5°C and the mean annual precipitation was 680 mm in both Karkkila and Lapinjärvi (Jokinen et al., 2021). None of the sites was an experiment established to study tree species effects, but the history of all stands was well known. Norway spruce was the dominant tree species at all sites, with grey alder admixture at all sites and birch (both silver birch [*Betula pendula* Roth] and downy birch [*Betula pubescens* Ehrh.]) admixture at three of the sites. Spruce stands were established on clear-cuts by planting in MT sites and by natural regeneration in OMT sites; alder and birch had self-propagated from seeds or sprouts (or both) in all study sites. Birches were of approximately the same age as spruces at each site, whereas alders were of different ages, but we assumed that an alder had existed throughout the whole rotation time at the same spot and regenerated by sprouting. Before planting the spruces, the site was prepared by spot mounding in MT-21 and probably by harrowing in MT-40. In MT-21, early clearing was carried out for the first time in 2006 and for the second time in 2012 to achieve a density of 2000 stems ha^{-1}. In MT-40, the first thinning took place in 2020. In OMT-62, the first thinning was probably carried out in 1987–1990 and the second thinning in 2002–2003. In OMT-63, hold--over trees were cut in 1976–1977 and the stand was thinned in 2010.

The admixed broadleaved trees were scattered evenly within the stands, and the individual trees selected for sampling were located relatively far apart from each other within each stand. The samples were collected from the close proximity (less than 1.5 m) of each tree

Table 1
Description of the study sites. Forest site type is based on Finnish forest site type classification according to Cajander (1949). In the order of increasing fertility: *Vaccinium myrtillus* type (MT) < *Oxalis acetosella* – *Vaccinium myrtillus* type (OMT). n is the number of trees (per tree species) close to which soil and microdialysis sampling was conducted at each site.

Study site	Site ID	Coordinates	Dominant tree species	Admixed tree species	Forest site type	Stand age (years)	n
Karkkila 2	MT−21	60° 37' N, 24° 6′ E	spruce	alder	MT	21	3
Karkkila 3	MT−40	60° 37' N, 24° 5′ E	spruce	alder, birch	MT	40	8
Latokartano	OMT−62	60° 39' N, 26° 7′ E	spruce	alder, birch	OMT	62	3
Mysmalm	OMT−63	60° 37' N, 26° 6′ E	spruce	alder, birch	OMT	63	3

individual selected for sampling. Soil and microdialysis samples were taken from the OMT-62 and OMT-63 sites in June 2020; microdialysis samples were taken from MT-21 in early June 2022 and from MT-40 in August-September 2022; soil samples were taken from MT sites in September 2022. At the time of sampling, soil temperature at the interface of forest floor and the mineral soil was 9.1 ± 0.4 °C in OMT-62 and 13.6 ± 0.3 °C in OMT-63. The gravimetric moisture content of the forest floor was 60 ± 3 % of fresh weight in OMT-62 and 47 ± 4 % of fresh weight in OMT-63 (no data from MT sites).

2.2. Soil sampling and particle size distribution

Soil samples were taken from the forest floor (organic horizon) and at 0—10 cm depth from the mineral soil at all study sites using a soil corer with a diameter of 5.8 cm, or 2.0 cm for mineral soil samples from OMT-63. The thickness of the forest floor was measured from each core. In the MT sites, a soil core was taken at 20–80 cm distance from tree stem and divided into layers; each soil core was analysed separately. In OMT sites, four soil samples were taken at different distances (approx. 40, 80, 120 and 150 cm) from each tree stem, divided into layers and combined into one composite sample per layer to determine C and N stocks of forest floor and mineral soil and particle size distribution of mineral soil.

To determine the particle size distribution in the 0—10 cm mineral soil layer, soil samples were air-dried, ground, and sieved through a 2 mm mesh sieve. All samples from the MT-21 site were pooled, while samples from other sites were pooled by species. The sieving and sedimentation method (ISO 11277:2020) was used. Mineral soil particles (< 2 mm) in the 0—10 cm layer consisted of 69 % sand, 25 % silt and 6 % clay at the MT-21 site, representing a sandy loam textural class (IUSS Working Group WRB, 2015b). At the other sites, where soil texture was determined from the pooled samples collected under each species, trees of different species appeared to grow on relatively similar spots based on soil texture (Table 2). The middle-fertile MT sites were located on coarser-textured soils, whereas the fertile OMT sites were located on finer-textured soils with relatively high clay-% of 21—38.

Table 2
Particle size distribution and textural class based on IUSS Working Group WRB (2015b) in mineral soil 0—10 cm layer for the pooled samples (air-dried, sieved < 2 mm) taken under different tree species. Sand 0.06-2 mm; Silt 0.002-0.06 mm; Clay < 0.002 mm.

Site ID	Alder	Birch	Spruce
MT−40			
Sand (%)	71	62	69
Silt (%)	25	30	21
Clay (%)	4	8	10
Textural class	*Sandy loam*	*Sandy loam*	*Sandy loam*
OMT−62			
Sand (%)	34	20	36
Silt (%)	45	51	34
Clay (%)	21	29	30
Textural class	*Loam*	*Clay loam – Silt clay loam*	*Clay loam*
OMT−63			
Sand (%)	12	8	13
Silt (%)	50	59	58
Clay (%)	38	33	29
Textural class	*Silt clay loam*	*Silt clay loam*	*Silt clay loam*

2.3. Determinations of SOC and N stocks

Soil samples were air-dried (40 °C) and ground (< 2 mm) for C and N analyses by dry combustion using a CHN analyser (Leco CHN-600). The SOC and N stocks in kg m^{-2} were calculated using the Eq. 1.

$$SOC(orN) = c \times BD \times LT \times (1 - CF), \quad (1)$$

where c is the SOC (or N) content in kg C (or N) kg^{-1} dry matter, BD is the bulk density in kg m^{-3}, LT is the layer thickness in m and CF is the coarse fraction (> 2 mm mineral soil particles, m^3 m^{-3} soil). The volume of the coarse fraction (V_{CF}) in m^3 was calculated as $V_{CF} = m_{CF}/\rho_p$, where m_{CF} is the mass of the CF in kg, and ρ_p is the particle density of the CF in kg m^{-3}, assuming a ρ_p of 2650 kg m^{-3} (Heiskanen, 1992). We used a pedo-transfer function from Nilsson and Lundin (2006) to estimate the bulk density (BD) in kg m^{-3} of the mineral soil samples taken with a 2 cm corer at OMT-63 using Eq. 2.

$$BD = 1.7262 \times \exp(-0.3632 \times SOC^{0.5}) \times 1000, \quad (2)$$

where SOC is the SOC content in % dry matter.

2.4. Microdialysis sampling and microplate analyses of N compounds

We used microdialysis to determine the induced diffusive fluxes of soil N *in situ* (Inselsbacher et al., 2011). The microdialysis system set-up and sampling protocol were the same as in Soronen et al. (2024). Briefly, four CMA 20 microdialysis probes with a semi-permeable membrane (molecular weight cut-off 20 kDa, length 30 mm, diameter 0.5 mm) were inserted vertically into the soil at a depth of 1.5 cm near the soil sampling spots of each sample tree. The probes were perfused with Milli-Q water at a flow rate of 5 µl min^{-1} for 52 min to collect the dialysates. In MT sites, the microdialysis samples were collected close to the soil sampling spot by distributing the four probes in an area of approximately 20 cm × 20 cm. In OMT sites, the four microdialysis samples were collected close to the soil sampling spots at approximately 40, 80, 120 and 150 cm distance from each sample tree (see Section 2.2). Before and after each field sampling event, blank samples were prepared by immersing the probes in Milli-Q water in the laboratory and sampling as in the field.

The concentrations of NH_4-N, NO_3-N, and total free amino acid -N (AA_{tot}-N) from each dialysate were analysed using a microplate reader. Four dialysates from each sampling spot in MT-40 site were pooled for microplate analyses to save sample for the measurement of individual amino acids by liquid chromatography-mass spectrometry (LC-MS; see Section 2.5). NH_4-N was determined using a colorimetric method based on the Berthelot reaction (Hood-Nowotny et al., 2010). NO_3-N was determined using a vanadium (III) chloride (VCl_3) and Griess method as described by Inselsbacher et al. (2011), based on Miranda et al. (2001). Total free amino acid -N (AA_{tot}-N) was determined by a fluorometric amino acid method based on o-phthaldialdehyde and β-mercaptoethanol (OPAME) as described by Darrouzet-Nardi et al. (2013), based on Jones et al. (2002). Leucine was used as the standard and therefore AA_{tot}-N results should be interpreted as Leu-N.

Diffusive fluxes rates (DFR) in nmol N m^{-2} s^{-1} of different N forms were calculated using Eq. 3.

$$DFR = \frac{\frac{c \times V}{A}}{t} \quad , \tag{3}$$

where c is the concentration of the compound in nmol l^{-1}, V in the sample volume in l, A is the surface area of the membrane (4.732×10^{-5} m^2), and t is the sampling time in s.

2.5. Measurement of amino acids with LC-MS (MT-40 site)

Microdialysis samples from the MT-40 site were further analysed for individual amino acid concentrations by LC-MS. First, 100 µl of each dialysate was lyophilised to dryness and stored at −18 °C until measurement. Samples were resuspended in 10 µl of 20 mM HCl, followed by the addition of 30 µl of AccQ-Tag™ Ultra borate buffer and 10 µl of AccQ-Tag™ derivatisation reagent using a Waters AccQ-Tag™ Ultra Derivatisation kit for amino acid analysis. The total volume of derivatised sample was 50 µl. The next derivatisation steps, equipment and instrument settings were the same as in Jämtgård et al. (2018). Separation was performed by reversed-phase LC on an HP 1290 UHPLC system (Agilent Technologies), and amino acid detection was performed on an Agilent 6460 triple quadrupole mass spectrometer. Detected amino acids were glycine (Gly), alanine (Ala), gamma-aminobutyric acid (GABA), serine (Ser), proline (Pro), valine (Val), threonine (Thr), isoleucine (Ile), leucine (Leu), asparagine (Asn), aspartic acid (Asp), glutamine (Gln), glutamic acid (Glu), methionine (Met), histidine (His), phenylalanine (Phe), arginine (Arg), tyrosine (Tyr), tryptophan (Trp), ornithine (Orn), lysine (Lys), cysteine (Cys).

2.6. Determination of monoterpenes in soil air (OMT sites)

Monoterpenes were collected from the soil air at the OMT-62 and OMT-63 sites, at approximately 1 m distance from each sample tree, using a chamber method as described by Smolander et al. (2013) based on Haselmann et al. (2000). Briefly, a cylindrical stainless-steel cap (diameter 19 cm, height 12 cm, volume 3.4 l) was hammered into the soil. A sorbent sampling tube (activated carbon) was connected to the chamber and soil air was sampled by pumping through the tube at a flow rate of 0.5 l min^{-1} for 6 min. Sampling tubes were stored at −18 °C until analysis. Monoterpenes were extracted from the sampling tubes with dichloromethane and measured using an Agilent 7890B gas chromatograph equipped with a DB-5MS capillary column (length 30 m x ID 0.25 mm x film 0.25 µm) and an Agilent 5977 A mass spectrometer. The temperature at the MS interface was 230°C. Compound identification was based on authentic reference compounds. Quantitative analyses were performed using internal standard 1-chlorodecane and external standard humulene.

2.7. Determinations of organic matter quality and microbial biomass and activities (OMT sites)

At the OMT-62 and OMT-63 sites, the forest floor was additionally sampled with the same cylindrical cap used for monoterpene sampling (see Section 2.6). Samples were stored at 4 °C and sieved through a 6 mm mesh sieve one to three days after sampling. Soil pH was measured from a soil-water suspension (15 ml of fresh soil: 25 ml of Milli-Q water). Dry matter and organic matter contents were determined by thermogravimetric analysis (TGA).

Net N mineralisation and net nitrification were measured in a 4-week aerobic laboratory incubation experiment (14 °C, 60 % water holding capacity) as described by Soronen et al. (2024). NH_4-N and NO_3-N concentrations were measured from non-incubated and incubated samples after extraction with 1 M KCl. C mineralisation rate was estimated as the mean CO_2-C production rate measured three times during incubation; bottles were sealed with gas-tight septa 24–28 h before each measurement using an Agilent 8890 gas chromatograph system with a thermal conductivity detector. The concentrations of microbial biomass C (MBC) and microbial biomass N (MBN) were determined by the fumigation-extraction method as described by Törmänen et al. (2018), based on the method of Vance et al. (1987), using the conversion formulae of Martikainen and Palojärvi (1990). Dissolved organic C (DOC) and dissolved N concentrations were determined from the concentrations of C and N in 0.5 M K_2SO_4-extracts of the non-fumigated samples.

Part of the sample was air-dried and ground (< 0.5 mm) to determine the concentration of condensed tannins as described by Smolander et al. (2005), based on the acid-butanol assay (Porter et al., 1985; Terrill et al., 1992). We used the previous standard curves prepared from extracted and purified condensed tannins from silver birch leaves and Norway spruce needles described by Adamczyk et al. (2011). Condensed tannins were measured from the samples using a Shimadzu UV-2401PC UV–VIS recording spectrophotometer.

2.8. Statistical analyses

We tested data for statistical differences in C and N stocks and C:N-ratios of forest floor and mineral soil and N fluxes near alder, birch and spruce trees based on a multivariate general linear model (GLM) with the following formula: soil properties ~ species + fertility + site + age, where soil properties represent the multivariate response, species (alder, birch, spruce) is the main covariate, and fertility (middle-fertile MT, fertile OMT), site, and age of the stand (as continuous covariate) are (fixed) nuisance covariates. A separate test was run for the differences in other forest floor properties measured only from the OMT sites (see Sections 2.6–2.7); for those properties, we ran a multivariate two-way ANOVA using the following model: soil properties ~ species + site, where soil properties represent the response variables, species (alder, birch, spruce) is the main factor and site is a (fixed) nuisance factor. Function 'graph.flm' from the R package GET was used to create graphical outputs of the test results, showing the pairwise comparisons (Myllymäki et al., 2017; Mrkvička et al., 2021; Myllymäki and Mrkvička, 2024). The function performs permutations using the Freedman-Lane procedure (Freedman and Lane, 1983), considered as one of the best strategies for permutation inference of linear models in the literature (Winkler et al., 2014). We accounted for multiple testing of several soil properties simultaneously using correction with false discovery rate control (Mrkvička and Myllymäki, 2023). R (R for Windows, version 4.4.0) was used for statistical analyses and data visualisation (R Core Team, 2024).

3. Results

3.1. C and N stocks and diffusive N fluxes

Forest floor C stock was approximately 0.8 kg m^{-2} higher near alder than near birch trees in the mixed Norway spruce -dominated stands (Fig. 1a). Forest floor N stock was higher near alders than near birches or spruces (Fig. 1b). Alder decreased forest floor C:N-ratio by about five units compared to spruce (Fig. 1c). The mineral soil C and N stocks did not differ significantly between species (Fig. 1: d-e), but both broad-leaved species decreased mineral soil C:N-ratio compared to spruce (Fig. 1f). We did not observe any significant differences between species in the diffusive fluxes of soil N (Fig. 1: g-i). The effect of the type of distribution and variation of the soil property on the power to detect significant differences by the statistical test applied is demonstrated with a simulation study (Supplementary material: Chapter 1, Supplementary Figs. 1–4).

Forest floor C concentration was highest near alder trees at all sites and forest floor was thinnest near birch trees at all three sites where birch was present (Supplementary Table 1), resulting in the higher forest floor C stocks observed near alder *versus* birch at both site types (Fig. 2a). Similar to N stocks, forest floor N concentration was highest near alder at all sites (Fig. 2b, Supplementary Table 1). Mineral soil C and N

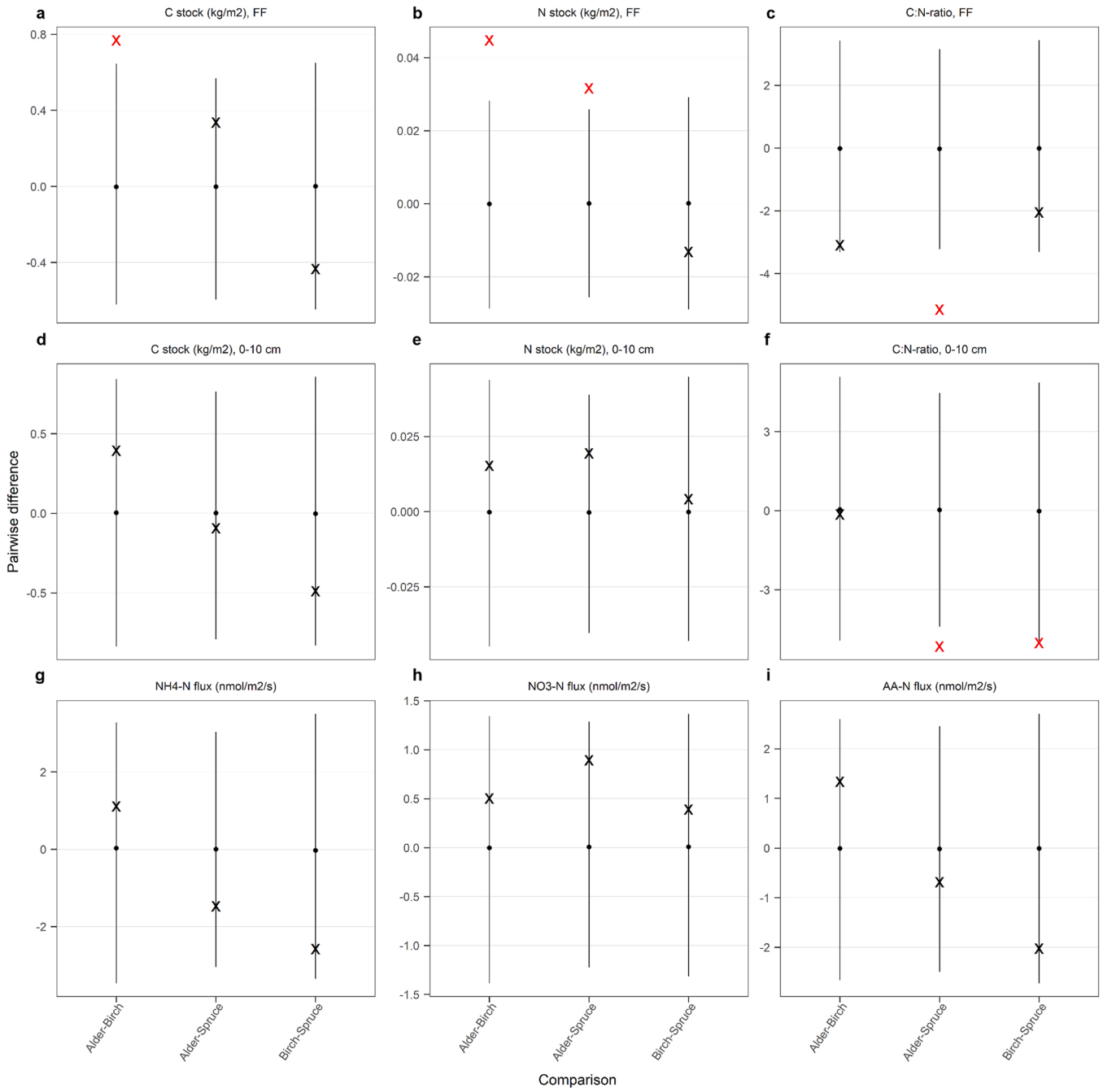

Fig. 1. Results of the statistical test (multivariate GLM: soil properties ~ species + fertility + site + age) for the null hypotheses that there are no differences in the properties between soils near alder, birch and spruce trees. Panels show the pairwise differences between tree species in (a) C and (b) N stocks and (c) C:N-ratio of the forest floor (FF), (d) C and (e) N stocks and (f) C:N-ratio of mineral soil (0–10 cm depth), and the fluxes of (g) ammonium-N (NH4-N), (h) nitrate-N (NO3-N) and (i) the total amino acid -N (AA-N). Data is from four mixed Norway spruce -dominated stands: MT-21, MT-40, OMT-62, and OMT-63 (n = 17 for alder and spruce, n = 14 for birch). The crosses show the observed pairwise differences between the species, and the whiskers represent the acceptance region of the test. Pairwise difference is significant if the cross is outside the acceptance region, marked with red colour.

concentrations were not consistently affected by tree species (Supplementary Table 2), and mineral soil C and N stocks exhibited high variation (Fig. 2: a-b). It must be noted that charcoal was found in mineral soil samples collected near six spruces but only near two alders and two birches in MT-40 site; this finding probably explains why spruce had on average highest C concentrations and stocks in mineral soil at this site (Supplementary Table 2, Fig. 2a). Of all soil properties, the most consistent tree species effects were observed in C:N-ratios of both forest floor and mineral soil; spruce had highest C:N-ratios in both layers and at all sites (Fig. 2c, Supplementary Tables 3,4). Lowest C:N-ratios were observed near alder, with the only exception being mineral soil in MT-40, where birch had on average lowest C:N-ratio.

As mentioned above, the diffusive fluxes of soil N showed high variation and no significant differences between species (Fig. 3). The total N flux (sum of NH_4-N, NO_3-N and AA_{tot}-N) was on average 7.0 $\pm$ 1.0 nmol m^{-2} s^{-1}, different N forms contributing on average 55 $\pm$ 5 % (AA_{tot}-N), 31 $\pm$ 4 % (NH_4-N), and 14 $\pm$ 3 % (NO_3-N) to the total flux.

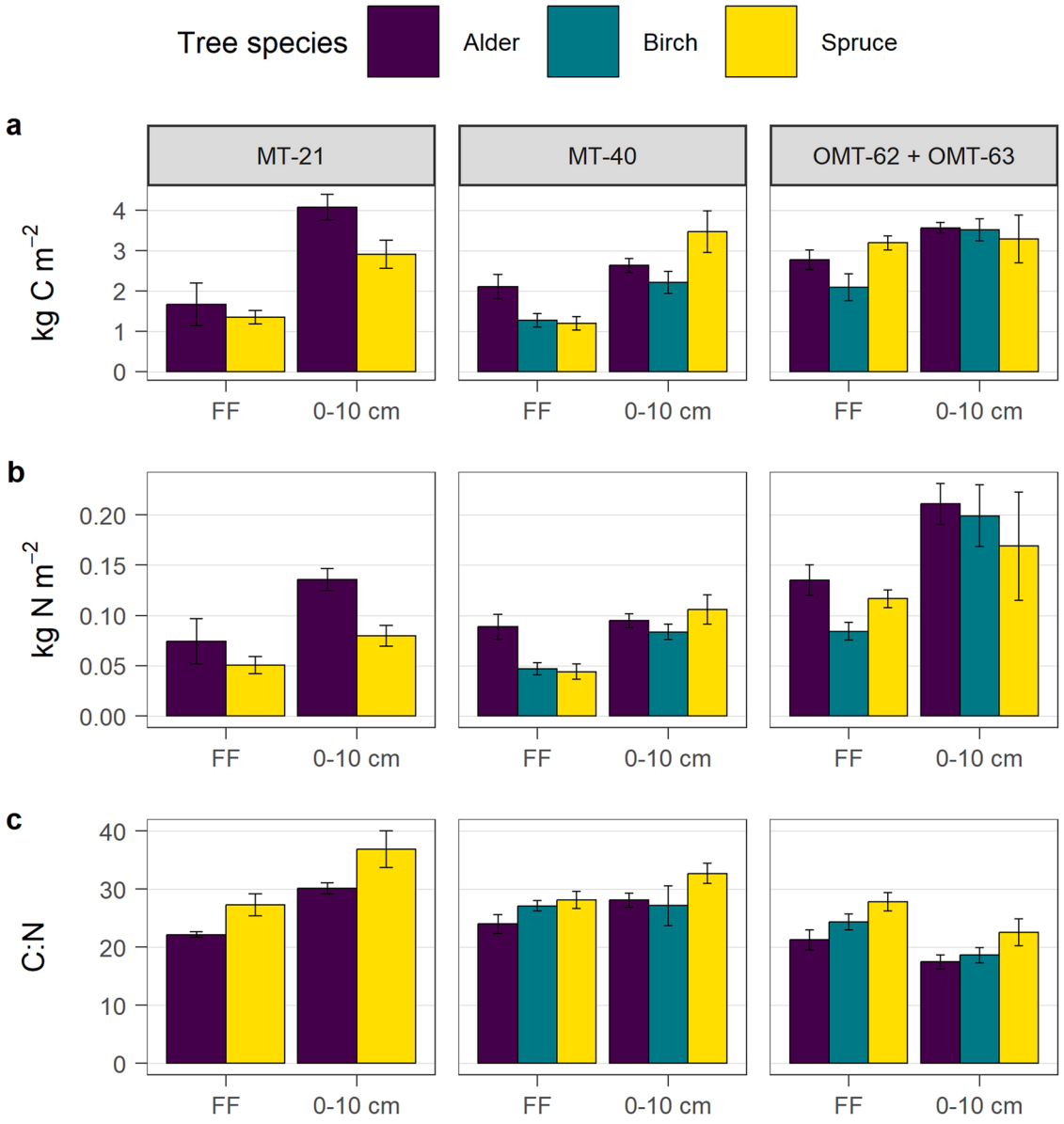

Fig. 2. (a) C stocks and (b) N stocks, and (c) C:N-ratios of the forest floor (FF) and the mineral soil 0–10 cm layer (0–10 cm) near alder, birch, and spruce trees. Bar represents mean ± SE. Data is from four mixed Norway spruce-dominated stands presented in the order of increasing fertility and age: middle-fertile 21-year-old stand (MT-21; n = 3), middle-fertile 40-year-old stand (MT-40; n = 8), and the mean values from two fertile 62—63-year-old stands (OMT-62 + OMT-63; n = 6).

3.2. Amino acid composition of the diffusive flux (MT-40 site)

The amino acid composition or the fluxes of individual amino acids, determined by LC-MS analysis from the microdialysis samples taken from the MT-40 site, did not show marked differences between tree species (Fig. 4, Supplementary Fig. 5). The dominant amino acids and their mean proportions of the AA_{sum}-N flux were Gln (28 %), Glu (20 %), Asp (12 %), Asn (11 %), Gly (7 %), Ala (6 %), Arg (5 %), and Ser (4 %).

3.3. Organic matter quality and microbial biomass and activities (OMT sites)

Additional forest floor characteristics reflecting organic matter quality, measured only from the fertile OMT-62 and OMT-63 sites, showed some statistically significant differences between tree species (Figs. 5 and 6). MBN was smaller near alder trees than near birch trees (Fig. 5h). Birches decreased the C:N-ratio of microbial biomass by about one unit compared to spruces (Fig. 5i). Alders decreased C mineralisation rate and DOC concentration compared to spruces (Fig. 5: j-k). Similarly, birches seemed to decrease DOC concentration, but the difference to spruce was not statistically significant (Fig. 6k). Net nitrification rates seemed to be higher under alders than under birches or spruces, but the differences were not statistically significant, and nitrification rate was generally low (Fig. 5d, Fig. 6d). Site-specific results regarding organic matter quality and microbial activities are presented in Supplementary material (Supplementary Tables 5–7).

The efficiency of the microbial population to mineralise forest floor C was on average highest under spruce, whereas the efficiency to mineralise N was highest under alder; lowest mineralisation efficiencies for both C and N were observed under birch (Table 3). The shares of MBC of total C and MBN of total N were on average highest under birch and lowest under alder.

The sum of monoterpene concentrations in soil air did not differ significantly between tree species due to the large variation (Fig. 7). Monoterpene composition was relatively similar under all tree species, dominated by α-pinene (55 ± 3 %), β-pinene (22 ± 2 %), and camphene

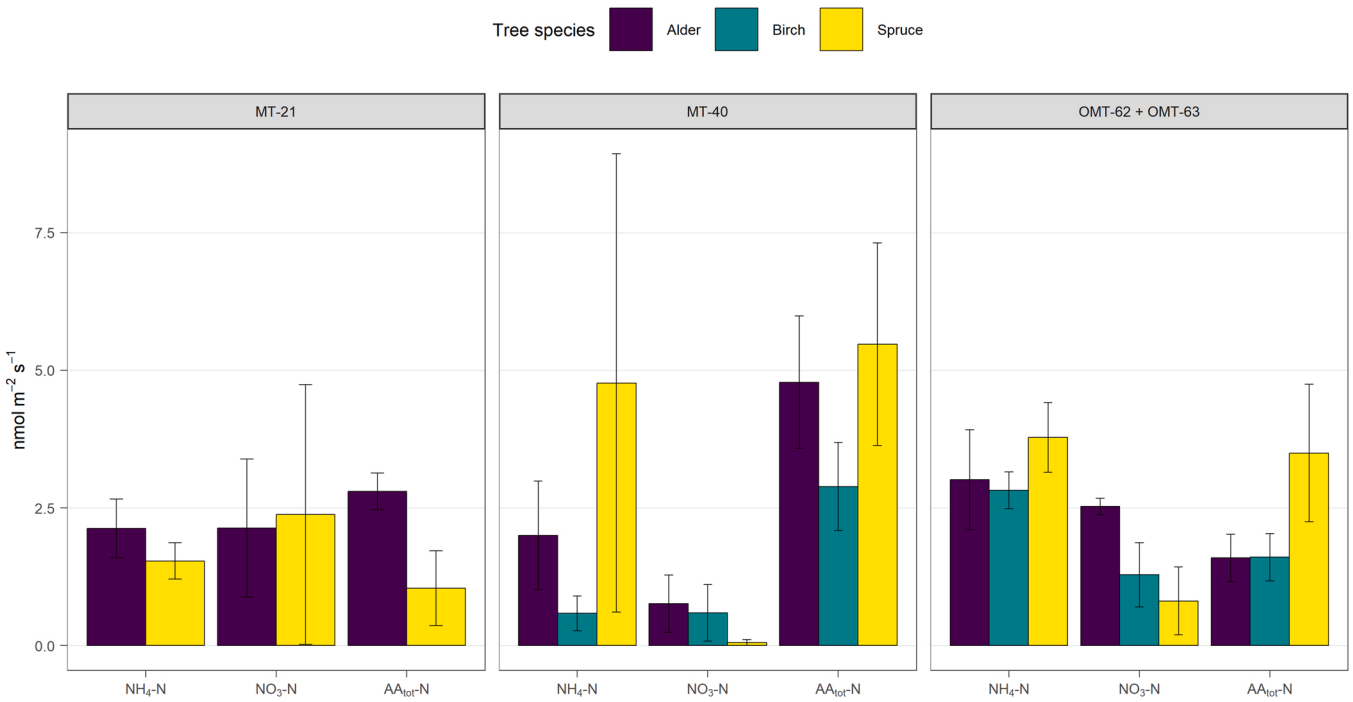

Fig. 3. Diffusive flux rates of ammonium-N (NH_4-N), nitrate-N (NO_3-N) and the total amino acid -N (AA_{tot}-N) near alder, birch, and spruce trees, sampled with microdialysis technique *in situ* and measured with a microplate reader in the laboratory. Bar represents mean ± SE. Data is from four mixed Norway spruce-dominated stands presented in the order of increasing fertility and age: middle-fertile 21-year-old stand (MT-21; n = 3), middle-fertile 40-year-old stand (MT-40; n = 8), and the mean values from two fertile 62—63-year-old stands (OMT-62 + OMT-63; n = 6).

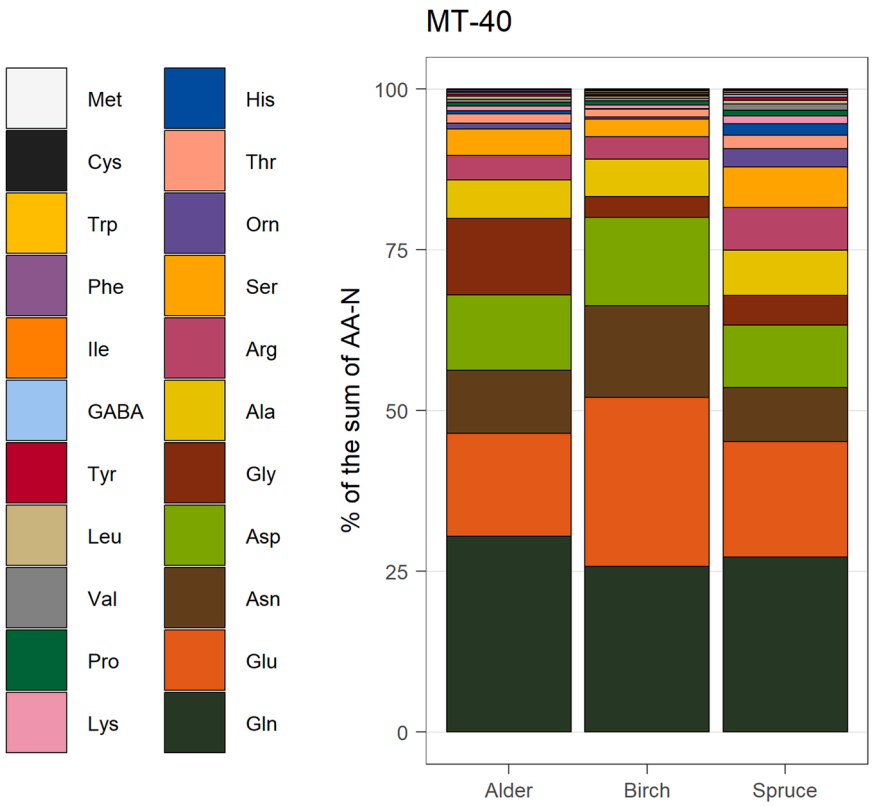

Fig. 4. Amino acid (AA-N) composition of the diffusive flux near alder, birch, and spruce trees, sampled with microdialysis technique *in situ* and measured with liquid chromatography – mass spectrometry (LC-MS) in the laboratory. Data is from a middle-fertile 40-yr-old mixed Norway spruce -dominated stand, MT-40 (n = 8 per tree species). Stacked bars represent mean percentages of 22 different amino acids, marked with different colours. Met = methionine; Cys = cysteine; Trp = tryptophan; Phe = phenylalanine; Ile = isoleucine; GABA = gamma-aminobutyric acid; Tyr = tyrosine; Leu = leucine; Val = valine; Pro = proline; Lys = lysine; His = histidine; Thr = threonine; Orn = ornithine; Ser = serine; Arg = arginine; Ala = alanine; Gly = glycine; Asp = aspartic acid; Asn = asparagine; Glu = glutamic acid; Gln = glutamine.

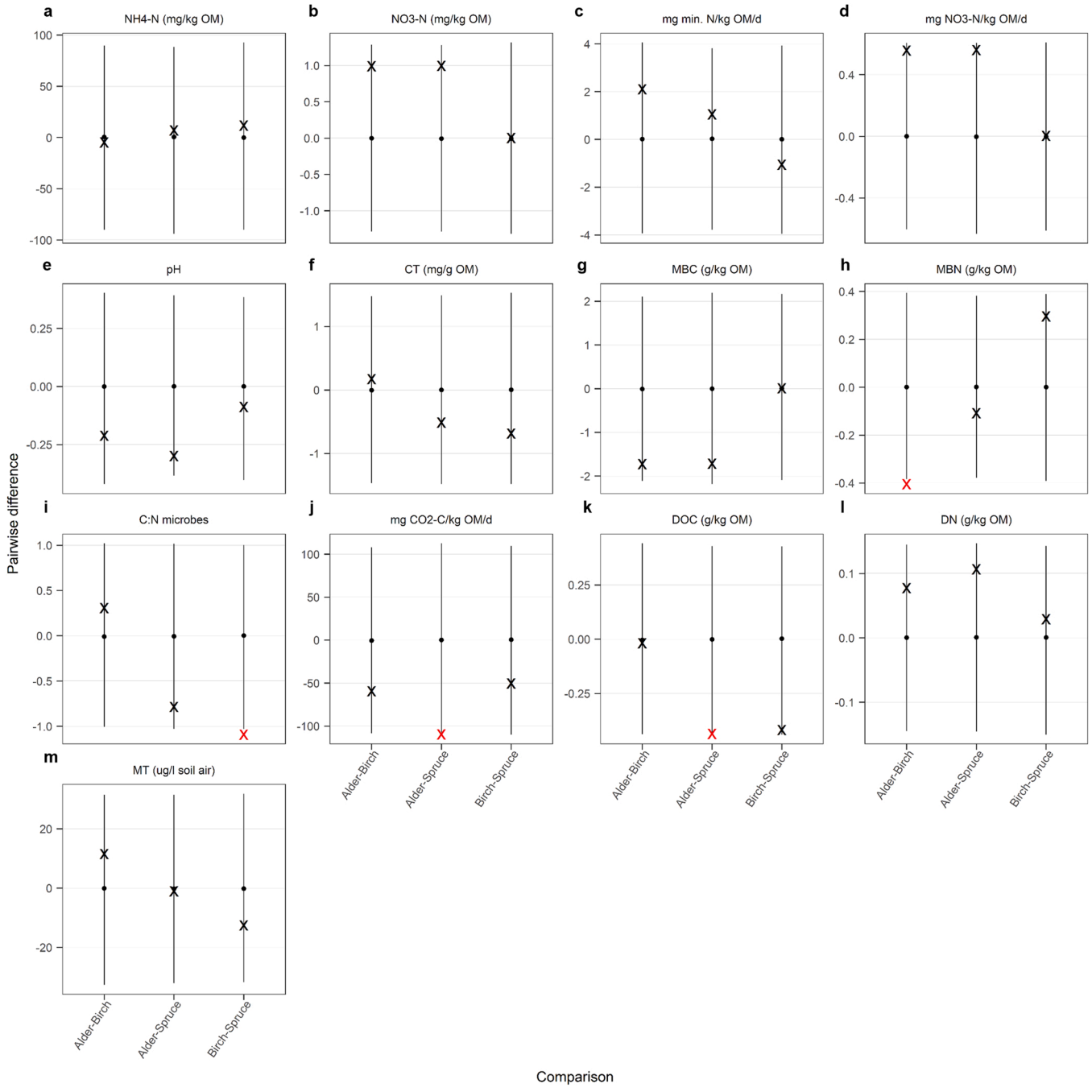

Fig. 5. Results of the statistical test (multivariate two-way ANOVA: soil properties ~ species + site) for the null hypotheses that there are no differences in the forest floor properties near alder, birch and spruce trees. Panels show the pairwise differences between tree species in the concentrations of (a) ammonium (NH4)-N and (b) nitrate (NO3)-N, measured from 1 M KCl – soil extracts, the rates of (c) net N mineralisation and (d) net nitrification, (e) pH, (f) concentrations of condensed tannins (CT), microbial biomass (g) C (MBC) and (h) N (MBN), (i) C:N-ratio of microbial biomass, (j) C mineralisation rate, concentrations of (k) dissolved organic C (DOC) and (l) dissolved N (DN), and (m) the concentrations of monoterpenes (MT) in soil air. Data is from two fertile mixed 62—63-yr-old Norway spruce -dominated stands, OMT-62 and OMT-63 (n = 6 per species). There was one missing value for monoterpenes of one spruce tree at OMT-63 – we replaced the missing value with the mean value of the other two spruces in OMT-63. The crosses show the observed pairwise differences between the species, and the whiskers represent the acceptance region of the test. Pairwise difference is significant if the cross is outside the acceptance region, marked with red colour. OM = organic matter; min. = mineral.

(11 ± 1 %).

4. Discussion

We examined the effects of grey alder, birch (both silver birch and downy birch), and spruce trees on the spatial variation of soil properties, focusing on SOC and N stocks and N availability, in Norway spruce -dominated stands. Several variables reflecting N availability (N fluxes and concentrations, N transformation processes) were not significantly affected by tree species in our study. Alder decreased C:N-ratios of both forest floor and mineral soil compared to spruce. Similarly, birch decreased mineral soil C:N-ratio. Thus, an increase of soil inorganic N availability in the proximity of broadleaved trees in spruce-dominated mixed stands (hypothesis I) was not supported by our results, but an

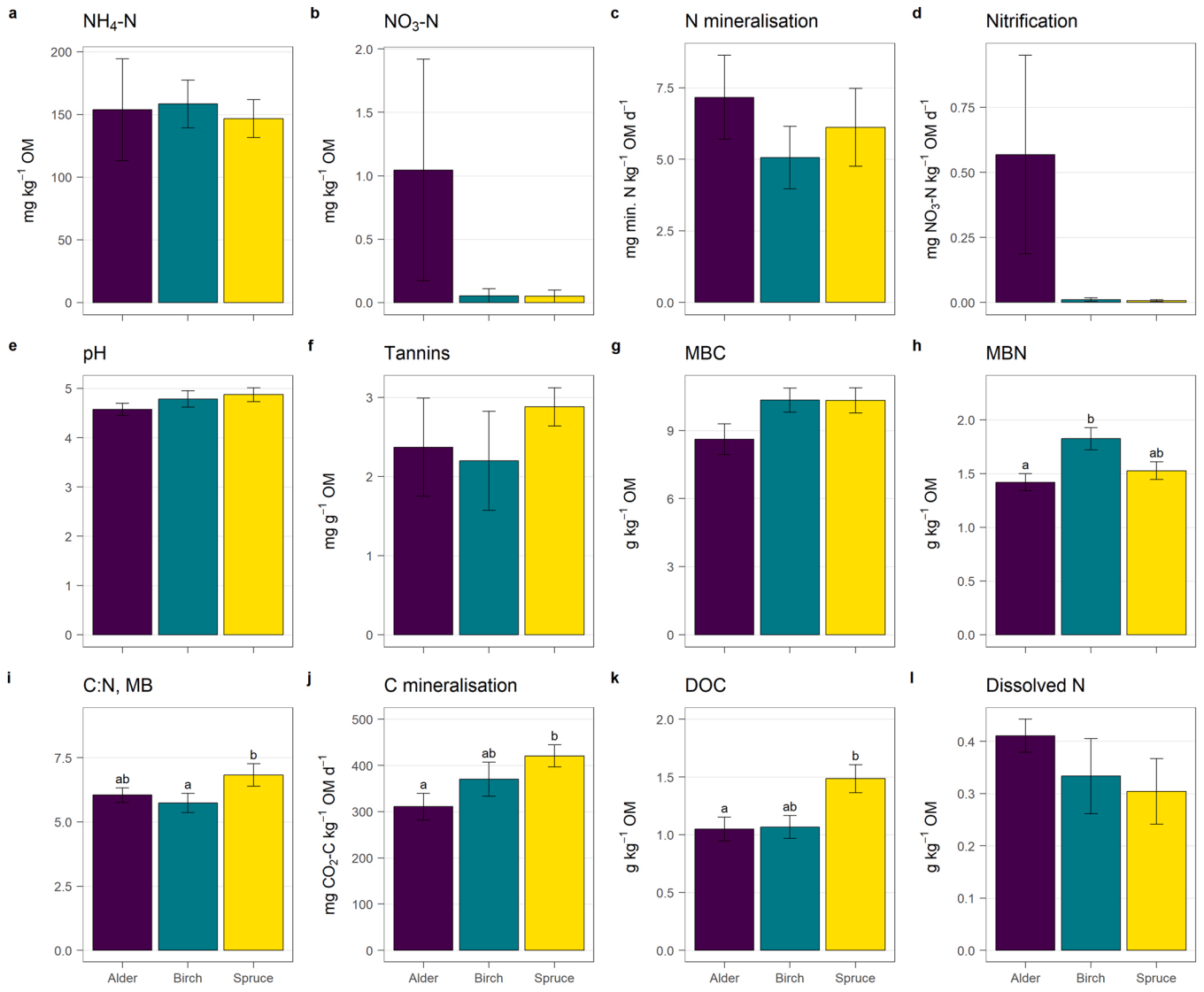

Fig. 6. The concentrations of (a) ammonium (NH_4)-N and (b) nitrate (NO_3)-N, measured from 1 M KCl – soil extracts, the rates of (c) net N mineralisation and (d) net nitrification, (e) pH, (f) concentrations of condensed tannins, concentrations of microbial biomass (g) C (MBC) and (h) N (MBN), (i) C:N-ratio of microbial biomass (MB), (j) C mineralisation rate, and the concentrations of (k) dissolved organic C (DOC) and (l) dissolved N in the forest floor near alder, birch and spruce trees. Bar represents mean ± SE. Data is from two fertile mixed 62—63-yr-old Norway spruce -dominated stands, OMT-62 and OMT-63 (n = 6 per tree species). OM = organic matter. Significant differences between tree species are denoted with different letters (no letters = no significant differences), based on the results of the statistical test presented in Fig. 5.

Table 3
Forest floor C and N mineralisation rates, expressed as per microbial biomass C (MBC) and N (MBN), and the shares of MBC and MBN of the total C and N contents near alder, birch, and spruce trees. Data is from two fertile 62—63-yr-old mixed Norway spruce -dominated stands, OMT-62 and OMT-63 (n = 6). min. N = mineral N.

	C mineralisation (mg CO_2-C g^{-1} MBC d^{-1})		Net N mineralisation (mg min. N g^{-1} MBN d^{-1})		Microbial biomass C (% of total C content)		Microbial biomass N (% of total N content)	
Tree species	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alder	36.2	5.0	5.0	2.2	1.7	0.3	6.1	1.2
Birch	35.5	4.2	2.7	1.2	2.4	0.7	10.0	2.7
Spruce	40.8	3.4	3.9	1.8	2.2	0.3	8.9	0.9

increase in overall N availability was observed near broadleaves, reflected as decreased C:N-ratios. We found higher forest floor C stocks near alder than near birch trees and higher forest floor N stocks near alder than near birch or spruce. However, broadleaves did not significantly alter C stocks of forest floor or mineral soil compared to spruce. Additional analyses of forest floor from two of our study sites (fertile, 60 yr) revealed that MBN was lower under alder *versus* birch, birch decreased microbial biomass C:N-ratio compared to spruce, and alder decreased C mineralisation rate and DOC concentration compared to spruce. Thus, soil N was elevated and C mineralisation rate lower close to alder, but against our expectation (hypothesis II), extra N under alder did not increase C stock in relation to spruce. We expected different C allocation patterns between birches and spruces (hypothesis III), but we found no significant differences in C stocks of forest floor or mineral soil between these species in our study sites.

The finding that grey alder increased forest floor N stocks and decreased the C:N-ratios compared to spruce was expected due to the N-fixation symbiosis of alder; this is in agreement with previous studies looking at the effects of mixture of red alder and conifers (Binkley et al., 1992) or spreading of grey alder litter in conifer stands (Mikola, 1966). Comparing the effects of alder and birch, our study agrees with the

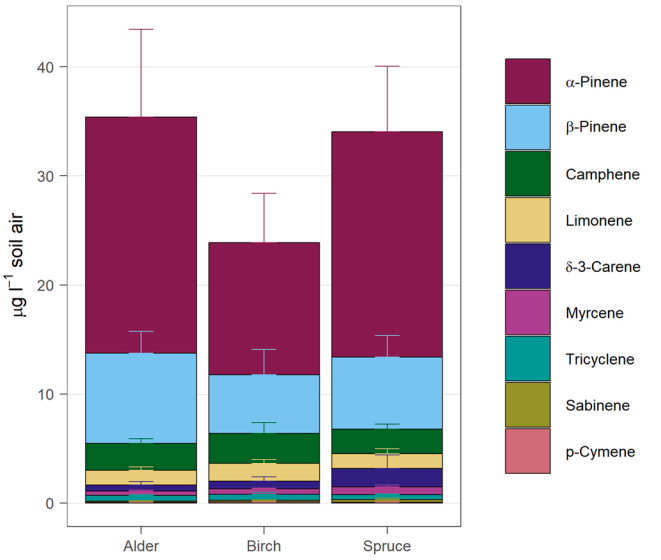

Fig. 7. Concentrations of monoterpenes in soil air in the forest floor near alder, birch, and spruce trees. Bar represents mean ± SE, different compounds marked with different colours. Data is from two fertile mixed 62—63-yr-old Norway spruce -dominated stands, OMT-62 and OMT-63 (n = 6). No significant differences between tree species were observed in total monoterpene concentrations, based on the results of the statistical test presented in Fig. 5.

findings of Mikola (1966); soil N content of coniferous stands increased particularly under alder litter after spreading alder or birch litter on the soil.

The differences between tree species in C and N dynamics or rooting depths may provide explanations for the higher C stocks in the forest floor near alder than near birch trees in our study. The N fixation symbiosis of alder increases N inputs through litter and may decrease decomposition of organic matter for N acquisition. Morozov et al. (2019) found twice as high leaf litter N input of a young grey alder stand compared to that of a young silver birch stand due to the higher N content of alder leaf litter. We found lower C mineralisation rate and DOC concentration near alders than near spruces in our fertile mixed spruce-dominated sites. We can only speculate whether the reduced C mineralisation rate was related to the additional N, to the lower efficiency of the microbial population to mineralise C, to potential differences in DOC chemistry or all of these factors combined. Leaf litter of both alder and birch are considered labile, providing easily degradable organic matter for soil fauna and microbes, whereas spruce litter contains more recalcitrant compounds. The forest floor of birch stands is typically thinner and contains less C than that of spruce stands (Hansson et al., 2011, Olsson et al., 2012). Besides faster decomposition rate of birch litter, forest floor C stock may be affected by the differences in rooting depth; alder and spruce have rather superficial rooting systems (Kalela, 1937), whereas birch roots grow deeper into mineral soil (Laitakari, 1935). Higher forest floor C stock under alder than under birch in our study sites can thus be the result of several processes: enhanced C accumulation due to reduced C mineralisation under alder, i.e., faster C turnover under birch, and differences in root C allocation to different layers. More information about C inputs (above- and belowground litter production), outputs (mineralisation, leaching), stabilisation and allocation into deeper soil layers under different tree species would be needed.

To our knowledge, microdialysis-based diffusive fluxes of soil N have not been compared between the tree species included in our study before. Due to the N-fixation symbiosis of alder and based on previous studies determining inorganic N concentrations and net N mineralisation with soil extractions and laboratory incubations (Mikola, 1966, Smolander and Kitunen, 2011), we expected higher inorganic N fluxes and lower AA_{tot}-N fluxes (due to faster N mineralisation) under broadleaves *vs.* spruce. Diffusive fluxes of NH_4-N, NO_3-N, or AA_{tot}-N did not differ between the study sites or tree species despite the fertility gradient in our study sites, which is probably related to the dynamic nature of the fluxes, exhibiting high spatial variation. This is consistent with the results of Oyewole et al. (2017); they found no difference in the diffusive fluxes between a nutrient-poor Scots pine stand and a nutrient-rich spruce stand. Our study sites represented two fertility groups; two study sites represented the MT forest site type, the most common type of spruce stands in southern Finland (Korhonen et al., 2021), and the other two represented a more fertile OMT type. It is possible that the mass flow flux would have revealed differences between site types or tree species in our study, as Oyewole et al. (2017) observed that when also mass flow flux was considered, the difference between the study sites became clear, mainly due to the higher mass flow fluxes of NO_3-N in the spruce stand. The results on the amino acid composition of the diffusive flux in MT-40 site agree with the study by Werdin-Pfisterer et al. (2009); Glu, Gln, Asp, Asn, Ala and His dominated the soil water-extractable amino acid pool in a successional gradient from willow to black spruce -dominated forests in Alaska, USA. All these amino acids except His were predominant in our samples; amino acid composition was rather similar under different tree species. Diffusive fluxes are spatially and temporally highly variable; the proportions of different compound groups of the flux can change over the growing season (Randewig et al., 2019), and thus, single sampling time from each of our study sites represents only a snapshot of the dynamics of N fluxes.

Previous studies conducted on single-species plots of tree species experiments have shown that birch increases soil pH and microbial biomass C and N and decreases soil C:N-ratios and concentrations of volatile monoterpenes and condensed tannins in relation to spruce (Priha, 1999; Smolander and Kitunen, 2011, 2021). However, tree species effects in mixed-species plots cannot always be directly predicted from the relative proportions of different tree species based on the values measured in single-species plots (Smolander and Kitunen, 2021). Within a mixed spruce-birch stand, Saetre et al. (1999) found a moderately but significantly higher MBC and C mineralisation rate under birch than under spruce. Furthermore, in a case study by Schua et al. (2015), individual birch trees increased forest floor pH, MBN content and basal respiration of a spruce stand. We observed significant differences between birch and spruce in C:N-ratio of both mineral soil and microbial biomass, which were lower under birch compared to spruce, suggesting higher N availability and a shift in microbial community composition. Several soil properties often clearly differing between birch and spruce monocultures did not differ between birch or spruce trees within the mixed stands in our study.

Monoterpenes and condensed tannins are groups of compounds of particular interest due to the marked differences observed between tree species and their effects on soil C and N processes (Smolander et al., 2012; Adamczyk et al., 2019; Smolander and Kitunen, 2021). Concentrations of these compounds have been observed to differ significantly between spruce and birch plots, with several times higher concentrations observed in the forest floor of spruce monocultures compared to birch monocultures. It has also been observed that grey alder leaf litter has a relatively low concentration of condensed tannins (Ikonen et al., 2002) and that monoterpene emissions from grey alder, as compared to birch, are also low (Hakola et al., 1999). To our knowledge, forest floor condensed tannin concentrations or monoterpene emissions have not been compared between grey alder and Norway spruce before, but we expected that these compounds would be enriched under spruce. However, we did not find any differences in these compound groups under the influence of different tree species in the mixed stands. Soil organic matter chemistry is affected by both coniferous and broadleaved vegetation in mixed stands, which is reflected as diluted concentrations of compounds typically enriched in forest soils under pure broadleaved or pure coniferous stands (Vancampenhout et al., 2009). We speculate that the similar concentrations of soil air monoterpenes and forest floor

condensed tannins under different tree species in our study may be the result of dispersal of both above- and belowground litter from different tree species across the stand, diluting the differences between species. Another possibility is differences in rates of degradation of the compounds by soil microbial populations adapted to the tree species. Similarities in ground vegetation under different tree species in mixed stands may also mitigate tree species effects. The effect of birch or alder on soil is not necessarily similar when growing together with other tree species in mixed stands, as compared to a situation where tree species is growing alone in monoculture; this is the case also with the effect of spruce.

Vesterdal et al. (2013) have pointed out the limitation of the approach we used; estimating tree species effects in mixed stands, where the mixed species occur naturally, has its disadvantages because we do not know if the soil properties were originally different in the spots where alder or birch occurred. How much this contributed to our results – compared to the effect of the tree species on soil properties - is unknown. However, soil textural analysis did not reveal any major differences in soil particle size distribution between the spots within sites where trees of different species occurred. Moreover, the lack of tree species experiments old enough established on forest soils, with mixed plots of the species under scrutiny, limits the ability to study mixed forests. Given the potential benefits of mixed forests for biodiversity, forest resilience, and C sequestration, we emphasise the need to establish new tree species experiments with mixed-species plots.

5. Conclusions

Our study demonstrates that individual broadleaved trees can significantly change some of the properties of forest floor and topmost mineral soil (0–10 cm depth) within mixed Norway spruce -dominated stands. Data from our four study sites showed that broadleaved trees, especially alder, can significantly increase N availability by decreasing C:N-ratios in the forest floor and mineral soil. However, the optimal density of broadleaved trees requires further investigation. We found that more C was stored near alder trees than near birch trees, potentially related to the differences in SOM quality and rooting depths. Tree species -induced changes in SOM quality and microbial biomass and activity were evidenced by decreased DOC concentration and C mineralisation near alders and altered microbial biomass properties between tree species. Tree species effects were mainly restricted to relatively stable soil properties, such as C:N-ratios and C and N stocks, whereas the more dynamic properties showed high variation and spatial heterogeneity in mixed stands. Our study highlights the complex interactions between tree species and brings new information about tree species -induced variation within mixed stands, particularly about topsoil C stocks under boreal tree species.

CRediT authorship contribution statement

Päivi Soronen: Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Aino Smolander:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Mari Myllymäki:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Sandra Jämtgård:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.123224.

Data availability

Data will be made available on request.

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