

RESEARCH ARTICLE

Ericaceous dwarf shrubs contribute a significant but drought-sensitive fraction of soil respiration in a boreal pine forest

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Abstract

1. Boreal forests often have a dense understorey of ericaceous dwarf shrubs with ecological adaptations that contrast those of the canopy-forming trees. It is therefore important to quantify contributions by understorey shrubs to ecosystem processes and disentangle shrub- and tree-driven responses to climatic factors.
2. We quantified soil respiration driven by the pine canopy and the ericaceous shrub understorey over 3 years, using a factorial pine root exclusion and shrub removal experiment in a mature *Pinus sylvestris* forest. Soil temperature and moisture-related responses of respiration attributed to autotrophs (shrubs, pine roots) and heterotrophs were compared. Additionally, we assessed effects of interactions between these functional groups on soil nitrogen availability and respiration.
3. Understorey shrubs accounted for $22\% \pm 10\%$ of total autotrophic respiration, reflecting the ericaceous proportion of fine root production in the ecosystem. Heterotrophic respiration constituted about half of total soil respiration. Shrub-driven respiration was more susceptible to drought than heterotrophic- and pine-driven respiration. While the respiration attributed to canopy and understorey remained additive, indicating no competitive release, the plant guilds competed for soil N.
4. *Synthesis.* Ericaceous understorey shrubs accounted for a small, yet significant, share of total growing season soil respiration. Overlooking understorey respiration may lead to erroneous partitioning and modelling of soil respiration mediated by functional guilds with contrasting responses to soil temperature and moisture. A larger contribution by heterotrophs and pine root-associated organisms to soil respiration under drought conditions could have important implications for soil organic matter accumulation and decomposition as the climate changes in boreal forests.

KEYWORDS

autotrophic respiration, ericaceous dwarf shrubs, Gadgil effect, mycorrhiza, nitrogen availability, *Pinus sylvestris*, root trenching, understorey

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1 | INTRODUCTION

Much of the boreal forest is a continuous expanse of ectomycorrhizal trees and ericoid mycorrhizal understorey shrubs (Romell, 1938, Lahti & Väisänen, 1987, Read et al., 2004, Kron & Lutyen, 2005), yet the potentially contrasting and interactive effects of these functional groups on below-ground processes are often overlooked. Multiple, global-scale analyses indicate that higher latitude ecosystems dominated by ectomycorrhizal trees accumulate more soil organic matter than lower latitude arbuscular mycorrhizal ecosystems (Averill et al., 2014; Read, 1991; Soudzilovskaia et al., 2019; Steidinger et al., 2019). However, within high latitude systems, there are multiple studies pointing to an increase in soil organic matter stocks along gradients from ectomycorrhizal to ericoid mycorrhizal dominance (Clemmensen et al., 2013, 2021; Friggens et al., 2020; Hartley et al., 2012; Read, 1991; Ward et al., 2021). In much of the boreal forest, ericaceous dwarf shrubs and trees coexist and have spatially overlapping root systems. Ericaceous roots are located largely in the organic horizon (mor layer; Persson, 1983) and their hair roots are typically colonized by a range of ascomycetous fungi including ericoid mycorrhizal species (Kohout et al., 2011; Lindahl et al., 2007). Tree fine roots are more extensively distributed across soil horizons, but typically with highest density at the interface between mineral and organic layers (Persson, 1983; Rosling et al., 2003). The ectomycorrhizal fungi associated with tree roots are primarily basidiomycetes with prominent mycelia extending into the surrounding soil (Rosling et al., 2003). Trees and ericaceous shrubs with their root-associated fungal communities have contrasting ecological niches, and appear to affect soil C and N dynamics in contrasting ways on both small and large scales (Clemmensen et al., 2015; Sietiö et al., 2018; Ward et al., 2021).

Soil respiration is the most important biological process that leads to losses of soil organic carbon from terrestrial ecosystems, and consequently, knowledge of how soil respiration is partitioned into autotrophic and heterotrophic components is vital to understand soil respiration dynamics and ecosystem carbon budgets (Chapin III et al., 2009; Högberg et al., 2001). An autotrophic component in this case, is defined as the respiration that directly depends on plant carbon allocation to roots, mycorrhizal fungi and other closely associated organisms. Heterotrophic respiration is defined as the respiration that is independent of recent below-ground plant allocation, and instead depends on the decomposition of organic matter by free-living organisms. In forests, plant-driven contributions to soil respiration have been estimated in experiments where the below-ground carbon flow was interrupted by stem girdling or root exclusion (i.e. trenching; Subke et al., 2006). One girdling study in a boreal forest demonstrated a decrease in soil respiration of 54% (within 1–2 months) with a steep decrease just 5 days after below-ground inputs were disrupted (Högberg et al., 2001). A meta-analysis of 25 independent experiments in boreal coniferous forests found, on average, higher heterotrophic (65%) than autotrophic (35%) contributions to soil respiration (Subke et al., 2006). However, in all these

studies, understorey shrubs were removed (see Bond-Lamberty et al., 2004; Haynes & Gower, 1995; Vogel et al., 2005), absent or ignored (Lavigne et al., 2003; O'Connell et al., 2003), despite their generally high abundance in boreal forests (Kron & Lutyen, 2005; Nilsson & Wardle, 2005; Romell, 1938).

Only a few studies have estimated contributions of ericaceous dwarf shrubs to soil respiration, and they have provided a wide range of results from almost no effect (Friggens et al., 2020; Kritzler et al., 2016) to contributions between 8% and 55% (Kopittke et al., 2013; Ryhti et al., 2021). Unaccounted respiration by dwarf shrubs could lead to an overestimation of the heterotrophic contribution in tree root exclusion studies, especially in boreal ecosystems where net primary productivity of the understorey may be comparable to trees (Nilsson & Wardle, 2005; Wardle et al., 2012). Furthermore, soil respiration mediated by saprotrophs, pine roots and ericaceous dwarf shrubs may elicit different responses to variation in soil moisture and temperature, since they occupy different parts of the soil profile (Lindahl et al., 2007; Persson, 1983), and harbour fungi with different ecological strategies (Clemmensen et al., 2015; Sietiö et al., 2018).

Nitrogen limitation is the primary constraint on plant growth in boreal forests (Tamm, 1991) and both ecto- and ericoid mycorrhizal symbioses have likely evolved to overcome low nutrient availability caused by retention of nutrients in the organic topsoil (Read & Perez-Moreno, 2003). Ericoid mycorrhizal fungi have been found to mobilize N from organic sources such as ¹⁵N-enriched ectomycorrhizal fungal necromass, both in laboratory microcosms (Kerley & Read, 1997) and after canopy girdling, indicating that relaxed competition for N benefitted the shrubs (Bhupinderpal Singh et al., 2003). More recently, it has been recognized that some boreal ectomycorrhizal fungi may be even more efficient in accessing organic N sources than ericoid mycorrhizal fungi, through their production of oxidative enzymes and Fenton reaction mechanisms (Bödeker et al., 2014; Lindahl & Tunlid, 2015; Rineau et al., 2012). Heterotrophic contributions however tend to be over-estimated in root exclusion and girdling experiments, as saprotrophic growth, decomposition and respiration are stimulated by the flush of recently cut mycorrhizal roots and mycelium (Comstedt et al., 2011; Hanson et al., 2000; Lindahl et al., 2010; Savage et al., 2018). A 'Gadgil effect' could additionally increase the activity of free-living decomposers because of a competitive release when ectomycorrhizal roots are excluded (Berg & Lindberg, 1980; Fernandez & Kennedy, 2016; Gadgil & Gadgil, 1971; Sterkenburg et al., 2018). Such a competitive release could be linked to increased soil moisture (Comstedt et al., 2011; Koide & Wu, 2003) and/or soil N availability (Fernandez & Kennedy, 2016). Thus, both free-living decomposers and mycorrhizal fungal guilds may experience a competitive release if N availability is increased after pine root exclusion or shrub removal.

Multi-year, in situ estimates of the contribution of understorey ericaceous dwarf shrubs to soil respiration in forested ecosystems, contextualized with soil N availability and microclimate dependencies, are still lacking. We therefore conducted a factorial root

exclusion and dwarf shrub removal experiment to assess the contributions and interactions among three respiration sources: pine roots, dwarf shrubs and heterotrophs, to total soil respiration over three growing seasons in an old-growth boreal pine forest. We hypothesized that dwarf shrubs and pine roots contributed to autotrophic respiration in proportion to their relative fine root production at the same site; 30% and 70% for shrubs and trees, respectively (Persson, 1983). Second, we hypothesized that excluding a plant guild would result in increased root-associated respiration and N uptake by the remaining guild, indicating a competitive release. Third, we expected that heterotrophic respiration would contribute, on average, <50% of soil respiration when also accounting for the understorey. Furthermore, we explored how respiration attributed to each of the three sources, that is, heterotrophs, tree and shrub-associated communities, responded to variation in soil moisture and temperature. Disturbed control plots were used to monitor treatment side effects and ectomycorrhizal root and mycelial re-establishment.

2 | MATERIALS AND METHODS

2.1 | Mature Scots pine forest

A 0.5-hectare study site was established in a well-documented forest in central Sweden, Jädraås, Ivantjärnsheden V (60°48'56.6"N, 16°30'43.2"E) at 185 m above mean sea level (Persson, 1983; Figure S1a). The forest of *Pinus sylvestris* L. (393 trees ha⁻¹) regenerated naturally after charcoal and tar burning in the 1850s and was thinned in 1962 (Bråkenhielm & Persson, 1980), resulting in a homogenous stand of evenly aged and distributed trees. The understorey (65% ground cover) consists mainly of the ericaceous dwarf shrubs *Vaccinium vitis-idaea* L. and *Calluna vulgaris* (L.) Hull, with a lower cover of *Empetrum nigrum* L. and *Vaccinium myrtillus* L. (Bråkenhielm & Persson, 1980). The bottom layer of mosses (c. 90% ground cover) and lichens (<10% ground cover) is mainly comprised of *Pleurozium schreberi* (Bridel) Mitten and *Cladonia rangiferina* (L.) F. H. Wigg. For this study, we avoided areas dominated by lichens. No grasses or herbs, but a few small spruces, were present in the study site. Earlier, 313 ± 15 gm⁻² of pine roots, 158 ± 11 gm⁻² of *Calluna* and 158 ± 6 *Vaccinium vitis-idaea* roots <10 mm in diameter were observed across mineral and organic horizons down to 30 cm in the same site (Persson, 1983). The soil profile consists of a glacial fluvial sandy podzol with a 10–15 cm thick mor layer (pH 3.0), and a pale eluvial horizon followed by a rust-red illuvial horizon of the mineral soil (pH 4.4–4.8; Bringmark, 1980). The bedrock is composed of older granites, sediments and volcanic rocks, which are widespread in Fennoscandia. The mean annual air temperature during the study period (2017–2019) was 4.8°C with an average daily maximum of 24.4°C in July 2018 and a minimum of -20.7°C in January 2017. The mean growing season precipitation was 300 mm, with 335, 227 and 364 mm in the three study years respectively. The approximate duration of snow cover is from late November to late April. The growing season, defined as the period

during which the daily average temperature was above 5°C for five consecutive days, typically lasts from the beginning of May to mid-October. In our study this accounted for 150, 161 and 171 days in 2017, 2018 and 2019 respectively.

2.2 | Experimental design

At the end of November 2016 during a period of light frost, we initiated a fully factorial, randomized-block experiment to distinguish respiration fluxes driven by dwarf shrubs, pine trees and free-living decomposers (Wardle & Zackrisson, 2005). The experiment included factorial combinations of pine root exclusion by trenching and ericaceous shrub removal. Eight replicate blocks with five treatment plots in each (1.2 m × 1.2 m, spaced by 5–10 m) were set up, giving a total of 40 plots (Figure S1). The five treatments encompassed a control (representing the sum of all soil respiration fluxes), shrub removal, pine root exclusion, combined pine root exclusion and shrub removal (presented as 'heterotrophic' respiration), and a disturbed control in which pine roots and associated mycorrhizal mycelium were severed but allowed to re-establish in the plot from the edges. The shrub removal was initiated in November 2016 and any re-sprouting shoots were removed regularly during the following growing seasons. This was done by gently pulling out rhizomes attached to above-ground shoots in the plots and in a 20 cm zone outside untrenched plots to inhibit any horizontal ingrowth from dwarf shrubs. Most rhizomes could be removed initially, without major disturbance in the lightly frozen organic soil, however, deeper rhizomes were not removed. The initially removed shrub biomass was split into species and further cut into above- and below-ground components at the uppermost lateral root and dried. The pine root exclusion was achieved by inserting open-ended, steel boxes with sharpened lower edges to 70 cm below the surface around each plot, using a backhoe to push the boxes into place with minimal disturbance incurred. Given the even tree distribution at the site, several trees were within 10 m of all plots, suggesting that several pine root systems overlapped in all plots. The efficacy of the barriers and potential side effects were monitored using a 'disturbed control' by cutting to 30 cm depth around the plots with a spade well below the densest part of the rooting zone in the organic mor layer (Persson, 1983). No permanent barrier was left in place in the disturbed control, enabling the tracking of re-establishment of roots and mycelium. Thus, we would expect to see similar respiration in the disturbed control as in the pine root exclusion plots directly after the treatments, but convergence to the control levels as roots and ectomycorrhizal mycelium re-established. Any large side effects caused by decomposition of severed roots and mycelium should result in respiration levels in the disturbed control that are elevated above the control levels. All plots without barriers were surrounded by boardwalks with an inner size of 1.4 × 1.4 m and height of 25 cm in May 2018 to minimize impact of trampling. Only the inner 1 × 1 m of each plot was used for sampling to minimize any edge effects.

2.3 | Soil respiration measurements

Soil respiration was measured at 3–5-week intervals (eight to nine times) throughout the growing season, over three consecutive years after the field manipulations had been implemented. Measurements were made with a portable, infrared gas analyser coupled to a 1296 cm³ dark chamber in a closed air circuit (EgM-4 with SRC-1 probe type; PP Systems). The chamber was pressed firmly to the soil surface and into the moss layer between any vascular plants in the same 10 cm diameter sampling area on each sampling occasion. This procedure ensured a tight seal and minimal below-ground disturbance with the intention that the measurements should reflect the plot-level treatments. Respiration was monitored between 11:00 and 18:00 CET once for 120 s per plot and calculated as a function of linear increases in CO₂ concentration in the chamber. The sampling was alternated block-wise each monitoring day to minimize temporal sampling bias. Measurement quality was ensured by automatic baseline calibration by the 'auto-zero' option at least every 20 min and by keeping the start CO₂ concentration in the chamber at ambient level (Parker et al., 2020). No measurements were made under high wind speeds, and a subset of plots was re-measured to ensure reproducibility during the same day. Cumulative growing season respiration was estimated by multiplying the average respiration rate of two adjacent measurements by the time passed, and these values were summed to obtain cumulative values for each growing season (Bhupinderpal Singh et al., 2003). This integration procedure assumes negligible effect of the shorter-term fluctuations in respiration, for example, those linked to short-term temperature and moisture fluctuations, on the overall average for each period.

2.4 | Soil moisture, temperature and nitrogen availability

Hourly measurements of soil volumetric water content (VWC) and temperature were monitored with a EM50 data logger (METER Group) in each block and Decagon 5TM sensors placed at 5 cm depth next to the respiration sampling area in each plot. Loggers were in place in blocks 2, 5 and 7 from December 2016, in blocks 3 and 8 from the middle of June 2017, and in the remaining three blocks from the middle of August 2017. Data were manually checked, and errors were removed, based on technical advice from the manufacturer.

We used ion-membrane exchange strips to capture changes in soil nitrogen availability. Blank anion and cation membrane sheets (Maltz Sales) were cut into 2 × 3 cm strips and charged in 0.5 M NaHCO₃ with a minimum of 10 ml solution per strip for 1 h. In May 2017, four charged strips (two anion, two cation) were inserted at approximately 5–10 cm depth in each plot. In July, the strips were collected and replaced with a new set, incubated until September. In 2018, two similar sets were incubated from July to September and from September to November. Upon collection, each set of four strips from a plot was pooled, rinsed in deionized H₂O and transferred to a 50 ml centrifugation tube. The adsorbed N was

extracted by vertical shaking for 4 h in 36 ml of 2 M KCl (Shaw & DeForest, 2013). Extracts were frozen until analysed for NH₄-N and NO₃-N using a flow injection analyser (FIAstar TM, FOSS Tecator). Values below the detection limit (0.02 mg NH₄-N or NO₃-NL⁻¹) were treated as zero values.

We also sampled four to five healthy, current-year *V. vitis-idaea* leaves per plot in October 2017 as an integrative indicator of N availability (Bhupinderpal Singh et al., 2003). When present, re-sprouted shoots in the shrub removals were included in the analysis. The leaves were dried (70°C, 24 h) and ground to a fine powder in a ball mill. Leaf C and N concentrations and isotopic ¹⁵N:¹⁴N ratios were analysed on an isotope ratio mass spectrometer (IRMS; Isoprime, Micromass-GV Instruments) coupled to a Eurovector CN elemental analyser.

2.5 | Verification of treatment efficacy

To verify that the shrub removal and tree root exclusion efficiently terminated below-ground autotrophic carbon flows, we incubated humus-filled mesh bags to monitor ericaceous root and ectomycorrhizal mycelial production in all plots over 17 months. Since ectomycorrhizal fungi depend on host trees for carbon, they should be sensitive indicators of below-ground carbon allocation. Mesh bags (2 × 8 cm) were constructed of 50 μm-mesh allowing the ingrowth of fungal mycelia and ericaceous fine roots, and filled with homogenized, oven-dried (40°C) humus from the organic layer. Bags were incubated at 5–10 cm depth of each plot from June 2018 until the end of November 2019. Upon collection, the 40 bags were kept intact at –20°C until the content was freeze-dried and ball milled. DNA was extracted from 50 mg of organic material using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany). Three samples from the original humus material were included to assess background levels of DNA. ITS2 rDNA markers were amplified by 18–30 cycles of duplicate polymerase chain reactions using the gITS7 and ITS4 primers elongated with sample-unique identification tags (Castaño et al., 2020; Clemmensen et al., 2016; Ihrmark et al., 2012). These primers were designed to target fungi, but they also match all four of the ericaceous plant species in our system. Adaptor ligation and sequencing on a Pacific Biosciences Sequel I platform were performed by SciLifeLab (NGI). Sequences shorter than 150 bp and with a mean quality score lower than 20, individual bases with a quality score lower than 10, or missing 3' or 5' tags were removed. Sequences were screened for primers by requiring a minimum match of 90%, and reverse complemented if necessary. This quality filtering removed 57.3% of the total sequences and an additional 10% unique genotypes were removed to reduce the incidence of sequencing errors. To obtain species level clusters, remaining sequences were run through pairwise comparisons with USEARCH (Edgar, 2010) followed by single linkage clustering with the minimum similarity to the closest neighbour set at 98.5% (Köljalg et al., 2013). In total, 349 species level clusters (hereafter species) were formed, based on the total of 60,974 sequences passing quality control. The most

abundant sequence from each species was selected as a representative and identified by BLAST comparisons with the UNITE (Kõljalg et al., 2013) and NCBI databases. Species occurring in more than 1% of any sample were manually annotated for ectomycorrhizal status. Dual ectomycorrhizal and ericoid mycorrhizal fungi were not included because of their uncertain association with dwarf shrubs or pine roots. After removal of non-fungal sequences (<3% of all sequences), the summed relative abundance of ectomycorrhizal fungi out of all fungal sequences was calculated for each sample. Plant species (2.5% of all sequences) were almost entirely composed of the four ericaceous shrubs present at our site, and their relative abundance was calculated as a percentage out of all sequences per sample.

2.6 | Statistical analyses

The soil respiration, integrated across each growing season, was tested for the effects of pine root presence, shrub presence and year using a linear mixed effects model in the NLME package in R (Pinheiro et al., 2019). Pine root and shrub presence were included as binary fixed factors. The random effect of plot, nested within block, accounted for the spatial dependency of the randomized block design. A log transformation was needed to obtain homoscedasticity of respiration data. We tested whether there was an interaction effect between pine root and shrub presence, with reduced respiration of either component in the presence of the other, which would be indicative of antagonistic suppression. The effects of year, pine roots and shrubs on growing season averages of soil moisture and temperature were tested separately with the same model setup.

The monthly soil respiration measurements were tested for effects of pine root presence, shrub presence, soil moisture, temperature and their interactions, with linear mixed effects models. Again, pine root and shrub presence were included as binary, fixed factors. Soil moisture and temperature were included in this model to account for differences in environmental conditions across plots and time. For this, soil moisture and temperature measurements were averaged over 4 days preceding each respiration measurement per plot to account for a lagged transfer of photosynthates to manifest (Ekblad et al., 2005). To obtain data corresponding to all respiration measurements, missing moisture and temperature plot level data in 2017 were gap-filled with treatment averages for each date, as moisture clearly depended on treatment. We regard this the best solution to avoid disregarding collected respiration data in the model. To account for potential dependencies across space and time, an error correlation matrix with decimal date was specified, and plot, nested by block, was included as a random effect. The final model excluded non-significant ($p > 0.2$) three- and four-way interaction terms by stepwise removal of the least significant term. Linear mixed models were also used to test the effects of pine roots, shrubs and year on the soil moisture and temperature measurements averaged over the 4 days preceding each respiration measurement (excluding gap-filled data).

The response of respiration to moisture and temperature was visualized for each respiration component using the GAMM4 package in R (Wood, 2017) based on data from the second and third study years. In these years, we assumed a negligible disturbance-related decomposition. Pine-associated respiration was calculated as the difference in respiration between control and pine root exclusion plots, while shrub-associated respiration was the difference in respiration between control and shrub removal plots (all calculated per block). Heterotrophic respiration was assessed as respiration in plots with pine roots excluded and shrubs removed. Mosses were not removed from the experiment and were assumed to have stable contributions in all treatments; thus, they were included in the heterotrophic respiration component and cancelled out in the estimate of pine- and shrub-associated respiration responses to soil moisture and temperature. Standardization rescaled the data to have a mean of zero and a standard deviation of one to visualize differences in environmental responses among functional groups. Soil moisture and temperature data were averaged across the eight control plots per time point to reflect the ambient conditions of the site as a basis for shrub and pine root-associated 'gamm4' visualizations.

3 | RESULTS

3.1 | Verifying pine root and shrub removals

The biomass of ericaceous shrubs removed during the initial set-up of the experiment amounted to $144 \pm 10 \text{ gm}^{-2}$ above-ground and $47 \pm 7 \text{ gm}^{-2}$ below-ground, with *Vaccinium vitis-idaea* contributing 60%, *Calluna vulgaris* 23%, *V. myrtillus* 9% and *Empetrum nigrum* 8% above-ground. After the 17 months of incubation, the ingrowth bags designed to capture new shrub root and ectomycorrhizal growth showed that the shrub removal efficiently reduced relative abundance of ericaceous DNA from $6\% \pm 2\%$ in the control to $0.5\% \pm 0.3\%$ after shrub removal ($p < 0.001$; Figure S2). Root trenching reduced the relative abundance of ectomycorrhizal fungal DNA in the ingrowth bags from $24\% \pm 4\%$ in the control to $0.6\% \pm 0.2\%$ after trenching ($p < 0.001$; Figure S2).

3.2 | Contributions by shrubs, pine roots and saprotrophs to soil respiration

The soil respiration integrated over each growing season was overall highest in the first year and lowest in the third year (Figure 1; Table 1). Pine root exclusion significantly reduced growing season soil respiration and shrub removal tended to lower soil respiration, but this depended on the year. Over the 3 years, we estimated that heterotrophic respiration (in combined pine root exclusion and shrub removal plots) contributed $53\% \pm 5\%$ of the total soil respiration in control plots (Figure 1; Table 1). Pine root-associated respiration (the difference between controls and pine root exclusions) contributed 41%–45% and shrub-associated respiration (the difference between

FIGURE 1 Soil respiration integrated across each growing season (g C m^{-2}) for control plots or for plots from which understorey shrubs, pine roots or both had been removed. Here, we used a linear mixed effects model to test the fixed and interactive effects of year, pine root and shrub presence on soil respiration, including plot nested within block as random factors ($df = 82$). Insignificant ($p > 0.2$) three-way interaction were removed. Bars are averages ± 1 standard error ($n = 8$)

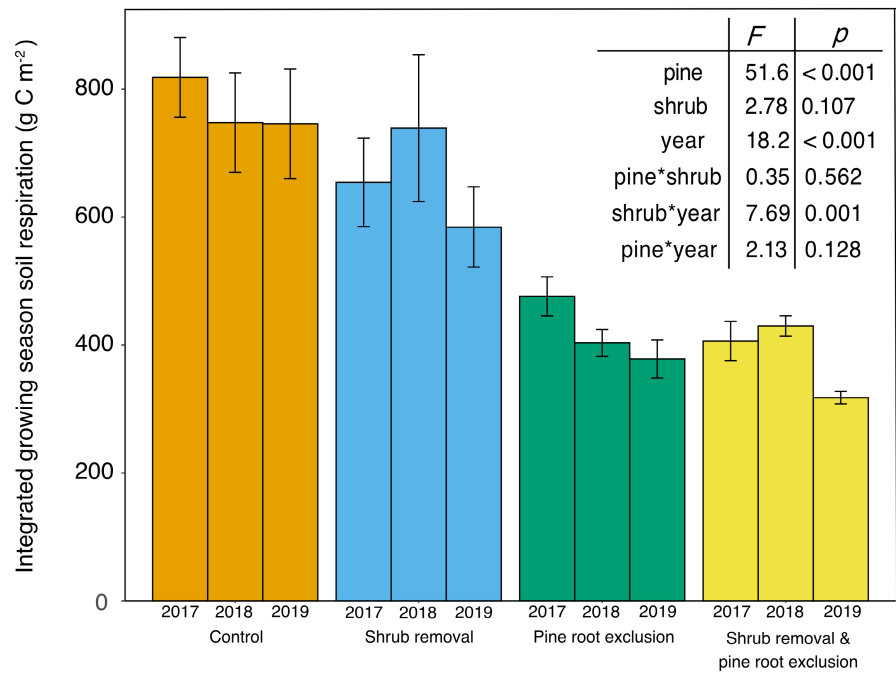


TABLE 1 Soil respiration attributed to shrubs, pine roots and saprotrophs in (a) absolute and (b) relative terms, as well as (c) the relative contributions of shrub and pine root guilds to autotrophic respiration, integrated over each growing season. Heterotrophic respiration is assessed as respiration after combined pine root exclusion and shrub removal. Pine-associated respiration is estimated as the difference between control and pine root exclusion plots, while shrub-associated respiration is estimated as the difference between the control and shrub removals. Average contributions to soil respiration ($\pm SE$) for each year are calculated across blocks ($n = 8$) while the average ($\pm SE$) across the 3 years is also given ($n = 3$)

(a) Soil respiration (g C m^{-2})	Shrub associated	Pine associated	Heterotrophic
2017	164 \pm 84	342 \pm 49	406 \pm 31
2018	8.5 \pm 140	344 \pm 86	429 \pm 16
2019	162 \pm 113	367 \pm 92	317 \pm 10
Average	111 \pm 51	351 \pm 8	384 \pm 59
(b) Soil respiration (% of control)	Shrub associated	Pine associated	Heterotrophic
2017	17 \pm 9	41 \pm 3	51 \pm 5
2018	0 \pm 19 ^a	42 \pm 6	63 \pm 8
2019	15 \pm 11	45 \pm 7	46 \pm 5
Average	11 \pm 9	43 \pm 1	53 \pm 5
(c) Guild respiration (% of total autotrophic)	Shrub associated	Pine associated	
2017	31	68	
2018	2	98	
2019	31	69	
Average	22 \pm 10	78 \pm 10	

^aThe shrub removals in some cases had higher respiration than their corresponding controls, so the average shrub-associated respiration was set to 0%.

controls and shrub removals) accounted for 0% to 17% of the total growing season soil respiration (Table 1). Thus, pine roots and shrubs contributed $78\% \pm 10\%$ and $22\% \pm 10\%$ of the total root-associated (i.e. autotrophic) respiration respectively (Table 1). There was no significant interactive effect of pine and shrubs on soil respiration (Figure 1).

3.3 | Variation in soil temperature and moisture

The daily average soil temperature at 5 cm depth ranged between -4.1 and 21.4°C over the 3 years, with an overall mean of 6.2°C (Figure 2c). The average growing season soil temperature varied among years ($p < 0.001$) with the highest average of 12.6°C in the

second year compared to 10.8 and 11.5°C for the first and third years respectively (Figure S3; Table S1). There was no effect of treatments on temperature (Figure 2c; Figure S3; Table S1).

Daily average soil moisture ranged from 1.9% to 29% (volumetric water content) with a mean of 13% over the 3 years (Figure 2b). The average growing season soil moisture was higher in the third year than previous years, and in 2018 (the second year) soil moisture was significantly higher in the combined pine root exclusion and shrub removal compared to the controls (Figure S3; Table S1). Living pine roots also decreased moisture levels across individual growing season point measurements, but the combined pine root exclusion and shrub removal increased soil water content disproportionately to single guild removals, resulting in a significant interaction term (Figure 2b; Table 2b). Shrubs had no main effect on soil moisture, but shrub removal (in the presence of pine roots) even seemed to reduce soil moisture levels in the second and third years (Figure 2b).

3.4 | Functional group responses to soil moisture and temperature

The effects of pine roots and shrubs on soil respiration were tested in a model that also included soil temperature and moisture as predictors across all measurements (Figure 2a). Soil temperature correlated positively, while soil moisture correlated negatively, with soil respiration (Table 2a). Pine roots increased soil respiration, and more so under warmer conditions, resulting in a synergistic interaction between pine presence and temperature. Shrubs did not affect respiration significantly but instead tended to interact negatively with temperature (Table 2a). There was no significant interaction between pine roots and shrubs on soil respiration (Table 2a), although the effects of both plant guilds on respiration tended to depend on the interaction between soil moisture and temperature (see three-way interactions in Table 2a).

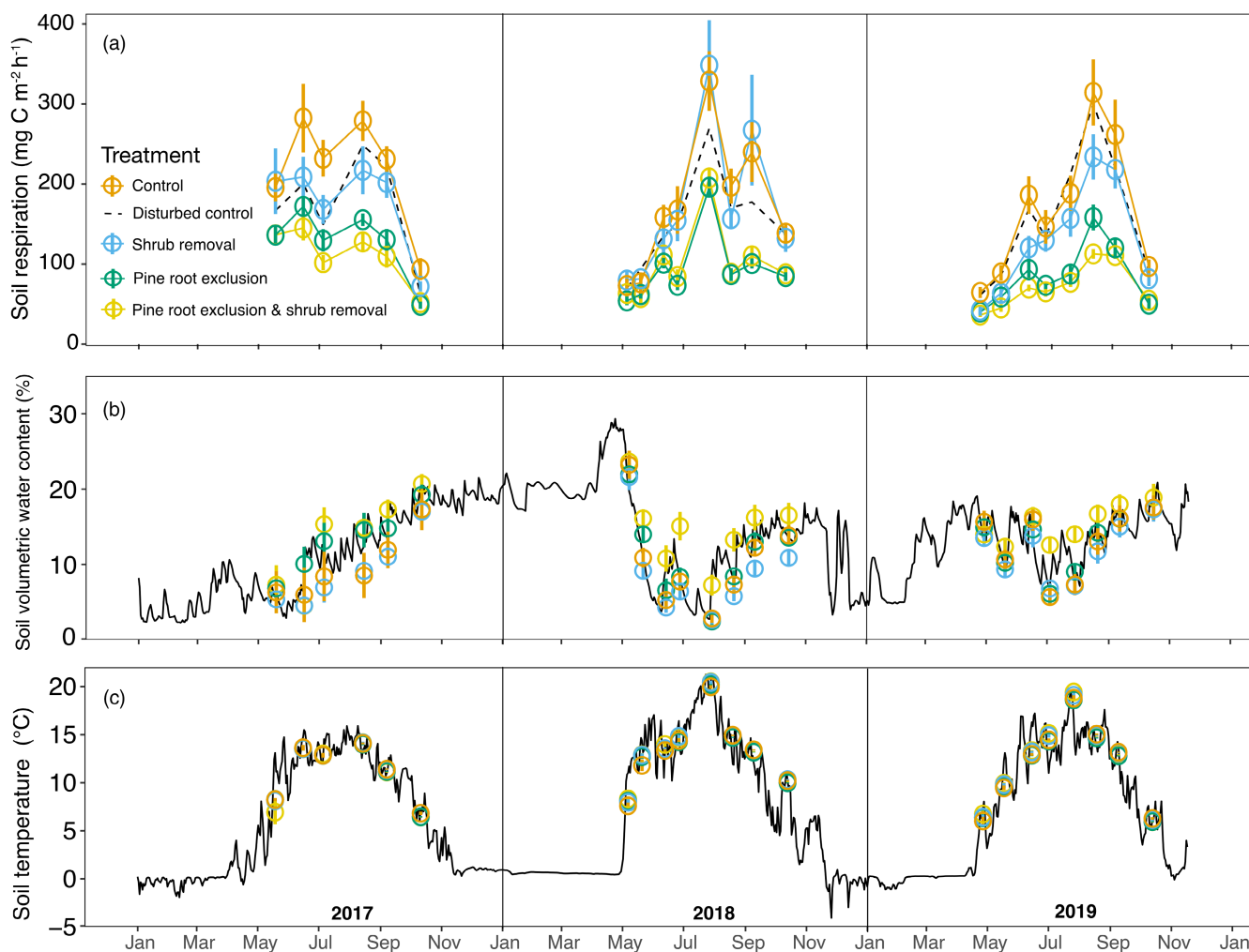


FIGURE 2 Growing season (a) soil respiration ($\text{mg C m}^{-2} \text{h}^{-1}$), (b) volumetric soil water content (%) and (c) soil temperature ($^{\circ}\text{C}$) in control plots and in plots from which shrubs or pine roots or both had been removed. Respiration was measured monthly over the growing seasons of 2017 to 2019. Soil water content and temperature averaged per treatment across the 4 days preceding each respiration measurement are shown as coloured circles, and the black trend lines represent daily averages across all treatments. Points are averages ± 1 standard error ($n = 7\text{--}8$)

TABLE 2 Linear mixed effects models of (a) soil respiration and (b) soil moisture (volumetric water content) and temperature point measurements. The individual and interactive effects of shrubs and pine roots, soil moisture and temperature on monthly measurements are shown. Pine root and shrub presence were coded as binary factors. Potential dependencies across space and time were accounted for and data from the disturbed control were not included. Insignificant ($p > 0.20$) three-way interaction terms were removed stepwise from the models. Values in bold ($p < 0.05$) have coefficient signs indicating positive or negative effects. Coefficient signs in parentheses indicate the direction of tendencies ($p < 0.10$)

	df	F	p	Coefficient sign
(a) Respiration ($\text{g C m}^{-2} \text{h}^{-1}$)				
Pine	(1,21)	47.5	<0.001	+
Shrub	(1,21)	2.22	0.151	
Moisture	(1652)	111	<0.001	-
Temp	(1652)	197	<0.001	+
Pine × shrub	(1,21)	1.28	0.270	
Pine × moisture	(1652)	1.40	0.237	
Shrub × moisture	(1652)	0.08	0.772	
Pine × temp	(1652)	9.47	0.002	+
Shrub × temp	(1652)	3.50	0.062	(-)
Moisture × temp	(1652)	3.04	0.082	(-)
Pine × moisture × temp	(1652)	2.84	0.092	(+)
Shrub × moisture × temp	(1652)	3.27	0.071	(+)
(b) Soil moisture (%)				
Shrub	(1,21)	1.29	0.269	
Pine	(1,21)	11.5	0.003	-
Year	(2670)	6.86	0.001	
Pine × shrub	(1,21)	5.79	0.025	+
(c) Soil temperature (°C)				
Shrub	(1,21)	0.086	0.772	
Pine	(1,21)	0.033	0.858	
Year	(2670)	10.7	<0.001	
Pine × shrub	(1,21)	0.003	0.954	

Generalized additive models described how respiration attributed to each guild had contrasting dependencies on soil moisture and temperature (Figure 3). Pine root-associated respiration increased with warmer conditions and was less sensitive to soil moisture (*adjusted (adj.)* $R^2 = 0.174$, $p < 0.001$; Figure 3a). Shrub-associated respiration, in contrast, was favoured by mid-range conditions and tended to decrease during wet and cold conditions and during drought conditions, but this was not well explained by the model (*adj.* $R^2 = 0.000$, $p = 0.085$; Figure 3b). Heterotrophic respiration varied the most across the soil moisture and temperature conditions experienced during the experiment and their GAMM models had the best fit (*adj.* $R^2 = 0.518$, $p < 0.001$), with particularly high respiration in warm and dry periods (Figure 3c).

3.5 | Soil nitrogen availability

Soil ammonium availability increased significantly in the absence of pine roots in the summer of 2017 and in both summer and fall periods of 2018 (Figure 4a). There was a significant interaction between the pine root and shrub presence in the second year with a large increase

in N availability in the combined removals (Figure 4a). Nitrate availability remained below detection levels in all treatments (data not shown). Nitrogen concentration of *V. vitis-idaea* leaves, used as an additional proxy for N availability during the first year, showed additive effects of plant groups; N concentrations were lowest when both plant groups were present, intermediate in the individual removals and highest in the combined removal (Figure 4b). There were no differences in leaf $\delta^{15}\text{N}$ between the control and any of the removal or exclusion plots, however, the disturbed control had significantly lower $\delta^{15}\text{N}$ values than the shrub removal while all other treatments were intermediary (Figure S4).

4 | DISCUSSION

4.1 | Soil respiration contributions by understory shrubs

Better estimates of the contribution by ericaceous dwarf shrub understories to soil respiration, relative to canopy-forming trees, should advance our understanding of environmental dependencies of soil respiration at the ecosystem level and why more soil

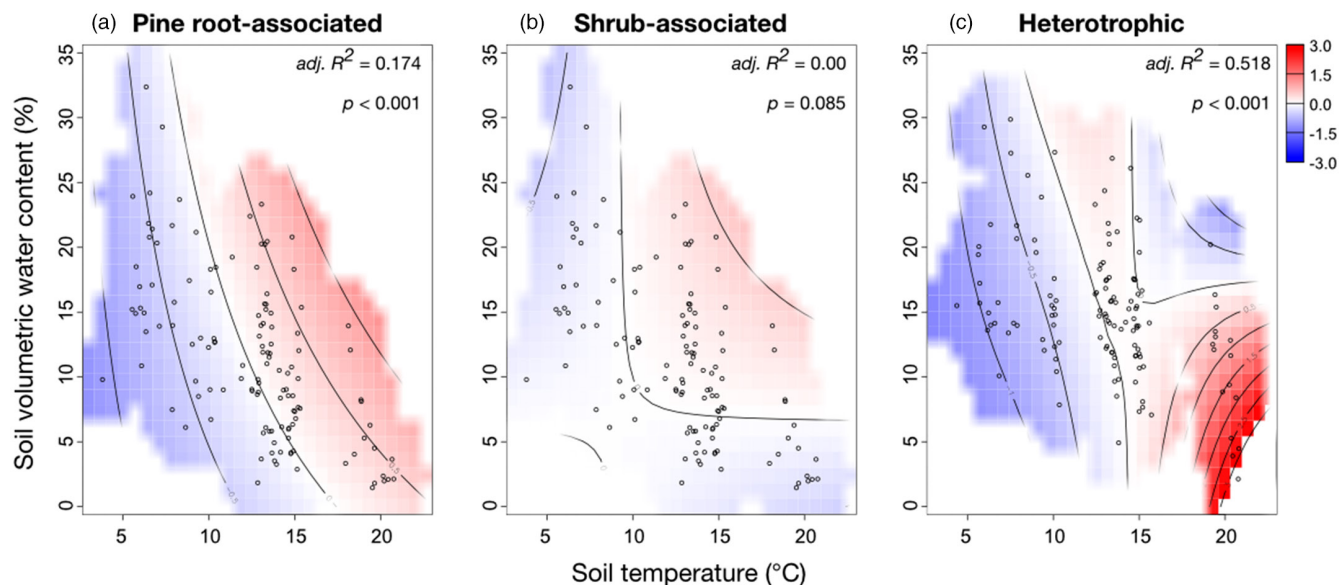


FIGURE 3 Generalized additive models of soil respiration (from blue = low to red = high) associated with (a) pine, (b) shrub and (c) heterotrophic sources in response to soil temperature and moisture (indicated by points) across the second and third growing seasons ($n = 112$). The respiration associated with each guild was rescaled to have a mean of zero and a standard deviation of one. Plot grid pixels that are extrapolated too far from the measured explanatory soil moisture and temperature data were excluded from the plot. Pine-associated respiration (a) is the difference between control and pine root removal plots, while shrub-associated respiration (b) is the difference between the control and shrub removals, all calculated per block and sampling date. Heterotrophic respiration (c) is assessed as respiration in the combined pine root exclusion and shrub removal treatment. Soil moisture and temperature data from the control plots were used for both pine root- and shrub-associated respiration. The zero-contour lines (the line between blue and red) represents the standardized mean for each of the three respiration sources

organic matter develops in ericoid dominated habitats (Clemmensen et al., 2015; Hartley et al., 2012; Tedersoo et al., 2020). We found that the ericaceous dwarf shrub understorey, on average, over 3 years, contributed 11% of the total soil respiration and 22% of autotrophic respiration. We hypothesized that the contribution of dwarf shrubs to autotrophic respiration would reflect their 30% share of fine root biomass (Persson, 1983), which closely matched our estimate of 31% if excluding the second growing season with drought conditions. Earlier studies in the boreal forest have estimated that autotrophic respiration contributes 35%–55% of total soil respiration (Comstedt et al., 2011; Hanson et al., 2000; Högberg et al., 2001; Subke et al., 2006), but only a few studies have monitored understorey contributions with girdling or trenching in forested ecosystems, and they provide conflicting evidence (Friggens et al., 2020; Grau-Andrés et al., 2020; Parker et al., 2020; Ryhti et al., 2021). In a southern Finnish boreal forest, dwarf shrubs and pine roots were estimated to contribute 8% and 48%, respectively, of total soil respiration over 3 years (Ryhti et al., 2021). These contributions are half of our estimate for shrubs in non-drought summer seasons (16%) and slightly higher than our pine root-associated contributions (43%). The currently available, albeit scarce, data suggest that shrubs can make a significant contribution to soil respiration, likely reflecting their contribution to primary productivity (Kulmala et al., 2019; Nilsson & Wardle, 2005), particularly below-ground. However, one potential process underlying ericaceous shrub accretion of soil organic matter may be a lower metabolic quotient (respiration per unit biomass) compared to pine roots, as demonstrated

in a microcosm experiment (Kulmala et al., 2018). This remains to be further tested in situ.

4.2 | Large, drought-related variation in shrub-associated respiration

The proportion of autotrophic respiration attributed to dwarf shrubs varied dramatically from 2% to 31% among growing seasons. The removal of ericaceous shrubs had only a marginal effect in the second year of the experiment, coinciding with a severe summer drought in 2018. In northern Sweden, a synchronized lack of response to shrub removals was found in an experiment where shrubs were removed continuously during 22 years in advance of the respiration measurements (Grau-Andrés et al., 2020). This suggests a direct effect of drought on shrub performance, rather than the influence of experimental side effects such as elevated heterotrophic decomposition of severed roots and mycelia, to have caused this lack of response to shrub removal. Plausibly, ericaceous dwarf shrubs closed their stomata completely to cope with the low moisture conditions in the upper soil horizons, where most of their roots are found (Persson, 1983). In addition, ericoid mycorrhizal fungi have maintained genetic capacities similar to free-living saprotrophs (Martino et al., 2018) and this apparent versatile ecology may be an adaptation to cope with unreliable below-ground allocation of photosynthates by shrubs. Pines, in contrast, have around half of their root biomass in deeper mineral soil horizons at our site (Persson, 1983). Accordingly, the pine-mediated respiration

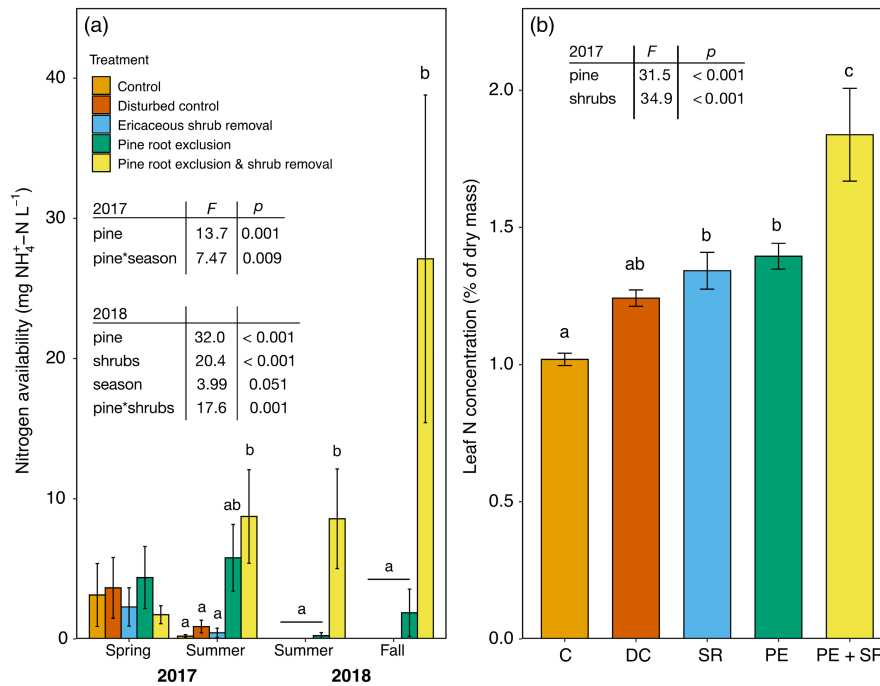


FIGURE 4 Nitrogen availability in (a) soil solution and (b) assessed by *V. vitis-idaea* leaf N concentration, as affected by pine root exclusion and understory shrub removal. In (a), soil ammonium availability during the first and second years of treatments was based on early and late incubation periods of ion exchange strips. Here, we analyzed the effect of pine and/or shrub presence (binary) and season as fixed factors in a linear mixed model, including plot nested in block as random factors. In (b), leaf N concentrations (% of dry mass) of *V. vitis-idaea* were analysed in the fall of the first year to indicate plant available N ($n = 7-8$, except the combined shrub removal and pine root exclusion for which $n = 3$). Treatments are indicated by C = control, DC = disturbed control, SR = shrub removal, PE = pine exclusion, PE+SR = combined pine root exclusion and shrub removal. Again, we tested pine and shrub effects on leaf N concentrations in a linear mixed model, including plot nested within block as random factors. Letters above bars indicate significant differences between treatments within each sampling period ($\alpha = 0.05$)

was much more consistent across years (41%–45%) and less affected by drought than the shrub-driven respiration. A study of pine root growth in southern Finland during the same drought season of 2018 found that the growth of pioneer pine roots was unaffected, while the growth of the fibrous, mycorrhizal side-roots ceased temporarily during the drought (Ding et al., 2020). Subarctic Scots pine stands have also been found to maintain higher respiration rates through extreme weather events, particularly dry and hot spells, than Norway spruce stands (Matkala et al., 2021), and the interannual variation in photosynthesis of the trees seemed to be more stable from year to year than understory shrubs in an ecosystem respiration budget (Kulmala et al., 2019). Further, diurnal fluctuations in respiration by the understory have not been well assessed and contrasted to those of canopies, although such differences may be important, especially during drought. Overall, the contrasting responses to soil temperature and moisture between autotrophic guilds signifies that there is a strong dependence on phenological attributes (Makita et al., 2018), and potentially different activity thresholds for the understory and canopy.

4.3 | Competition or coexistence between dwarf shrubs and pine roots

Our second hypothesis predicted that dwarf shrubs and pines would compete for scarce resources in this ecosystem, and that

the exclusion of both functional groups would lead to a larger increase in soil N availability than predicted from the single plant type eliminations. The N concentration of *V. vitis-idaea* leaves, however, increased additively from single to the combined exclusion in the first year. This was congruent with soil ammonium availability over the summer period of the first year, where treatments may have led to increased N mineralization from decomposing roots and mycelium (Hanson et al., 2000). As a reflection of this, we expected that the $\delta^{15}\text{N}$ signatures in the leaves would increase, as mycorrhizal mycelium is enriched in ^{15}N compared to plant pools (Bhupinderpal Singh et al., 2003). Instead, we found little effect of the pine root exclusion relative to the controls and a clear increase in the shrub removal treatment relative to the disturbed control. While most of the dwarf shrub rhizomes were pulled out of the soil, many fine roots harbouring the root-associated fungi were likely left to decompose in the plot, and ericoid mycorrhizal fungi strongly fractionate stable N isotopes by keeping more ^{15}N in their mycelium during transfer of N to their plant partner (Emmerton et al., 2001). Consequently, the N pool that became available after shrub removals may have been a particularly ^{15}N -enriched pool in our system, leading to the elevated leaf $\delta^{15}\text{N}$ signatures. In the second year, there was a large and non-additive increase in soil ammonium availability in the combined pine root exclusion and shrub removal treatment, indicating that low N availability persisted with only one remaining plant group present because of increased N uptake after a competitive release. Uptake

by plants and mycorrhizal fungal associates thus represented a major sink for soil N in the summer and fall. This evidence for strong N limitation and competition between pine and shrub roots was, however, not strongly tied to increased below-ground respiratory activity, which remained additive for the two plant groups throughout the experiment. Potentially, roots may have increased their carbon-use efficiency with higher N availability in this N-limited system (Forsmark et al., 2021; Robinson et al., 1999). Remaining plants could thus have increased root N uptake and growth without a corresponding increase in respiration.

4.4 | Heterotrophic respiration and experimental side effects

We expected that saprotrophs would contribute less than half of soil respiration when accounting for both autotrophic sources; however, heterotrophic contributions, on average, remained around 50% of total soil respiration. Even so, as visualized in the response functions, heterotrophic respiration varied the most across the soil moisture and temperature conditions during the experiment, with particularly high values in warm and dry periods. As a result, the cumulative heterotrophic respiration was higher and contributed more to total soil respiration (63%) in the second drought year, when understorey shrubs were hampered. It is quite surprising that free-living decomposers seemed unabated by low moisture, but a meta-analysis of warming experiments also indicated a stronger positive dependency of heterotrophic respiration on temperature than on water content, especially in northern forested ecosystems (Wang et al., 2014). Furthermore, mosses may have increased their respiration differentially across the treatments, which we are unable to evaluate in the present experiment.

The permanent barriers succeeded in excluding pine roots and ectomycorrhizal fungi. The respiration in the disturbed controls rebounded from trenched to control respiration levels in the second growing season. This indicates that C flow to roots, ectomycorrhizal mycelium and other root-associated organisms had, at least partially, been re-established in the disturbed controls. In permanently trenched plots, soil respiration remained at low levels throughout the 3 years, and no ectomycorrhizal DNA was found in the humus-filled mesh bags, confirming that pine below-ground C allocation did not re-establish in the plots with barriers.

Treatment side effects, such as increased soil moisture and inputs of necromass from severed roots and mycorrhizal mycelium, may boost saprotrophic activities (e.g. respiration, decomposition and mineralization) in trenching experiments (Hanson et al., 2000). In our forest site, $313 \pm 15 \text{ g m}^{-2}$ of living pine roots <10 mm in diameter have been quantified down to 30 cm (Persson, 1983). Dead pine roots (1–10 mm diameter) have been shown to lose 10%–20% of mass over one growing season when the most labile compounds were degraded, and close to 40% mass loss over three growing seasons in our forest (Berg, 1984). Patterns of ^{14}C distribution in soil profiles of nutrient poor pine forest indicated that 10% of root-derived C

was lost during a year, and 60% was lost during a 10-year period (Kyaschenko et al., 2019). There is likely to be $15\text{--}50 \text{ g m}^{-2}$ of ectomycorrhizal mycelium in coniferous boreal forest soils (Finlay & Clemmensen, 2017), which would mostly turn over during 1 year in an older pine forest (Hagenbo et al., 2016), besides a smaller recalcitrant fraction. Based on these estimates, decomposition of severed roots and ectomycorrhizal mycelium during the first year corresponded to $44\text{--}83 \text{ g C m}^{-2}$ which may have inflated the heterotrophic respiration (of $406 \pm 31 \text{ g C m}^{-2}$) by up to 10%–20% over the first growing season. However, because of the timing of trenching, mycelial decomposition could have happened before respiration measurements began. For example, mycelium has been shown to disappear within 5 days under optimal growing conditions (Finlay & Read, 1986), and more than 50% mass loss was observed after 7 days under field conditions (Ryan et al., 2020). Further, the respiration in the disturbed controls never exceeded that in control plots in the second or third years where pine roots and ectomycorrhizal mycelium re-established. Thus, the flux from severed roots and mycelium in any of our trenched plots was likely not very large compared to the estimated autotrophic and heterotrophic fluxes. Additionally, the treatments with severed below-ground pine inputs had lower cumulative respiration in the third year than in the first year. This could be linked to the induced flush from severed pine roots and mycelium in the first year, which was sequentially exhausted, but the reduction in respiration over time could also be exaggerated by ceased rhizosphere inputs (via a decreased 'priming effect') from mycelial and root production. We suggest that facilitation and interdependence among saprotrophs and root-driven inputs are likely to be important and should be emphasized in future experiments.

5 | CONCLUSIONS

Taken together, over 3 years, we estimated that autotrophic and heterotrophic respiration each contributed around half of total soil respiration in an old-growth pine forest. Shrub-associated respiration was about a third of total autotrophic respiration under non-drought conditions, reflecting their share of fine root production. We found evidence of competition for N between pine roots and shrubs, but contrary to our expectations, tree root- and shrub-associated respiration showed no clear sign of a competitive release (i.e. they remained additive throughout the 3 years). Pine root-associated and heterotrophic respiration persisted, and even increased in relative terms, through periods of drought. In contrast, shrub-associated respiration almost ceased during drought, indicating that relative contributions of ericaceous dwarf shrubs to soil respiration may shift dramatically with changing environmental conditions. It remains to be investigated whether the simultaneous inactivity of dwarf shrubs and augmented activity of free-living saprotrophs and pine roots during drought have important consequences for soil organic matter accumulation in the long term. Disparate trade-offs in respiratory versus growth responses between trees and understorey shrubs should be a future research priority.

AUTHORS' CONTRIBUTIONS

The field experiment was initiated and designed by K.E.C., B.D.L., R.D.F. and A.E. and the specific study was conceived by all authors. The field and laboratory work was performed by L.A.M. and K.E.C. Isotope ratio mass spectroscopy was performed by A.E. Data were analysed by L.A.M. The first draft of the manuscript was written by L.A.M. and all authors contributed to the data interpretation and revisions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this work.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.13927>.

DATA AVAILABILITY STATEMENT

All data are available in Dryad Digital Repository <https://doi.org/10.5061/dryad.1ns1rn8wt> (Mielke et al., 2022), apart from raw sequence data, which can be found under the Sequence Read Archive (SRA) with BioProject accession number PRJNA834027.

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