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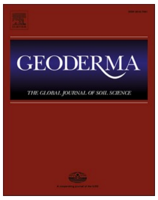

Non-symbiotic N_2 fixation is less sensitive to changes in temperature than carbon mineralization in Northern forest soils

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ABSTRACT

Northern forests are characterized by low temperatures that play a key role in the whole ecosystem functioning. However, Northern forests are expected to experience the largest temperature increase of all forest biomes in the next decades, which could affect central ecosystem processes, such as carbon (C) mineralization and N_2 fixation. Aiming to clarify the temperature-dependence of non-symbiotic N_2 fixation and C mineralization in Northern forest soils, we quantified the rates of both processes in soils of Scots Pine (*Pinus sylvestris*) forests located along a temperature gradient in Sweden in laboratory incubations at different temperatures (5, 12 and 20 °C). Our results show that N_2 fixation by free-living bacteria in the organic layer of these forest soils ranges between 2 and 10 kg N ha^{-1} yr^{-1} which highlights the importance of non-symbiotic N_2 fixation in Northern forest soils. We found a positive correlation between non-symbiotic N_2 fixation per area and mean annual temperature (MAT). This relationship was caused by the positive relationship between the organic layer stock and MAT rather than by the direct effect of temperature on the process rate. In contrast, C mineralization per g of soil was negatively related to MAT. Furthermore, our results show that C mineralization is more sensitive to changes in incubation temperature (it increased by a factor of 2.2 from 5 to 12 °C as well as from 12 to 20 °C) than non-symbiotic N_2 fixation that was not significantly affected by incubation temperature. Taken together, while N_2 fixation responded little to changes in incubation temperature, our results suggest that the higher N_2 fixation rate per area at sites with higher MAT is beneficial for primary production and organic matter inputs to soil leading to larger organic layer stocks. Hence, there is a positive, temperature-dependent feedback among non-symbiotic N_2 fixation, primary production, and the organic layer formation in Northern forests.

1. Introduction

The Northern forests of boreal and boreo-nemoral regions form one of the largest biomes on Earth contributing considerably to global carbon (C) storage, water, and climate regulation, and providing a relevant proportion of wood to global markets (Gauthier et al., 2015; Pan et al., 2011). Northern forests grow in high-latitude environments of the northern hemisphere characterized by a short growing season and severe winters with freezing temperatures for several months. Thus, low temperature plays a key role in the whole ecosystem functioning (Gauthier et al., 2015; Jarvis and Linder, 2000; Jungqvist et al., 2014). Temperature controls tree growth and indirectly, the organic matter accumulation in the soil organic layer *via* plant litter inputs and organic matter decomposition (Jarvis and Linder, 2000; Spohn and Stendahl, 2022). Northern forests are expected to experience the largest temperature increase of all forest biomes, particularly during the winter period, according to the current global warming projections (Intergovernmental Panel on Climate Change, 2023).

Increasing temperatures in Northern forests can affect different biogeochemical processes to different extents. Future climate scenarios suggest an increase in soil C mineralization due to the temperature increase, and hence a decrease in C stocks stored in the organic layer of Northern forest soils (Bond-Lamberty and Thomson, 2010; Davidson and Janssens, 2006; Kurz et al., 2013; von Lützow and Kögel-Knabner, 2009). This increase in C mineralization can be particularly important in the coldest areas where stocks of recalcitrant C could be mineralized

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upon temperature increase (Biasi et al., 2005; von Lützow and Kögel-Knabner, 2009). However, the temperature sensitivity of soil C mineralization in cold regions remains still under debate because different feedbacks and environmental constrains can shape the response of C mineralization to increasing temperatures at the ecosystem level (Allison and Treseder, 2008; Bond-Lamberty and Thomson, 2010; Rousk et al., 2016; Salazar et al., 2020; Vanhala et al., 2008).

Increasing temperatures can also affect N cycling and N availability in Northern forests (Alvarenga and Rousk, 2021; Rousk et al., 2016; Salazar et al., 2020). Primary production in Northern forests is strongly N limited (Bergh et al., 2014; Högberg et al., 2021, 2017; Sponseller et al., 2016; Tamm et al., 1999). An important source of N to Northern forests might be atmospheric N_2 fixed by diazotrophs (N_2-fixing bacteria living free or associated with soil crust, mosses, or lichens) (DeLuca et al., 2008; Gundale et al., 2012a; Rousk et al., 2015, 2013). N_2 fixation is an enzymatic process that increases with raising temperature between approximately 5 and 26°C and then declines at higher temperatures (Houlton et al., 2008). This positive relationship between N_2 fixation and temperature may explain the positive correlation between mean annual temperature (MAT) and N stocks of the organic layer in forest in Sweden (Spohn and Stendahl, 2022). The impact of warming on N_2 fixation by cyanobacteria in association with a common feather moss has received great attention during the last years due to the potential impact on ecosystem N availability (Alvarenga and Rousk, 2021; Gundale et al., 2012b, 2012a; Rousk et al., 2013; Sorensen and Michelsen, 2011). However, less attention has been paid to the relationship of temperature and N_2 fixation performed by free living N_2 fixing bacteria in soil (hereafter, non-symbiotic N_2 fixation in soils) even though the non-symbiotic N_2 fixation rate in soils (0.4 to 1.4 kg N ha^{-1} during the growing season (Nohrstedt, 1985)) can be similar to those achieved by cyanobacteria-moss association in Northern forest (Huss-Danell et al., 2011; Nohrstedt, 1985; Rousk et al., 2015). Higher temperatures could increase the non-symbiotic N_2 fixation in soils by enhancing nitrogenase activity as well as by increasing the stocks of organic layer accumulated in forest (i.e., larger soil organic layer stocks, higher N_2 fixation per area). Yet, no study has evaluated this so far.

Although there are studies describing the temperature sensitivity of non-symbiotic N_2 fixation and C mineralization (Houlton et al., 2008; von Lützow and Kögel-Knabner, 2009), the sensitivity of the two processes to changes in temperature has not been compared yet, although both processes are central to element cycling in terrestrial ecosystems and are tightly connected (Spohn, 2016). Indeed, the sensitivity to temperature of non-symbiotic N_2 fixation could also affect the sensitivity of C mineralization and *vice-versa*. Therefore, comparing the temperature-dependence of non-symbiotic N_2 fixation and C mineralization in Northern forest soils seems to be crucial to understand the ecosystem functioning due to the currently raising temperatures. The aim of this study was to determine the temperature-dependence of non-symbiotic N_2 fixation and C mineralization in Northern Scots Pine (*Pinus sylvestris*) forests located along a temperature gradient in Sweden based on laboratory incubations at different temperatures (5, 12 and 20 °C). We tested the following hypotheses.

i) Non-symbiotic N_2 fixation and C mineralization are both positively correlated with MAT (along a temperature gradient ranging from −0.05 to 7.23 °C MAT).
ii) The rates of non-symbiotic N_2 fixation and C mineralization increase similarly in response to increases in incubation temperature.

2. Material and methods

2.1. Study sites

The 15 forest sites included in the study are dominated by *Pinus sylvestris* L. and are distributed along a 1,300 km latitudinal gradient in Sweden (56–67 °N) (Fig. 1). Forest of *Pinus sylvestris* were selected due to their large expanse in the region and for spanning a larger latitudinal gradient than Norway spruce (the other most common forest tree in the region) (Bergh et al. 2014). The selected sites span a gradient of mean annual temperature (MAT) between −0.05 and 7.23°C. The mean temperatures across the 15 sites of the coldest (January) and the warmest months (July) are −6.52 and 15.76 °C, respectively (Table S1). Mean annual precipitation (MAP) ranged between 588 and 801 mm during the period 1972–2022 (Swedish Meteorological and Hydrological Institute, https://www.smhi.se/data, last access: May 2023) (Table 1).

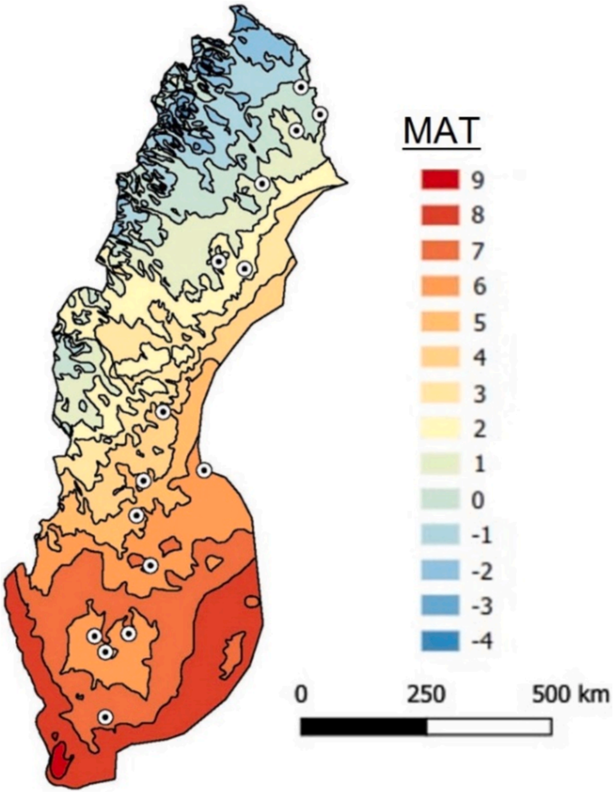

Fig. 1. Map of Sweden depicting mean annual temperature (MAT) and the site locations (dots).

The 15 sites are part of a long-term forest management experiment established between 1967 and 1981 that aims to study the effect of thinning and fertilization on forest biomass production (Bergh et al., 2014). The experiments were established in successful regenerations at the time of the first thinning (canopy closure and dominant height of 12–18 m) which (depending on the pedoclimatic conditions of the sites) occurred when the stands were between 32 and 53 years old. For the present study, we selected the control plots of the experiment without fertilization. The size of each experimental plot was approximately 0.1 ha (25 x 40 m), and the plots were surrounded by a buffer zone of at least 10 m. At the time of sampling in 2022, the experiments were between 41 and 55 years old and the forest stands were between 76 and 102 years old. During the experiment, the sites were monitored, and tree growth and biomass volume were regularly quantified as described in Bergh et al. (2014).

2.2. Soil sampling and processing

Soil sampling at the 15 sites was performed between May and July 2022. Aiming to collect all the samples at a similar vegetative stage (late spring), we started the sampling campaign at the southernmost site and finished in the northernmost site. We sampled the organic layer at each site by pooling 16 soil cores (3.5 cm diameter) collected every 2 m along two transects of 16 m, each connecting two opposite corners of the plots.

Table 1
Characteristics of the 15 *Pinus sylvestris* forests distributed across Sweden.

Site ID	Experiment Establishment (yr.)	Latitude (°N)	Longitude (°E)	MAT (°C)	MAP (mm)	Tree Biomass (m^3/ha)	Organic layer thickness (cm)	Organic layer pH
994	1978	67.48	23.1	−0.05	611.05	2307	2.8	3.73
951	1975	66.95	23.8	0.84	588.44	3572	3.9	3.60
952	1975	66.72	22.64	1.11	619.76	3245	2.9	3.86
1000	1981	65.84	20.87	1.12	647.79	4253	3.8	3.95
991	1976	64.35	19.82	1.88	680.83	4031	4.4	3.60
946	1976	64.51	18.77	1.96	629.91	4427	3.0	3.94
936	1974	61.86	16.33	3.93	652.88	3773	3.8	3.77
902	1969	60.01	15.3	4.94	807.47	3899	5.2	3.48
918	1970	60.63	15.56	5.30	665.26	4841	6.0	3.52
923	1973	57.84	13.87	6.02	820.82	4819	6.3	3.45
787	1973	60.79	17.79	6.07	612.19	6923	4.8	4.32
922	1967	57.89	15.02	6.20	698.71	4236	4.1	3.56
931	1973	57.56	14.24	6.29	796.94	4367	3.8	3.54
933	1973	59.11	15.78	6.53	646.51	2864	5.6	3.68
940	1969	56.39	14.26	7.23	801.13	3571	7.3	3.54

MAT, Mean annual temperature (period 1972–2022); MAP, Mean annual precipitation (period 1972–2022). Tree biomass was estimated using allometric functions based on plot average tree stem diameter and height.

Living plants and litter (Oi horizon) were removed before sampling. After extracting each soil core, the underlying mineral horizon, and larger roots (> 2 mm) were discarded, and the thickness of the organic horizon (Oe and Oa) was measured, and the material pooled in a plastic bag. Soils were classified as Podzols at all sites with an organic horizon between 2.8 and 7.3 cm thick and a pH ranging between 3.45 and 4.32 (Table S2).

In the laboratory, the organic soil samples were homogenized, weighed and the gravimetric water content determined to calculate the dry weight of organic layer per ha based on the sum of the area of the 16 sampled cores. Soil water holding capacity (WHC) of each soil sample was estimated as the gravimetric water content upon 24 h of free drainage of saturated samples in the laboratory. Soil pH was measured in H_2O using a subsample of air-dried soil (1:5 w:v).

2.3. Non-symbiotic N_2 fixation

We determined non-symbiotic N_2 fixation in the organic layer in the laboratory at three different incubation temperatures (5, 12 and 20 °C). We selected the three temperatures aiming to span a representative temperature range for N_2 fixation in the region. While 20°C was selected as the highest incubation temperature because higher temperatures are rare in the region (≈ 5 days per year with a mean daily temperature > 20°C at the coldest sites), the lowest incubation temperature was set at 5 °C because according to Houlton et al. (2008) no significant nitrogenase activity can be observed below 5 °C.

The non-symbiotic N_2 fixation by free-living microorganisms in soil was determined by the $^{15}N_2$ assimilation method in incubations of soil samples with 99.8 atom% $^{15}N_2$ according to the method described by Zechmeister-Boltenstern (1996). Briefly, a total of 12 serum flaks of 30 ml per soil sample were filled with fresh soil (1.5 g dry-mass equivalent) adjusted to 80 % WHC. The serum flasks were closed with a rubber lid and four flasks of each sample were incubated in the dark at each incubation temperature (5, 12 and 20 °C) for 24 h as a pre-incubation period. After pre-incubation, each flask was ventilated and closed with an aluminum crimp seal with PTFE/butyl septa (Product 854979, Supelco Inc., Bellefonte, PA, USA). Then, each sample flask was flushed with argon, evacuated, and finally filled with 25 ml of 99.8 atom% $^{15}N_2$ (gas lot MBBD1096, Sigma Aldrich co., St. Louis, MO, USA) and 3.6 ml O_2 with a syringe. After filling with the $^{15}N_2$ enriched atmosphere, all the flaks were incubated in the dark for 48 h at the three incubation temperatures (5, 12 and 20 °C). Afterwards, the flasks were opened, ventilated, and freeze-dried. The Certificate of Analysis of the $^{15}N_2$ gas lot that we used (MBBD1096 produced by Sigma Aldrich in 2021) shows that the N_2 gas is 99.9 % pure and contains < 15 ppm N_2O (see the Certificate of Analysis of the gas lot in the Supplementary Material). This is a lower level of N_2O contamination than the level certificated for the low contamination gas lot of Sigma (MBBB0968V, produced by Sigma Aldrich in 2014) analyzed by Dabundo et al. (2014).

Soil samples exposed to the $^{15}N_2$ enriched atmosphere as well as non-exposed samples incubated at 12 °C with a non-modified atmosphere (control) were milled and analyzed for ^{15}N using a continuous-flow isotope ratio mass Spectrometry of Flash EA 2000 via ConFlo IV open split interface to a Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) at the Stable Isotope Laboratory at SLU. The C and N concentration and the $\delta^{13}C$ and $\delta^{15}N$ values of the non-exposed samples were considered as the C and N concentration of the soil.

The non-symbiotic N_2 fixation was calculated using the isotope mixing model following Zechmeister-Boltenstern (1996):

$$^{15}N_2\ fixation\ rate\left(ng\ N\ g^{-1}h^{-1}\right) = TN\left(mg\ N\ g^{-1}\right)x\frac{\left(^{15}N\ labeled(atom\%)\ -\ ^{15}N\ control(atom\%)\right)}{100\ x\ t(h)}x10^{6}$$

where TN is the total soil N of sample, $^{15}N_{labeled}$ is the percentage of ^{15}N atoms in the labeled subsample, $^{15}N_{control}$ is the percentage of ^{15}N atoms in the control subsample and t is the incubation time in hours (48 h). All the non-symbiotic N_2 measurements were performed within a week after the sampling to limit soil alteration.

2.4. Carbon mineralization

Carbon (C) mineralization in the organic layer was determined as the CO_2–C emitted during 48 h at the same conditions under which non-symbiotic N_2 fixation was determined. Briefly, 12 serum flaks of 100 ml per sample were filled with fresh soil (2 g dry-mass equivalent) and adjusted to 80 % WHC. After weighting, the serum flasks were closed with a rubber lid and four flasks of each sample were incubated in the dark at each incubation temperature (5, 12 and 20 °C) for 24 h. This pre-incubation period allowed the equilibration of soil samples after sampling and processing. After the 24 h of pre-incubation, each flask was ventilated and closed with an aluminum crimp seal with PTFE/butyl septa and all the flaks were incubated in the dark for 48 h at the three temperatures (5, 12 and 20 °C). After 48 h, the CO_2 concentration in the headspace of each flask was measured using a Picarro CO_2 analyzer (G2201-i, Picarro Inc., USA). For each temperature, additional empty jars were incubated to account for the CO_2 background following the same procedure. Gas concentrations in the headspace was converted to fluxes according to the ideal gas law, the flask volume, the amount of dry soil incubated, and the incubation time.

2.5. *Calculations and statistical analysis*

The non-symbiotic N_2 fixation and C mineralization were converted to μg N $m^{-2}h^{-1}$ and mg C $m^{-2}h^{-1}$, respectively by multiplying the N_2 fixation and C mineralization rates per mass soil by the organic layer stock. Similarly, the C and N stocks of the organic layer were calculated as the C and N concentrations multiplied by the organic layer stock. The response of non-symbiotic N_2 fixation and carbon mineralization to increased incubation temperature from 5 to 20 °C were normalized by the rate at 5 °C by dividing the difference between rates at 20 °C and the rates at 5 °C by the rates at 5 °C.

The impact of incubation temperature, MAT, and their interaction on the non-symbiotic N_2 fixation rates and C mineralization were analyzed statistically using a linear mixed model in SPSS 27 (IBM SPSS, Inc., Chicago, USA). A model including incubation temperature (5, 12 and 20 °C) as fixed factor, MAT as a site-level covariate and their interaction was used. Data were tested for normal distribution (Shapiro-Wilk-test, $p > 0.05$), and log-transformed when needed prior further analyses. Bivariate correlations between variables were determined using the Pearson's correlation test ($p < 0.05$).

3. Results

3.1. *Non-symbiotic N_2 fixation and C mineralization rates per area*

Non-symbiotic N_2 fixation per area determined at the three incubation temperatures correlated positively with MAT of the sites ($R^2 = 0.53$, $R^2 = 0.58$ and $R^2 = 0.58$ at 5, 12 and 20 °C, respectively; Fig. 2A). Non-symbiotic N_2 fixation per area determined at 12 °C ranged between 48.1 and 192.0 μg N $m^{-2}h^{-1}$ (at sites with a MAT of 1.96 and 7.23 °C, respectively; Fig. 2A). We observed no significant effect of incubation temperature on the non-symbiotic N_2 fixation per area ($p = 0.812$), and the means across the 15 sites were 100.5, 98.8 and 96.4 μg N $m^{-2}h^{-1}$ at 5, 12 and 20 °C incubation temperature, respectively (Fig. 2A). No significant interaction between incubation temperature and MAT was observed ($p = 0.910$; Fig. 2A) for non-symbiotic N_2 fixation per area.

The mean C mineralization per area across the 15 sites increased by a factor of 2.2 for both incubation temperature differences (from 10.2 mg C $m^{-2}h^{-1}$ at 5 °C to 22.0 and 48.8 mg C $m^{-2}h^{-1}$ at 12 and 20°C, respectively; Fig. 2B). C mineralization was only significantly correlated with MAT at 20 °C ($R^2 = 0.41$ at 20 °C, Fig. 2B). The C mineralization per area determined at 12 °C ranged between 12.0 and 34.6 mg C $m^{-2}h^{-1}$ (at sites with a MAT of −0.05 and 6.53 °C, respectively) (Fig. 2A). No significant interaction between incubation temperature and MAT was

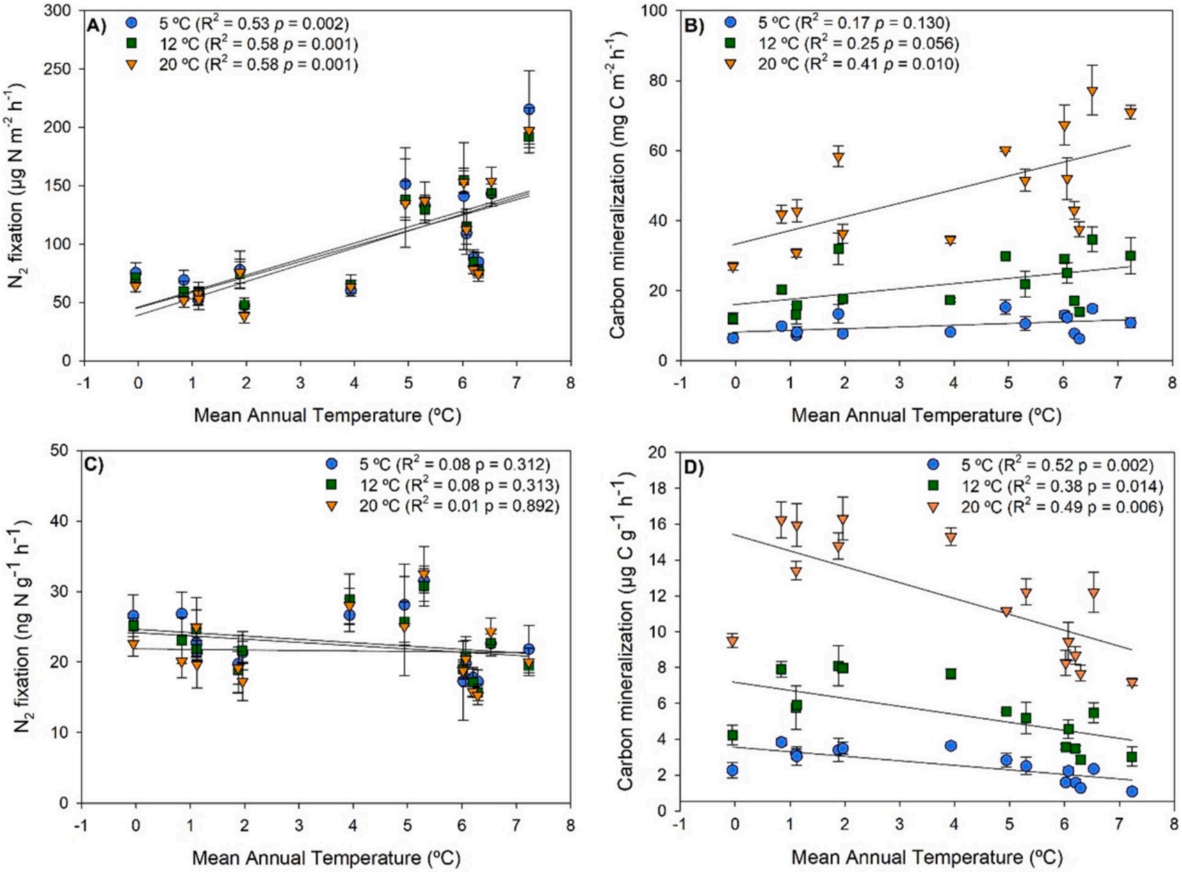

Fig. 2. Non-symbiotic N_2 fixation (A) and carbon mineralization (B) per area and non-symbiotic N_2 fixation (C) and carbon mineralization (D) per mass of soil in the soil organic layer at three incubation temperatures (5, 12 and 20 °C) as a function of mean annual temperature in 15 *Pinus sylvestris* forests distributed across Sweden. Symbols show means ($n = 4$), and error bars depict the standard deviation. The R^2 and p-value of Pearson's correlation test is indicated in the left upper corner.

observed ($p = 0.640$; Fig. 2B) for C mineralization per area.

3.2. Non-symbiotic N_2 fixation and C mineralization rates per mass of soil

Non-symbiotic N_2 fixation per g of soil organic layer was not significantly affected by the incubation temperature ($p = 0.179$; Fig. 2C, S1A). The mean non-symbiotic N_2 fixation rate per g of soil organic layer across the 15 sites was 22.8, 22.4 and 21.6 ng N g soil^{-1}h^{-1} at 5, 12 and 20 °C, respectively (Fig. 2C, S1A). In contrast, C mineralization per g of soil organic layer increased significantly by a factor of 2.2 for each increment in incubation temperature ($p < 0.001$), from 2.57 µg C g soil^{-1}h^{-1} at 5°C to 5.42 and 11.9 mg C m^{-2}h^{-1} at 12 and 20°C, respectively (Fig. 2D, S1B). In addition, the response of C mineralization per g of soil to the increase in incubation temperature from 5 to 20°C normalized by the C mineralization at 5°C correlated positively with MAT ($R^2 = 0.17$; Fig. 3).

Incubation temperature and MAT did not interact significantly ($p = 0.430$; Fig. 2C, S1A) in their relationships with non-symbiotic N_2 fixation per g of soil organic layer, but a MAT was significantly correlated with non-symbiotic N_2 fixation per g of soil ($p = 0.008$; Fig. 2C, S1A). C mineralization per g of soil organic layer decreased with increasing MAT ($p < 0.001$; Fig. 2D, S1B). This negative relationship between C mineralization and MAT is particularly evident in the incubations at 5 and 20°C ($R^2 = 0.52$ and $R^2 = 0.49$; Fig. 2D).

3.3. Impact of MAT on soil properties along the gradient

The soil organic layer stock increased significantly with MAT of the sites ($R^2 = 0.63$; Fig. 4A). The mean organic layer stock across the 15 sites was 45.5 t ha^{-1} and ranged between 22.2 and 98.7 t ha^{-1} (at sites with MAT 1.96 and 7.23°C, respectively). The N concentration of the organic layer increased significantly with MAT ($R^2 = 0.27$; Fig. 4B), whereas the C:N ratio ($R^2 = 0.68$; Fig. 4C) decreased with increasing MAT. The mean C:N ratio of the soil organic layer across the 15 sites was 43.3 and ranged between 32.9 and 53.9 (sites with MAT 6.07 and 0.84°C, respectively). The C and N stocks of the organic layer were positively correlated with the MAT (Fig. S2). In addition, the C stock of the organic layer was positively correlated with the non-symbiotic N_2

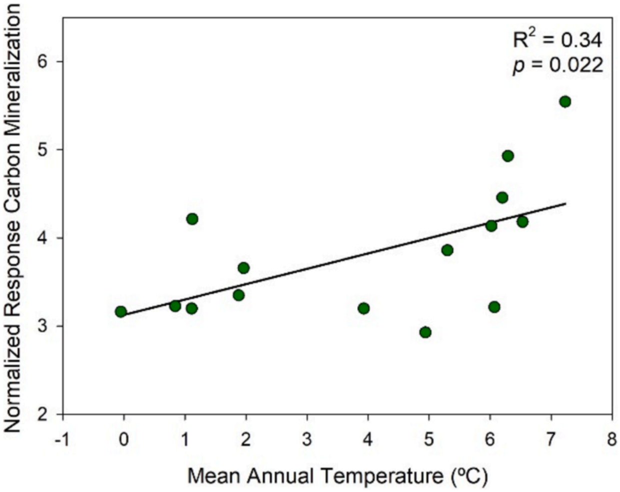

Fig. 3. Response of C mineralization per g of soil to the increase in incubation temperature from 5 to 20°C normalized by the C mineralization at 5°C, as a function of mean annual temperature in 15 *Pinus sylvestris* forests distributed across Sweden. The R^2 and p-value of Pearson's correlation test is indicated in the right upper corner. The response of C mineralization per g of soil to the increase in incubation temperature from 5 to 20°C normalized by the C mineralization at 5°C was calculated by dividing the difference between the carbon mineralization at 20 °C and the carbon mineralization at 5 °C by the carbon mineralization at 5 °C.

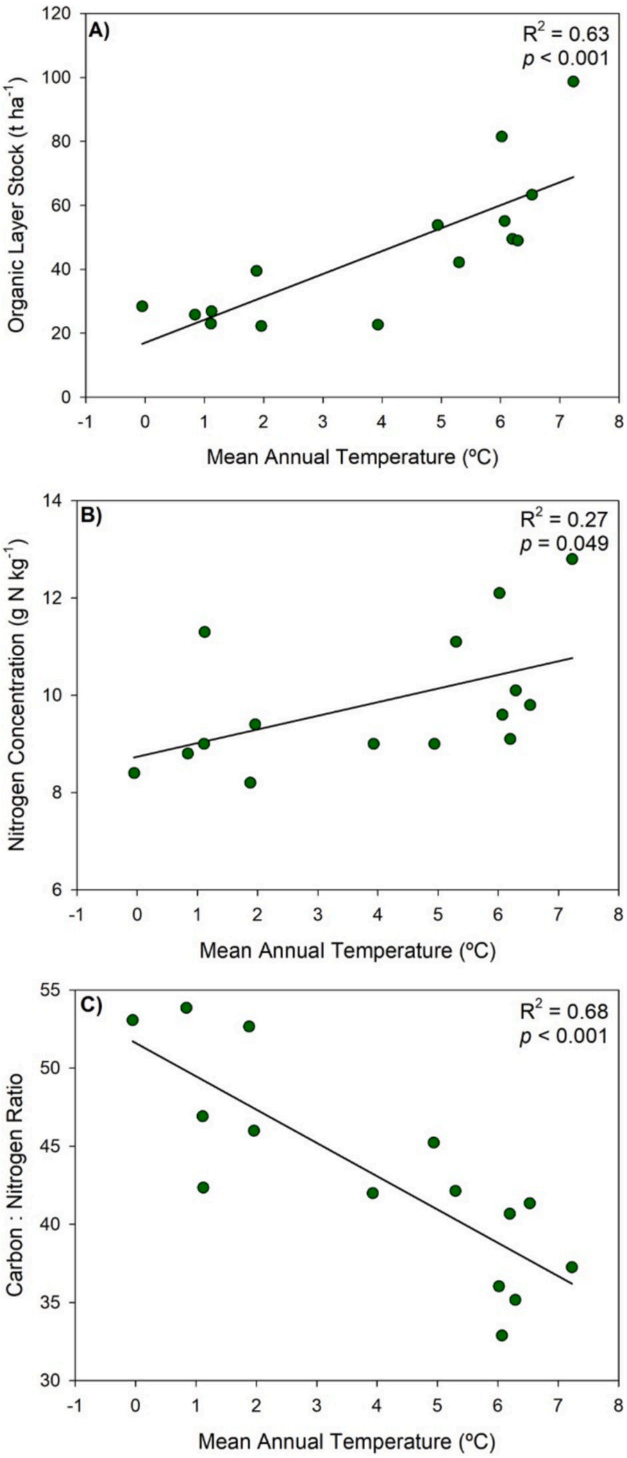

Fig. 4. The organic layer stock (A), the nitrogen concentration (B) and the carbon-to-nitrogen ratio (C) of the organic layer as a function of mean annual temperature in 15 *Pinus sylvestris* forests distributed across Sweden. The R^2 and p-value of Pearson's correlation test is indicated in the right upper corner.

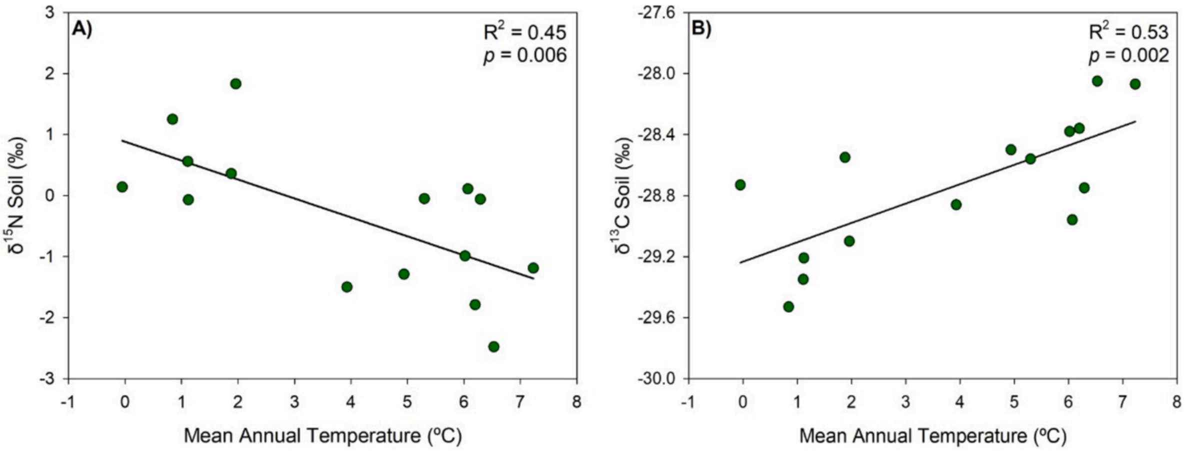

Fig. 5. The δ^{15}N (A) and δ^{13}C (B) of the organic layer as a function of mean annual temperature of 15 *Pinus sylvestris* forests distributed across Sweden. The R^2 and p-value of Pearson's correlation test is indicated in the right upper corner.

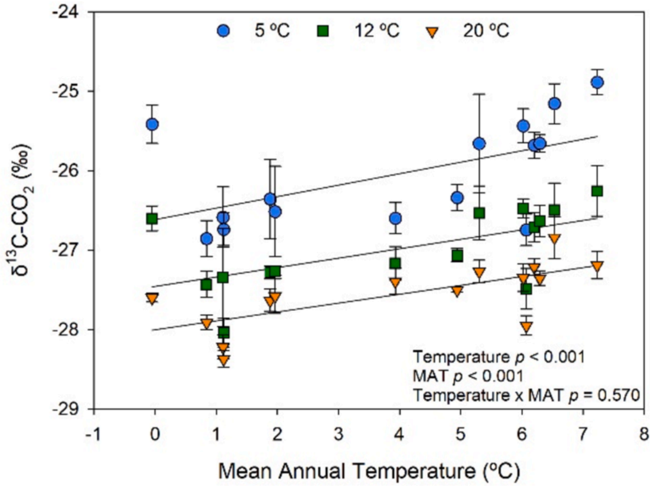

Fig. 6. δ^{13}C-CO$_2$ of the organic layer at three incubation temperatures (5, 12 and 20 °C) as a function of mean annual temperature of the site in 15 *Pinus sylvestris* forests distributed across Sweden. Bars show means (n = 4), and error bars depict the standard deviation. Statistical significance of the effects of incubation temperature and mean annual temperature (MAT) as well as their interaction is indicated in the right bottom corner.

fixation per area ($R^2 = 0.85$, $R^2 = 0.85$ and $R^2 = 0.83$ at 5, 12 and 20 °C, respectively; Fig. S3).

The δ^{15}N of the soil organic layer was negatively correlated with MAT ($R^2 = 0.45$; Fig. 5A) and ranged between −2.48 and 1.25 ‰ (sites with a MAT of 6.53 and 0.84 °C, respectively). In contrast, the δ^{13}C of the organic layer was positively correlated with MAT ($R^2 = 0.53$ Fig. 5B).

3.4. *Impact of MAT and incubation temperature on δ^{13}C-CO$_2$ of mineralized C*

The δ^{13}C of the CO$_2$-C formed during the incubation of the soil organic layer decreased significantly with increasing incubation temperature ($p < 0.001$) from −26.04 ‰ at 5°C to −26.98 and −27.56 ‰ at 12 and 20°C, respectively (Fig. 6). In addition, the δ^{13}C of CO$_2$-C was positively correlated with MAT ($p < 0.001$; Fig. 6).

4. Discussion

4.1. *The organic layer stock causes differences in non-symbiotic N$_2$ fixation per area along a temperature gradient*

We found a positive relationship between MAT and the non-symbiotic N$_2$ fixation rate per area, i.e., per m^2 (Fig. 2A). This supports our first hypothesis of a positive relationship between non-symbiotic N$_2$ fixation and MAT. However, the increase in non-symbiotic N$_2$ fixation per area with increasing temperature is caused by the positive relationship between MAT and the organic layer stock rather than by the direct effect of temperature on the process rate. While the non-symbiotic N$_2$ fixation per g of soil organic layer was similar along the temperature gradient (Fig. 2C), the higher organic layer stocks at the sites with higher MAT (Fig. 4A) caused elevated non-symbiotic N$_2$ fixation per area. This positive relation between MAT and organic layer stocks in Northern forests has been previously described (Akselsson et al., 2005; Högberg et al., 2021; Spohn and Stendahl, 2022). This relationship suggests that plant productivity and organic matter inputs to soil increase with increasing MAT more strongly than decomposition (Akselsson et al., 2005; Högberg et al., 2021; Spohn and Stendahl, 2022).

Our results suggest that the higher N$_2$ fixation rates per area at the sites with higher MAT contribute to the accumulation of organic C in soils as observed in the strong positive correlation between N$_2$ fixation rates per area and soil C stocks (Fig. S3). Increased N$_2$ fixation at the sites with high MAT leads to increased soil N contents (as also indicated by the lower C:N ratio), which in turn is beneficial for primary production, and thus organic matter inputs to soil. Hence, there is a temperature-dependent positive feedback (high N$_2$ fixation leading to high primary production, leading to high soil organic matter stocks, leading to high N$_2$ fixation) (Graphical abstract). This loop might also contribute to the positive correlation between MAT and N stocks of the organic layer in forest in Sweden (Spohn and Stendahl, 2022).

The proposed feedback of N$_2$ fixation and organic C accumulation is supported by previous studies showing a positive effect of N addition on the sequestration of C in plant biomass and soils of Northern forests (Gundale et al., 2014; Jörgensen et al., 2021; Maaroufi et al., 2015; Sponseller et al., 2016). Northern forests are strongly N limited due to the low N inputs and slow organic N mineralization (Högberg et al., 2017; Sponseller et al., 2016; Tamm et al., 1999), thus increased inputs of N can alleviate plant N limitation and enhance primary productivity and C accumulation (Maaroufi et al., 2015; Sponseller et al., 2016). However, further studies should confirm the proposed feedback of N$_2$ fixation and organic C accumulation.

Based on our data it is possible to estimate the annual N input to the forest soils by non-symbiotic N_2 fixation. We estimated the N input based on the number of days per year with temperatures above 5 °C (Table S1) using the non-symbiotic N_2 fixation per g of soil determined at 5 °C (no significant differences were detected among the three incubation temperatures, see section 4.2). The annual N input by non-symbiotic N_2 fixation in soils ranged between 2 and 10 kg N ha^{-1} yr^{-1} at the coldest and warmest end of the temperature gradient, respectively. The magnitude of our estimate is higher than the commonly observed N_2 fixation rates by the moss-cyanobacteria association in boreal regions (1–4 kg N ha^{-1} yr^{-1}) (DeLuca et al., 2002; Lindo et al., 2013; Rousk et al., 2013) and is similar to the estimates of N accumulation in boreal forest that range between 3–3.5 kg N ha^{-1} yr^{-1} (Berg and Dise, 2004) and 7 kg N ha^{-1} yr^{-1} (Korhonen et al., 2013). This highlights the importance of non-symbiotic N_2 fixation in Northern forest soils.

The $\delta^{15}N$ of the soil organic layer was negatively correlated with MAT ($R^2 = 0.45$; Fig. 5A), which may suggest higher N inputs at the warmer sites, as previously described for another set of forest soils in Sweden (Spohn and Stendahl, 2023). At the warmer sites, the higher N inputs from N_2 fixation (with $\delta^{15}N \approx 0$ ‰) can decrease the $\delta^{15}N$ of the soil organic layer. In contrast, at the colder sites, the lower N inputs promote recycling of N by biota, causing the ^{15}N enrichment of the organic layer since mycorrhiza provide plants preferentially with the lighter N isotope (Spohn and Stendahl, 2023). Altogether, our results suggest that the non-symbiotic N_2 fixation in soils plays a key role in the increase in N stocks with increasing MAT, and hence, in the entire N cycle in Northern forest soils.

4.2. Non-symbiotic N_2 fixation is less sensitive to changes in temperature than C mineralization in Northern forest soils

In contrast to our second hypothesis, the non-symbiotic N_2 fixation per g of soil was not affected by the incubation temperature (Fig. 2C). This contrasts with previous studies describing that nitrogenase activity is temperature-dependent (Houlton et al., 2008). This lack of effect of incubation temperature on N_2 fixation suggests that other factors rather than temperature limited the non-symbiotic N_2 fixation in the soils. Our findings also contrast with the positive (although moss-specie dependent) response of N_2 fixation by cyanobacteria-moss association in Northern forests to increasing temperature (Rousk et al., 2013) which indicates that the drivers of both processes differ. The pH in our study was very low ranging between 3.45 and 4.32 (Table 1) which could have limited the response of non-symbiotic N_2 fixation per g of soil to differences in incubation temperature. Previous studies have shown a strong limiting effect of acid soil pH on the non-symbiotic N_2 fixation in forest soils as well as a positive effect of liming (Limmer and Drake, 1996; Nohrstedt, 1985). Liming affects the soil pH and thus the growth and stability of the diazotrophic communities (Fan et al., 2018; Limmer and Drake, 1996; Yang et al., 2022) and the bioavailability of molybdenum, a key component of the nitrogenase enzyme that is scarce in acid boreal soils (Barron et al., 2008; Rousk et al., 2017).

C mineralization per g of soil increased strongly with raising incubation temperature (Fig. 2D) which confirms the sensitivity of C mineralization to temperature (Bond-Lamberty and Thomson, 2010; Davidson and Janssens, 2006; Kurz et al., 2013; von Lützow and Kögel-Knabner, 2009). We observed an increase in C mineralization by a factor of 2.2 for the rise of incubation temperature from 5 to 12°C and from 12 to 20°C, which slightly exceeds the rule-of-thumb of doubling rates of soil organic matter mineralization for every 10° rise in temperature (Davidson and Janssens, 2006). This clear positive effect of increasing incubation temperature on C mineralization per g of soil contrasts with the lack of effect on non-symbiotic N_2 fixation per g of soil, indicating that the drivers of both processes differ. Similarly, a recent study showed a lower sensitivity of N_2 fixation than of C mineralization to changes in soil water content (Seuss et al., 2022). However, to our knowledge this is the first study to show the different sensitivity of C mineralization and non-symbiotic N_2 fixation to changes in temperature and it suggests a divergent effect of increasing temperatures due to climate change in these two central ecosystem processes.

The $\delta^{13}C$ of the CO_2-C formed during the incubation of the soil organic layer decreased significantly with increasing incubation temperature ($p < 0.001$; Fig. 6). The ^{13}C depletion with increasing temperature confirms the results obtained in previous studies (Andrews et al., 2000; Biasi et al., 2005; Bol et al., 2003) and indicates a shift in the C pool being mineralized with increasing incubation temperature. More complex compounds, such as lignin are ^{13}C depleted compared to more labile compounds, such as cellulose or proteins (Andrews et al., 2000; Biasi et al., 2005; Fernandez et al., 2003). Thus, our $\delta^{13}C$-CO_2 results suggest that elevated incubation temperatures promote the mineralization of more complex C pools. Therefore, the predicted future increase in temperature could promote the mineralization of complex C compounds that have been preserved in the organic layer of Northern forests until now due to temperature constraints.

4.3. Mean annual temperature strongly affects the C mineralization along a temperature gradient

C mineralization per g of soil organic layer decreased with increasing MAT ($p < 0.001$; Fig. 2D). This could be explained either by a larger fraction of slow-turnover C compounds at the warmer sites (Bringmark et al., 2011; Laganière et al., 2015) that limits C mineralization (von Lützow and Kögel-Knabner, 2009) or by a higher N availability (i.e., lower C:N ratio) at the warmer sites that reduces C mineralization (Jörgensen et al., 2022, 2021; Maaroufi et al., 2015). The first explanation assumes that accumulated C mineralization is generally higher at sites with higher MAT (more warm days per year) where the soils are relatively enriched in slow-turnover C compounds as the fast-turnover C compounds are quickly mineralized (Bringmark et al., 2011; Laganière et al., 2015). This explanation is supported by the positive correlation of $\delta^{13}C$ of the organic layer with MAT ($R^2 = 0.53$ Fig. 6B) that could be interpreted as a relatively greater accumulation of slow-turnover C compounds at the warmer sites, similar as discussed above for the effect of incubation temperature. When decomposing soil organic matter, microbes preferentially respire the lighter ^{12}C, which causes an enrichment of ^{13}C in the soil (kinetic fractionation) (Fernandez et al., 2003; Högberg et al., 2004). Thus, higher $\delta^{13}C$ in soil organic layer might be interpreted as enhanced C mineralization and the relative accumulation of slow-turnover C. However, we are cautious with this interpretation because previous studies have also shown differences in the plant δ ^{13}C along temperature gradients which can also affect the soil $\delta^{13}C$ (Ortiz et al., 2016).

The second explanation of the decrease in C mineralization with increasing MAT (higher N availability at sites with higher MAT) is based on the classical N mining hypothesis for organic matter mineralization (Craine et al., 2007) that has also been observed in Northern forest upon N addition (Jörgensen et al., 2022, 2021; Maaroufi et al., 2015). As N availability increases, the need for microbial exploitation of organic stocks for N acquisition decreases reducing the C mineralization (Janssens et al., 2010). Therefore, as the C:N ratio is negatively correlated with MAT ($R^2 = 0.68$; Fig. 5C), a lower microbial N mining (i.e, microbial decomposition driven by need for N) can be expected at the warmer sites. The relationship between C:N ratio and MAT have been previously described in Swedish forests and have been related to higher atmospheric N deposition at the southern sites (Högberg et al., 2021; Spohn and Stendahl, 2022) and a positive relationship between MAT and N_2 fixation as observed in our data.

The response of C mineralization per g of soil to the increase in incubation temperature from 5 to 20°C normalized by the C mineralization at 5°C correlated positively with MAT (Fig. 4). This indicates that the sensitivity of C mineralization to temperature changes was higher at sites with higher MAT. This can be explained by the C quality-temperature hypothesis that proposes that the temperature sensitivity of decomposition is higher for recalcitrant C compounds than for labile ones (Davidson and Janssens, 2006). Because recalcitrant C compounds are likely positively correlated with MAT (Bringmark et al., 2011; Laganière et al., 2015) as indicated by the $\delta^{13}C$ of our soils, a greater effect of increasing temperature on C mineralization can be expected at the sites with higher MAT. Our results (higher normalized response of C mineralization to higher incubation temperatures with higher MAT) contrast with previous studies about boreal regions reporting no difference in C mineralization response among sites with contrasting MAT (Laganière et al., 2015; Vanhala et al., 2008). The contrasting results among our study and previous studies could be caused by the fact that we studied a wider MAT gradient (7.3°C in our study versus 5.6 and 4.5°C in the previous studies, respectively).

5. Conclusions

Our results show that C mineralization is more sensitive to changes in incubation temperature in a range of 5 to 20°C than non-symbiotic N_2 fixation. To our knowledge, this is the first study showing the different sensitivities of C mineralization and non-symbiotic N_2 fixation to short-term changes in temperature. In addition, we observed a strong negative relationship between MAT and C mineralization that could be caused either by a relatively enrichment of the soil with slow-turnover C compounds or a higher N availability at the warmer sites.

We found a positive relationship between MAT and the non-symbiotic N_2 fixation per area, and this was mainly caused by the positive relationship between MAT and the organic layer stock rather than by the direct effect of temperature on the process rates. In contrast, no relationship was observed between MAT and non-symbiotic N_2 fixation when expressed per g of soil. Our results suggest that the higher non-symbiotic N_2 fixation rates per area at the sites with higher MAT can contribute to the accumulation of organic C in soils through a positive effect on primary production. Increased N_2 fixation at the sites with high MAT leads to increased soil N contents, which in turn is beneficial for primary production and organic matter inputs to soil, and thus higher soil organic matter stocks and higher N_2 fixation. In addition, the increased N availability at warmer sites due to non-symbiotic N_2 fixation may also promote the C accumulation by limiting the C mineralization (lower N mining).

Finally, the magnitude of our annual estimates of non-symbiotic N_2 fixation in soil (2 and 10 kg N ha^{-1} yr^{-1} at the coldest and warmest end of the temperature gradient) is higher than the commonly observed N_2 fixation rates by the moss-cyanobacteria association in boreal regions which highlights the importance of non-symbiotic N_2 fixation in Northern forest soils. Future studies should improve these annual estimates of non-symbiotic N_2 fixation in soil by incorporating measurements across different periods of the season, including periods with temperatures below 5 °C. This would help to identify the minimum temperature threshold for non-symbiotic N_2 fixation in the soils of Northern forests.

CRediT authorship contribution statement

Eduardo Vázquez: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marie Spohn:** Writing – original draft, Validation, Supervision, Project administration, Investigation, Funding acquisition, Data curation, 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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2024.117128.

Data availability

Data will be made available on request.

References

Akselsson, C., Berg, B., Meentemeyer, V., Westling, O., 2005. Carbon sequestration rates in organic layers of boreal and temperate forest soils — Sweden as a case study. Glob. Ecol. Biogeogr. 14, 77–84. https://doi.org/10.1111/J.1466-822X.2004.00133.X.

Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob. Chang. Biol. 14, 2898–2909. https://doi.org/10.1111/J.1365-2486.2008.01716.X.

Alvarenga, D.O., Rousk, K., 2021. Indirect effects of climate change inhibit N2 fixation associated with the feathermoss Hylocomium splendens in subarctic tundra. Sci. Total Environ. 795. https://doi.org/10.1016/j.scitotenv.2021.148676.

Andrews, J.A., Matamala, R., Westover, K.M., Schlesinger, W.H., 2000. Temperature effects on the diversity of soil heterotrophs and the δ13C of soil-respired CO2. Soil Biol. Biochem. 32, 699–706. https://doi.org/10.1016/S0038-0717(99)00206-0.

Barron, A.R., Wurzburger, N., Bellenger, J.P., Wright, S.J., Kraepiel, A.M.L., Hedin, L.O., 2008. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. Nature Geoscience 2009 2:1 2, 42–45. https://doi.org/10.1038/ngeo366.

Berg, B., Dise, N., 2004. Calculating the long-term stable nitrogen sink in northern European forests. Acta Oecologica 26 (1), 15–21. https://doi.org/10.1016/j.actao.2004.03.003.

Bergh, J., Nilsson, U., Allen, H.L., Johansson, U., Fahlvik, N., 2014. Long-term responses of Scots pine and Norway spruce stands in Sweden to repeated fertilization and thinning. For. Ecol. Manage. 320, 118–128. https://doi.org/10.1016/J.FORECO.2014.02.016.

Biasi, C., Rusalimova, O., Meyer, H., Kaiser, C., Wanek, W., Barsukov, P., Junger, H., Richter, A., 2005. Temperature-dependent shift from labile to recalcitrant carbon sources of arctic heterotrophs. Rapid Commun. Mass Spectrom. 19, 1401–1408. https://doi.org/10.1002/RCM.1911.

Bol, R., Bolger, T., Cully, R., Little, D., 2003. Recalcitrant soil organic materials mineralize more efficiently at higher temperatures. J. Plant Nutr. Soil Sci. 166, 300–307. https://doi.org/10.1002/JPLN.200390047.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature 2010 464:7288 464, 579–582. https://doi.org/10.1038/nature08930.

Bringmark, E., Bringmark, L., Sonesten, L., Mjöfors, K., Johansson, M.B., 2011. Long-term monitoring of scots pine litter decomposition rates throughout sweden indicates formation of a more recalcitrant litter in the south. Ambio 40, 878–890. https://doi.org/10.1007/S13280-011-0202-0/FIGURES/6.

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1.

Dabundo, R., Lehmann, M.F., Treibergs, L., Tobias, C.R., Altabet, M.A., Moisander, P.H., Granger, J., 2014. The contamination of commercial 15N2 gas stocks with 15N–labeled nitrate and ammonium and consequences for nitrogen fixation measurements. PLoS One 9, e110335. https://doi.org/10.1371/JOURNAL.PONE.0110335.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 2006 440:7081 440, 165–173. https://doi.org/10.1038/nature04514.

DeLuca, T.H., Zackrisson, O., Nilsson, M.C., Sellstedt, A., 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 2002 419:6910 419, 917–920. https://doi.org/10.1038/nature01051.

DeLuca, T.H., Zackrisson, O., Gundale, M.J., Nilsson, M.C., 2008. Ecosystem feedbacks and nitrogen fixation in boreal forests. Science (1979) 320, 1181. https://doi.org/10.1126/science.1154836.

Fan, K., Weisenhorn, P., Gilbert, J.A., Shi, Y., Bai, Y., Chu, H., 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. Soil Biol. Biochem. 121, 185–192. https://doi.org/10.1016/J.SOILBIO.2018.03.017.

Fernandez, I., Mahieu, N., Cadisch, G., 2003. Carbon isotopic fractionation during decomposition of plant materials of different quality. Global Biogeochem. Cycles 17. https://doi.org/10.1029/2001GB001834.

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A.Z., Schepaschenko, D.G., 2015. Boreal forest health and global change. Science 1979 (349), 819–822. https://doi.org/10.1126/science.aaa9092.

Gundale, M.J., Nilsson, M., Bansal, S., Jäderlund, A., 2012a. The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. New Phytol. 194, 453–463. https://doi.org/10.1111/j.1469-8137.2012.04071.x.

Gundale, M.J., Wardle, D.A., Nilsson, M.C., 2012b. The effect of altered macroclimate on N-fixation by boreal feather mosses. Biol. Lett. 8, 805–808. https://doi.org/10.1098/rsbl.2012.0429.

Gundale, M.J., From, F., Bach, L.H., Nordin, A., 2014. Anthropogenic nitrogen deposition in boreal forests has a minor impact on the global carbon cycle. Glob. Chang. Biol. 20, 276–286. https://doi.org/10.1111/GCB.12422.

Högberg, P., Ekblad, A., Nordgren, A., Plamboeck, A.H., Ohlsson, A., Bhupinderpal-Singh, H., 2004. Factors determining the 13C abundance of soil-respired CO2 in boreal forests. Stable Isotopes and Biosphere - Atmosphere Interactions: Processes and Biological Controls 47–68. https://doi.org/10.1016/B978-012088447-6/50004-0.

Högberg, P., Näsholm, T., Franklin, O., Högberg, M.N., 2017. Tamm review: on the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2017.04.045.

Högberg, P., Wellbrock, N., Högberg, M.N., Mikaelsson, H., Stendahl, J., 2021. Large differences in plant nitrogen supply in German and Swedish forests – implications for management. For. Ecol. Manage. 482. https://doi.org/10.1016/j.foreco.2020.118899.

Houlton, B.Z., Wang, Y.P., Vitousek, P.M., Field, C.B., 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454, 327–330. https://doi.org/10.1038/nature07028.

Huss-Danell, K., Lundquist, P.-O., Ohlsson, H., 2011. N2 fixation in a young Alnus incana stand, based on seasonal and diurnal variation in whole plant nitrogenase activity. https://doi.org/10.1139/b92-193 70, 1537–1544. https://doi.org/10.1139/B92-193.

Intergovernmental Panel on Climate Change, 2023. Future Global Climate: Scenario-based Projections and Near-term Information. Climate Change 2021 – The Physical Science Basis 553–672. https://doi.org/10.1017/9781009157896.006.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 2010 3:5 3, 315–322. https://doi.org/10.1038/ngeo844.

Jarvis, P., Linder, S., 2000. Constraints to growth of boreal forests. Nature 2000 405: 6789 405, 904–905. https://doi.org/10.1038/35016154.

Jörgensen, K., Granath, G., Lindahl, B.D., Strengbom, J., 2021. Forest management to increase carbon sequestration in boreal Pinus sylvestris forests. Plant and Soil 466, 165–178. https://doi.org/10.1007/S11104-021-05038-0/FIGURES/5.

Jörgensen, K., Granath, G., Strengbom, J., Lindahl, B.D., 2022. Links between boreal forest management, soil fungal communities and below-ground carbon sequestration. Funct. Ecol. 36, 392–405. https://doi.org/10.1111/1365-2435.13985/SUPPINFO.

Jungqvist, G., Oni, S.K., Teutschbein, C., Futter, M.N., 2014. Effect of climate change on soil temperature in swedish boreal forests. PLoS One 9, e93957. https://doi.org/10.1371/JOURNAL.PONE.0093957.

Kurz, W.A., Shaw, C.H., Boisvenue, C., Stinson, G., Metsaranta, J., Leckie, D., Dyk, A., Smyth, C., Neilson, E.T., 2013. Carbon in Canada's boreal forest-A synthesis. Environ. Rev. 21, 260–292. https://doi.org/10.1139/ER-2013-0041/ASSET/IMAGES/LARGE/ER-2013-0041F10.JPEG.

Laganière, J., Podrebarac, F., Billings, S.A., Edwards, K.A., Ziegler, S.E., 2015. A warmer climate reduces the bioreactivity of isolated boreal forest soil horizons without increasing the temperature sensitivity of respiratory CO2 loss. Soil Biol. Biochem. 84, 177–188. https://doi.org/10.1016/J.SOILBIO.2015.02.025.

Limmer, C., Drake, H.L., 1996. Non-symbiotic N2-fixation in acidic and pH-neutral forest soils: aerobic and anaerobic differentials. Soil Biol. Biochem. 28, 177–183. https://doi.org/10.1016/0038-0717(95)00118-2.

Lindo, Z., Nilsson, M.C., Gundale, M.J., 2013. Bryophyte-cyanobacteria associations as regulators of the northern latitude carbon balance in response to global change. Glob. Chang. Biol. https://doi.org/10.1111/gcb.12175.

Maaroufi, N.I., Nordin, A., Hasselquist, N.J., Bach, L.H., Palmqvist, K., Gundale, M.J., 2015. Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. Glob. Chang. Biol. 21, 3169–3180. https://doi.org/10.1111/GCB.12904.

Nohrstedt, H.O., 1985. Nonsymbiotic nitrogen fixation in the topsoil of some forest stands in central Sweden. Canadian Journal Forest Research 15, 715–722. https://doi.org/10.1139/X85-116.

Ortiz, C., Vázquez, E., Rubio, A., Benito, M., Schindlbacher, A., Jandl, R., Butterbach-Bahl, K., Díaz-Pinés, E., 2016. Soil organic matter dynamics after afforestation of mountain grasslands in both a Mediterranean and a temperate climate. Biogeochemistry 131, 267–280. https://doi.org/10.1007/S10533-016-0278-5/FIGURES/6.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 1979 (333), 988–993. https://doi.org/10.1126/SCIENCE.1201609/SUPPL_FILE/PAPV2.PDF.

Rousk, K., Jones, D.L., DeLuca, T.H., 2013. Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00150.

Rousk, K., Sorensen, P.L., Lett, S., Michelsen, A., 2015. Across-habitat comparison of diazotroph activity in the subarctic. Microb. Ecol. 69, 778–787. https://doi.org/10.1007/s00248-014-0534-y.

Rousk, K., Michelsen, A., Rousk, J., 2016. Microbial control of soil organic matter mineralization responses to labile carbon in subarctic climate change treatments. Glob. Chang. Biol. 22, 4150–4161. https://doi.org/10.1111/GCB.13296.

Rousk, K., Degboe, J., Michelsen, A., Bradley, R., Bellenger, J.P., 2017. Molybdenum and phosphorus limitation of moss-associated nitrogen fixation in boreal ecosystems. New Phytol. 214, 97–107. https://doi.org/10.1111/NPH.14331.

Salazar, A., Rousk, K., Jónsdóttir, I.S., Bellenger, J.P., Andrésson, Ó.S., 2020. Faster nitrogen cycling and more fungal and root biomass in cold ecosystems under experimental warming: a meta-analysis. Ecology 101. https://doi.org/10.1002/ECY.2938.

Seuss, I., Scheibe, A., Spohn, M., 2022. N2 fixation is less sensitive to changes in soil water content than carbon and net nitrogen mineralization. Geoderma 424, 115973. https://doi.org/10.1016/J.GEODERMA.2022.115973.

Sorensen, P.L., Michelsen, A., 2011. Long-term warming and litter addition affects nitrogen fixation in a subarctic heath. Glob. Chang. Biol. 17, 528–537. https://doi.org/10.1111/j.1365-2486.2010.02234.x.

Spohn, M., 2016. Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic Appl. Ecol. 17, 471–478. https://doi.org/10.1016/J.BAAE.2016.05.003.

Spohn, M., Stendahl, J., 2022. Carbon, nitrogen, and phosphorus stoichiometry of organic matter in Swedish forest soils and its relationship with climate, tree species, and soil texture. Biogeosciences 19, 2171–2186. https://doi.org/10.5194/BG-19-2171-2022.

Spohn, M., Stendahl, J., 2023. Spatial patterns of nitrogen isotope ratios in forest soils are related to latitude and soil phosphorus concentration. Biogeochemistry 165, 43–56. https://doi.org/10.1007/S10533-023-01058-3/FIGURES/7.

Sponseller, R.A., Gundale, M.J., Futter, M., Ring, E., Nordin, A., Näsholm, T., Laudon, H., 2016. Nitrogen dynamics in managed boreal forests: recent advances and future research directions. Ambio 45, 175–187. https://doi.org/10.1007/s13280-015-0755-4.

Tamm, C.O., Aronsson, A., Popovic, B., Flower-Ellis, J., 1999. Optimum nutrition and nitrogen saturation in Scots pine stands.

Vanhala, P., Karhu, K., Tuomi, M., Björklöf, K., Fritze, H., Liski, J., 2008. Temperature sensitivity of soil organic matter decomposition in southern and northern areas of the boreal forest zone. Soil Biol. Biochem. 40, 1758–1764. https://doi.org/10.1016/J.SOILBIO.2008.02.021.

von Lützow, M., Kögel-Knabner, I., 2009. Temperature sensitivity of soil organic matter decomposition-what do we know? Biol. Fertil. Soils 46, 1–15. https://doi.org/10.1007/S00374-009-0413-8/TABLES/2.

Yang, Z., Chen, X., Hou, J., Liu, H., Tan, W., 2022. Soil texture and pH exhibit important effects on biological nitrogen fixation in paddy soil. Appl. Soil Ecol. 178, 104571. https://doi.org/10.1016/J.APSOIL.2022.104571.

Zechmeister-Boltenstern, S., 1996. Non-symbiotic nitrogen fixation. Pages 122–134 in F. Schinner, R. € Ohlinger, E. Kandeler, and R. Margesin, editors. Methods in soil biology. First edition. Springer, Heidelberg, Germany.