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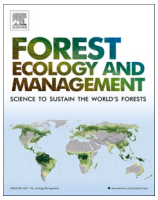

Nitrogen fertilization increases N_2O emission but does not offset the reduced radiative forcing caused by the increased carbon uptake in boreal forests

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ABSTRACT

Net primary production in boreal coniferous forests is generally severely limited by N deficiency. Nitrogen fertilization has thus the potential to strongly increase forest tree biomass production in the boreal region and consequently increase the biosphere uptake of atmospheric CO_2. Increased N availability may though increase the production and emission of soil N_2O, counteracting the climate mitigation potential from increased forest biomass production. Studies in the boreal region on the net effect on the climate mitigation potential from N fertilization are scarcer than in other biomes. Therefore, we explored how N affected soil GHG fluxes in two boreal field N-loading experiments, of which one is a long-term experiment (40 years), and the other established 6 years before investigation. We also estimated whether the increased soil N_2O emission could offset the N-driven increased C sequestration by the trees. Nitrogen additions affected the soil GHG fluxes in both stands. Soil N_2O emission was enhanced by N addition at every fertilization rate, though marginally compared to the reduced soil CO_2 emission and the increased atmospheric CO_2 uptake and biomass production. The estimated annual uptake of CH_4 by soil under long-term N addition increased. The magnitude of soil CH_4 uptake was on the same order of magnitude as the increase in soil N_2O emissions caused by N addition, when compared as CO_2 equivalents. In conclusion, forest N fertilization in boreal areas increased the GHG net uptake and, thus, provides a means to mitigate increasing atmospheric concentrations of GHG.

1. Introduction

Boreal forests are the largest forest biome on Earth and, as such, play a major role in the global carbon (C) cycle (Artaxo et al., 2022). However, in many regions of the boreal climate zone, the tree growth is limited by nitrogen (N) availability (Högberg et al., 2017; Tamm, 1991). The main natural source of N in boreal forests is the biological N fixation, which, according to the current best estimate, provides 1.3 kg N ha^{-1} yr^{-1} (range 0.6–2.5 kg N ha^{-1} yr^{-1}) to the soil (Du and de Vries, 2018). The current background N deposition in Northern Sweden is around 2 kg N ha^{-1} yr^{-1} and, consequently, forest tree growth is considered N-limited (Binkley and Högberg, 2016; Högberg et al., 2017). The Swedish Forest Agency recommends forest fertilization as a common practice to increase biomass yield (Nohrstedt, 2001). The general practice is to fertilize with ~ 150 kg N ha^{-1} yr^{-1} two to four times during a rotation at intervals of at least 10 years (Nohrstedt, 2001). Indeed, From et al. (2015) report that the fertilization does not affect forests only during 10 years from the application, as previously thought, but causes residual long-lasting effects that may last over the stand rotation. The concept of increasing N fertilization in boreal forest stands have recently been proposed as an efficient way to mitigate climate change by enhancing ecosystem C sequestration (Jorgensen et al., 2021).

Many experiments have studied the effect of N addition as means to

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increase both forest biomass growth and ecosystem C sequestration. In general, moderate to low N addition has positive effects on forest productivity, since it enhances C sequestration and biomass accumulation (De Vries et al., 2006; Jorgensen et al., 2021). In general, N-addition may increase stem wood production has been documented in several field experiments in boreal forests (e.g. Saarsalmi and Mälkönen, 2001), but the comprehensive response on ecosystem carbon sequestration is uncertain. For example, Lim et al. (2015) conclude that, although the above ground net primary productivity (NPP) in a Scots pine stand increases as a response to N addition, reductions in below ground allocation and root biomass production may counteract stem wood increment. From an ecosystem perspective, however, Zhao et al. (2022) that showed that the C sink capacity of the forest stand in the same experiment increased after long-term N addition to a new steady state. In addition, long-term fertilization has been shown to increase soil organic matter, carbon and nitrogen contents (Saarsalmi et al., 2014). However, N-addition may also lead to less desirable effects, such as impacts on soil greenhouse gas (GHG) flux dynamics, including CO_2, methane (CH_4), and nitrous oxide (N_2O) (Oertel et al., 2016). Indeed, Zaehle et al. (2011) indicate that the increasing soil N_2O emission, because of N-addition, may counteract a mitigating effect on atmospheric radiative forcing from increased ecosystem C sequestration, potentially impacting the sustainability of N fertilization to increase stem wood production.

The N_2O is a powerful GHG, with a lifetime of 121 years and a 100-year global warming potential (GWP100) 273-times higher than CO_2 (IPCC, 2021). N_2O is formed in soil by several different biogeochemical processes and pathways involving N transformation (cf. Hu et a, 2015). This includes denitrification (Tidje, 1988), nitrification-related pathways (including ammonium oxidation and nitrifier denitrification; Wrage et al., 2001), and dissimilatory nitrate reduction to ammonium (Rutting et al., 2011). The N_2O exchange in northern forest ecosystems are believed to be rather small and soils are either small sources or small sinks for atmospheric N_2O (Kim et al., 2013). Soil N_2O production and emission is known to highly variable in both time and space. Environmental factors, such as temperature, soil moisture, O_2 regime, and soil pH influences N_2O production rates. In addition, the variability is further enhanced because different N_2O forming processes respond differently to changes in controlling factors. In N poor systems the availability of N plays an important role in regulating N_2O production and N deposition and N-fertilization may very distinctly increase soil N_2O emission (Jassal et al., 2011; Li et al., 2019; Sitaula et al., 1995; Xu et al., 2017). So far, studies exploring the influence of N fertilization on soil N_2O emission in boreal forests are scarce (Aronson et al., 2012). Recent measurements, however, are contradictory and, e.g., Rütting et al. (2021) conclude that soil N_2O emission from both N-loaded (50 kg N ha^{-1} yr^{-1}) plots as well as control plots in a boreal Norway spruce stand remains low. At a global scale, modelling studies (Deng et al., 2020; Liu and Greaver, 2009; Tian et al., 2015; Zaehle and Dalmonech, 2011) suggest that increased N availability by N deposition may counteract the increment of biomass accumulation by increasing soil N_2O emission (Zaehle et al., 2011). However, field-based experimental data is needed to validate these model predictions. Thus, despite importance of net GHG dynamics on the overall impact of forest N fertilization, empirical proof is lacking, currently preventing evaluation of an integrated effect on the ecosystem level.

The CH_4 is another most powerful atmospheric GHG, with a warming potential 28-times higher (GWP100) than CO_2 (IPCC, 2021). Soil worldwide is an important sink of CH_4, especially in forest ecosystems, with an estimated annual uptake of 17–44 Tg CH_4 (Dutaur and Verchot, 2007). Earlier studies suggest N addition reduces CH_4 uptake (e.g., Liu and Greaver, 2009; Yang et al., 2017). However, litterateur reports on the long-term effect on soil CH_4 oxidation are inconclusive and, while some investigations report negative or no effect on oxidation rates (Börjesson and Nohrstedt, 2000), others, in fact, see a positive correlation between inorganic N availability and CH_4 oxidation rates (Goldman et al., 1995; Castro et al., 1995).

The soil CO_2 emission deriving from autotrophic and heterotrophic respiration is the major C loss in forest ecosystems. The forest C and N cycles are strongly coupled (Zaehle and Dalmonech, 2011), but the influence of N addition on soil CO_2 respiration is uncertain. For instance, previous studies have shown both a decrease (Allison et al., 2008; Bowden et al., 2019; Janssens et al., 2010; Olsson et al., 2005), or an increase in soil CO_2 emission (Jassal et al., 2011; Shrestha et al., 2015; Sitaula et al., 1995; Zhang et al., 2017) in response to N addition.

The research about the effect of N addition on GHG fluxes from boreal forest ecosystems is still quite limited. For example, Deng et al. (2020) present a compilation of studies on the change in CO_2 emission under altering N deposition, considering 202 reports from the temperate forests and only 24 from the boreal forests, and analyses on impacts on N_2O and CH_4 exchange are even scarcer. To address this shortcoming, we investigated the response of soil fluxes of CO_2, N_2O, and CH_4 in boreal pine (*Pinus sylvestris*) forest stands to increased N loading. The aim of this study was to provide empirical proof guiding future decisions on forest N fertilization practices. We hypothesized that long term N addition to *P. sylvestris* stands would:

- Increase C-sequestration and biomass production
- Decrease soil respiration rates
- Decrease soil CH_4 oxidation rates
- Increase soil N_2O emissions

Overall, when evaluating GHG fluxes from the perspective of radiative forcing as CO_2 equivalents, the increased N_2O emissions and reduced CH_4 oxidation and soil respiration rates, would counteract the increased ecosystem C sequestration.

2. Methods

2.1. Experimental sites

The study was conducted in two experimental sites designed to investigate the effects of N addition on boreal forest ecosystems. The first site is a boreal Scots pine (*Pinus sylvestris* (L.)) stand close to Norrliden, Västerbotten, Sweden (64° 21′ N, 19° 46′ E, 260–275 m a.s.l.). The annual mean temperature is 1.2 °C and the mean annual precipitation is 595 mm. The soil is glacial till with fine sand as the dominant fraction. The forest was previously an old spruce-dominated stand, which was clear-cut in 1951 and prescriptively burnt in 1952 (common practise for site preparation during that time) and planted with 2-year-old Scots pine seedlings in 1953. More information about the site and the experiment can be found in Tamm et al. (1999). The original purpose of the experiment was to investigate the impact of high levels of atmospheric N deposition on tree vitality and growth. The N addition experiment was commenced in 1971 at four different levels of N addition (N0, N1, N2, and N3), as NH_4NO_3, in a randomized block design (n = 3) (Table 1), N0 being the control. Each treatment was randomly assigned to three 30 × 30 m plots. The N was applied on the forest floor as fertilizer pellets in June every year. In N1 plots, N-addition is still ongoing, while in N3 and N2 plots, it was terminated in 1990 and 2008, respectively. Although no longer receiving N-additions these two treatments were included in the study as they offer an opportunity to study the forest stand recovery rate with respect to soil GHG exchange following termination of long-term N addition.

The second site is in the Rosinedal experimental forest (64° 10′ N, 19° 45′ E), near Vindeln, Västerbotten, Sweden (Lim et al., 2015; Metcalfe et al., 2013). The forest is a 90-year-old *Pinus sylvestris* stand. At the Svartberget field station 8 km from the study site, the mean temperature in the period 1981–2010 was 1.8 °C, and the annual mean precipitation in the same period was 614 mm. During the investigated year (2011), the annual mean temperature and precipitation were 3.5 °C and 647 mm, respectively, i.e. the annual air temperature was two times the 30-year average while the precipitation was close to the average. The

Table 1
N loading rates (kg N ha^{-1} yr^{-1}) and total N addition (kg N ha^{-1}) in Norrliden between 1971 and 2011, and for Rosinedal 2006–2011. At Norrliden the N3 treatment was terminated in 1990 and the N2 treatment was terminated in 2008 (Högberg et al., 2014).

Norrliden	**Treatment**			
Year	**N0**	**N1**	**N2**	**N3**
1971-1973	0	60	120	180
1974-1976	0	40	80	120
1977-1990	0	30	60	90
1991	0	0	0	0
1992	0	60	120	0
1993-2008	0	30	60	0
2009-2011	0	30	0	0
Total	**0**	**1350**	**2520**	**2160**
Rosinedal	**Treatment**			
Year	**Control**	**LN**	**HN**	
2006-2011	0	20	100	
Total	**0**	**120**	**600**	

background N deposition is about 2 kg N ha^{-1} $year^{-1}$.

The soil is fine sandy and silty glacial outwash sediments (Lim et al., 2017). The case study consisted of three un-replicated plots of 13 ha each: one plot acted as control (C), the second plot mimicked atmospheric N deposition at 20 kg N ha^{-1} yr^{-1} (hereafter referred to as low nitrogen, LN; Table 1) and the third plot was subjected to 100 kg N ha^{-1} yr^{-1} (hereafter referred to as high nitrogen, HN; Table 1). The N was applied on the forest floor in one annual application in mid-June, using Skog-Can fertilizer (Yara, Sweden) containing NH_4^+ (13.5%), NO_3^- (13.5), Ca (5%), Mg (2.4%), and B (0.2%) (Lim et al., 2015). Both sites are snow covered during the winter (approximately 150 days) with maximum snow depth of about 60 cm.

2.2. Greenhouse gas (GHG) sampling

The soil fluxes of CO_2, N_2O, and CH_4 were measured using manual dark static chambers during the snow-free season and by snow concentration gradients during winter. The measurements were started within one week of the N-addition. At Norrliden, fluxes were measured from July 2nd, 2010, to June 29th, 2011 at 28 measurement occasions. At Rosinedal, the measurements occurred from June 16th, 2011, to February 25th, 2013, at 43 measurement occasions. At Norrliden, permanent frames (0.48 ×0.48 m) for mounting chambers were installed in May 2010 (one frame per treatment plot, giving a replication following that of the original randomized block design of n = 3). At Rosinedal, six permanent frames (0.48 ×0.48 m) were installed in each of the three plots. The CO_2 fluxes were measured using a portable IRGA system (PP-systems) connected to a dark chamber (70 L). Emissions were based on the initial, linear, CO_2 increase during 3–5 min of measurements. For the N_2O and CH_4 measurements, gas samples were collected in pre-evacuated glass vials (22 ml) equipped with a gas-tight septum. A 30 ml of sample was taken from the chamber headspace and transferred to the GC-vials. At Norrliden, gas samples were taken from the chambers (volume 15 L) directly after closure and then at 15, 30, 45, and 60 min, while at Rosinedal the intervals were 17, 35, 52, and 70 min. The N_2O and CH_4 fluxes were analysed using a GC-FID/ECD (PerkinElmer Autosystem Gas Chromatograph). Separation was carried out on a Haysep N column using N_2 (40 ml min^{-1}) as carrier gas. The N_2O and CH_4 flux rates were calculated from the linear change of the gas concentration in the headspace of the chamber over time.

During the snow-covered seasons, the GHG flux estimates were derived from gas concentration measurements in the snow profile. Snow gas was sampled at the same locations as the permanent frames were installed (see above) using a stainless-steel probe (diameter 3 mm, length 100 cm) connected to a syringe. Thirty ml of gas was sampled and transferred to a pre-evacuated GC vial (22 ml) equipped with a gas-tight septum after which the pressure was equilibrated. Samples were taken at the interface between soil and snow and directly above the snowpack (Maljanen et al., 2009), which was documented to give a good approximation of the gas concentration gradient. GHG concentrations were analysed using GC-FID/ECD (as described above), equipped with a methanizer that reduced CO_2 to CH_4, which then entered in the GC. At each sampling point, the snow depth was recorded, and a snow core (diameter 15 cm) was extracted to determine the porosity of the snowpack. The intact snow samples were weighed for determining the average porosity of the profile using the density of pure ice (0.9168 g cm^{-3}). Calculations of the gas diffusion rates through the snowpack were done in accordance with Maljanen et al. (2003) and Sommerfeld et al. (1993), using diffusion coefficients of 0.14 cm^2 s^{-1} for N_2O and CO_2 and 0.25 cm^2 s^{-1} for CH_4. In Rosinedal, the measurements during the snow covered season of 2011/2012 were few due to technical problems. Therefore, a supplementary sampling campaign was carried out in the winter of 2012/2013, assuming that winter GHG fluxes remain relatively constant across years.

2.3. C stock in the standing biomass and net primary production (NPP)

The average C stock accumulation in tree biomass at Norrliden for the 1953–2010 period was estimated based on data from an inventory collected in June 2010, including biomass removal with stem wood in thinning in 1984 and 2000; the C content of that biomass was estimated based on biomass samples from different tree fractions sampled in October 2011. Biomass in different tree fractions (stem wood, bark, needles, living branches, dead branches) were estimated based on established biomass equations for Scots pine with tree diameter at 1.3 m height as explanatory variable (Marklund, 1988), while an equation by Petersson and Stahl (2006) was used for stump biomass including roots $\geq$ 2 mm. Carbon content in different tree fractions was based on biomass sampled and analysed for C content from two sample trees per plot randomly selected among the trees within the range of the mean diameter + /− 1 standard deviation. No biomass samples were taken from dead branches and stumps and roots. Carbon content in dead branches was, therefore, assumed to be the same as for living branches. Carbon content in stumps and roots were based on C content in stem wood, bark, and living branches with the weights 63%, 7%, and 30% of the biomass, respectively. With small differences between treatments, C content varied from 51% in needles up to almost 57% in the bark. N-induced C-sequestration was obtained by calculating the difference in C-stock between N1 and N0 plots. Data of NPP from the Rosinedal experiment was obtained from Lim et al. (2015). Their NPP estimates were based on destructive sampling of trees including stumps and coarse roots sampled before (five trees per plot) and six years after (six trees per plot) the fertilization commenced. Biomass data for different tree fractions were then used for site specific (treatment specific for foliage and cones) allometric equations that, together with analyses of C content in each fraction, were used for NPP estimates. For estimates of foliage and cones see detailed description in appendix A in Lim et al. (2015). The values were originally reported in g C m^{-2} yr^{-1} and were transformed in g CO_2 m^{-1} yr^{-1} to allow for comparison with the GHGs as CO_2-equivalents. NPP estimates were only available for the control and HN plots, and the N-induced NPP was obtained by calculating the difference in average annual C-sequestration between these treatments.

2.4. Statistical analysis and calculations

The statistical analysis was performed with R (version 3.6.1). The analysis of variance (ANOVA) was performed (base package), with the GHG fluxes as dependent variable and the date and treatment as independent variables. Normal distribution and homogeneity of variance were checked. When homogeneity of variance was not met, ANOVA was corrected with the weighted least squares.

Estimates of daily GHG fluxes were obtained by linear interpolation for each chamber between measurement points and were then summed

up to generate annual GHG budgets. The variance for the interpolated daily values included the effect of co-variance between the measurement occasions and included when propagating the SEs for the annual estimates. The cumulative flux for each chamber replicate was averaged for each treatment and analysed using ANOVA with Tukey's post hoc tests to compare treatment effects. The malfunction of the IRGA instrument during the latter part of the chamber measurements at Rosinedal made it impossible to construct a reliable annual CO_2 emission budget. In addition, technical problems with CH_4 analysis at Norrliden, during the first growing season (2010), prevented estimates of the annual CH_4 budget at this site.

3. Results

3.1. Norrliden

The N addition strongly influenced the N_2O emission (Fig. 1a). The average measured N_2O fluxes (μg m^{-2} d^{-1}) were 49 ± 4.35 (mean ± SE) in N0, 92 ± 8.52 in N1, 47 ± 17.77 in N2, and − 13 ± 16.27 in N3. The estimated cumulative annual fluxes (mg N_2O m^{-2} yr^{-1}) from N0, N1, N2, and N3 were 5.8 (2.5), 24.6 (7.8), 15.1 (9.2), and 2.1 (3.0), respectively. The annual N_2O emissions in N1 were significantly higher than in the control and N3. (Fig. 2a). The N_2O emission in N1 corresponded to ca. 0.5% of the annual N-addition (Table 2).

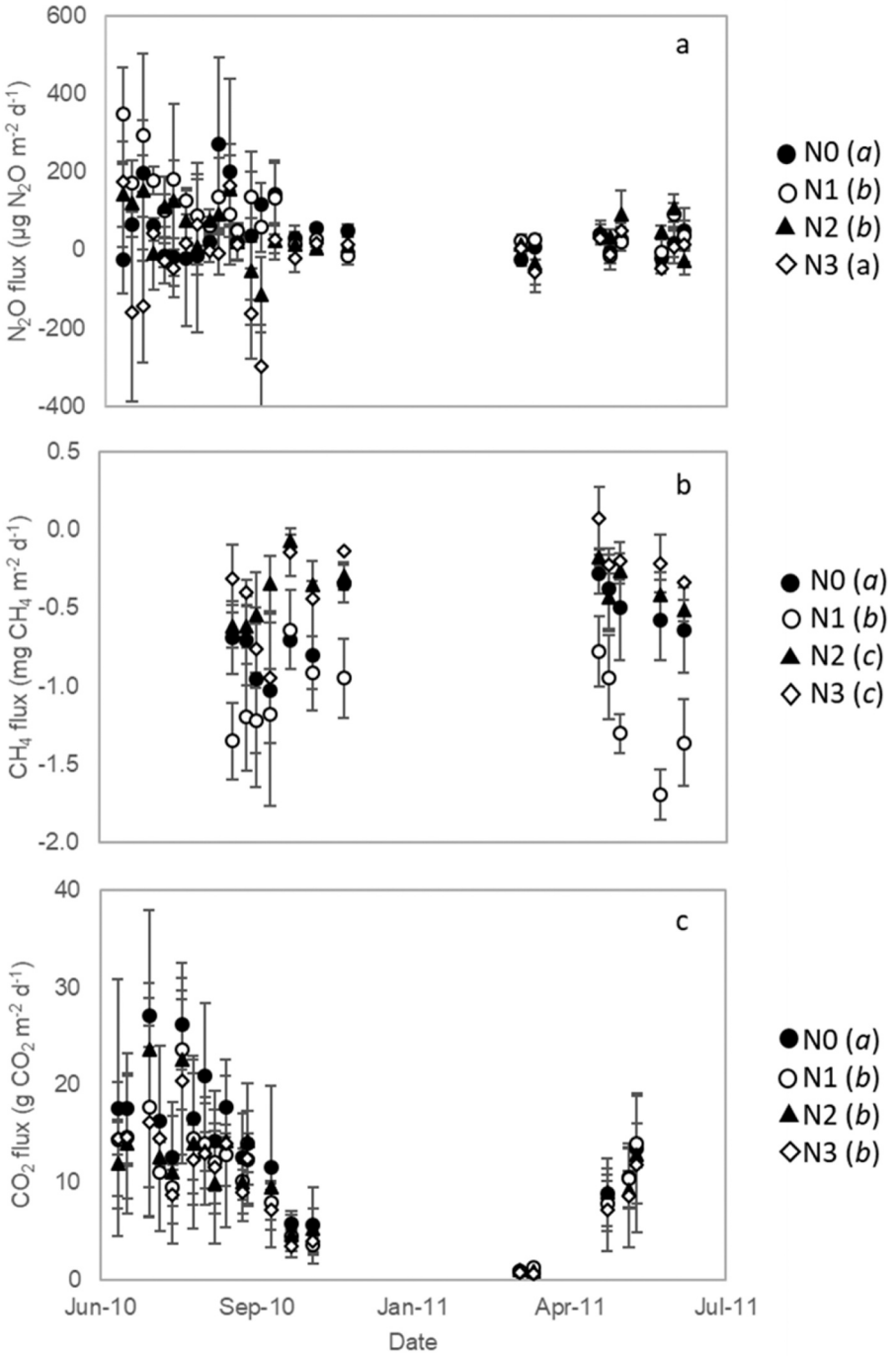

Fig. 1. Fluxes of (a) N_2O, (b) CH_4, and (c) CO_2 fluxes in Norrliden for N0, N1, N2, and N3 plots (2010–2011). Values are means; bars represent ± SE (n = 3). Significant differences among treatments are indicated by different letters ($p < 0.05$).

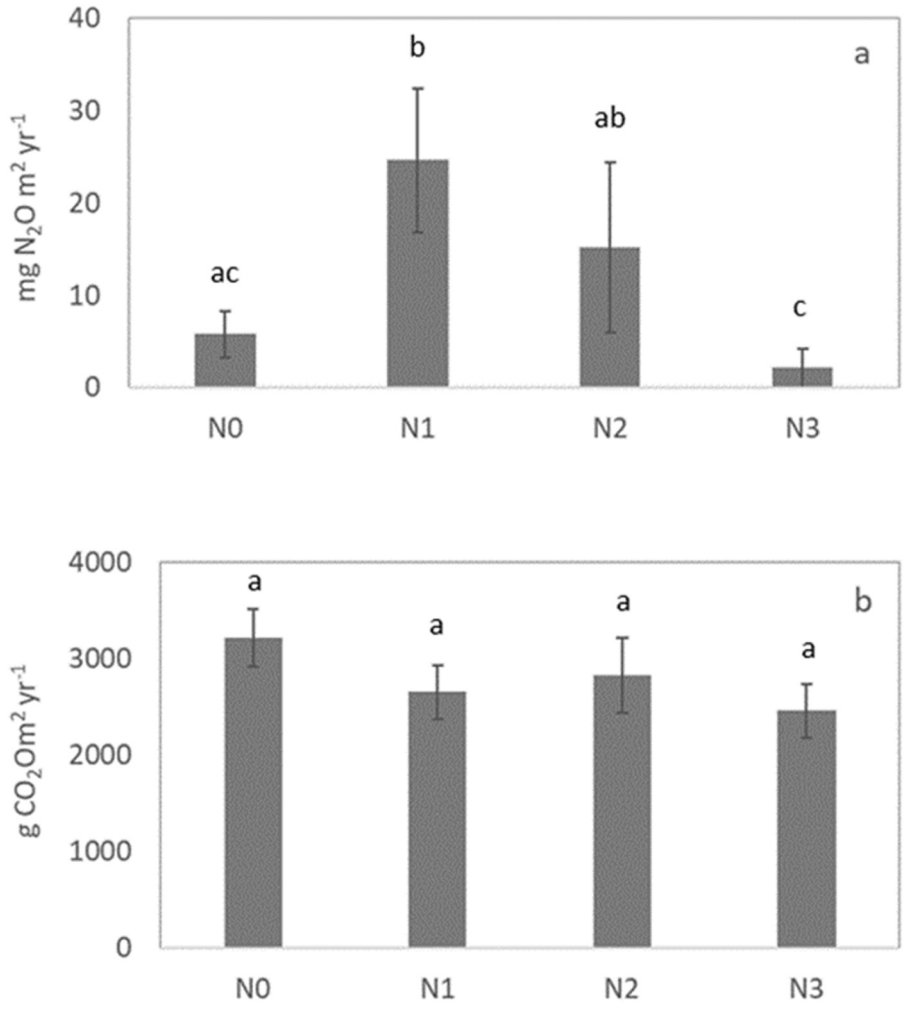

Fig. 2. Annual cumulative flux of (a) N_2O and (b) CO_2 in Norrliden for N0, N1, N2, and N3 plots. Significant differences among treatments are indicated by different letters ($p < 0.05$). Bars represent ± SE ($n = 3$).

Table 2
Comparison between annual emission of CO_2; annual emission of N_2O 2010–2011, expressed as g CO_2 equivalent m^{-2} yr^{-1} and kg N ha^{-1} yr^{-1}; average annual tree NPP (C sequestered in tree biomass during the period 1953–2010 (g CO_2 equivalent m^{-2} yr^{-1}); annual N addition; N emission factor as the ratio between N_2O emission and fertilization rate, in Norrliden.

	Unit of measurement	N0	N1	N2	N3
CO_2	g m^{-2} yr^{-1}	3204	2641	2823	2452
N_2O	CO_2-equivalent, g m^{-2} yr^{-1}	1.770	7.506	4.582	0.654
Tree NPP (1953-2010)	g CO_2 m^{-2} yr^{-1}	629.9	852.7	775.3	654.1
Δ Tree NPP (1953-2010)	g CO_2 m^{-2} yr^{-1}	-	222.8	145.4	24.2
N_2O emission	kg N ha^{-1} yr^{-1}	0.0369	0.1566	0.0962	0.0136
N addition rate	kg N ha^{-1} yr^{-1}	0	30	0	0
N emission factor	%		0.52		

All three treatment levels at Norrliden, as well as the control plots, were CH_4 sinks. The average rates (mg CH_4 m^{-2} d^{-1}) of the CH_4 flux were − 0.63 ± 0.26 (mean ± SE) in N0, − 1.13 ± 0.25 in N1, − 0.39 ± 0.1 in N2, and − 0.34 ± 0.15 in N3 (Fig. 1b). Significantly higher CH_4 uptake was observed in N1 than in N0, N2 and N3, while the CH_4 uptake did not significantly differ between N2 and N3 (Fig. 1b).

The rate of CO_2 emission was negatively affected by N-addition (Fig. 1c). Average CO_2 emission rates (g CO_2 m^{-2} d^{-1}) were 13.5 ± 2.0 (mean ± SE) in N0, 10.9 ± 1.9 in N1, 11.2 ± 2.0 in N2, and 10.2 ± 2.1 in N3 (Fig. 1c). The CO_2 emission was significantly higher in N0 than in the other treatments, while N1, N2, and N3 did not significantly differ from each other. No significant differences among treatments were detected for the annually estimated CO_2 emission (Fig. 2b).

The annual GHG budget in Norrliden is not fully complete, since the measurement period of CH_4 exchange was short (see above), such that it did not cover a year. Nonetheless given the low contribution of CH_4 to the annual budget on the occasions it was determined, suggests that the influence on the total is small. Comparing CO_2 and N_2O emissions, however, revealed that CO_2 emission was the main contributor to the annual GHG budget, while N_2O emission in CO_2-equivalents was small in comparison, despite the N-induced increase (Table 2).

The mean sequestration of C in tree biomass from planting in 1953 until the last revision in June 2010 in N0 corresponded to 629.9 g CO_2 m^{-2} yr^{-1}, and both N1 and N2 plots showed an increase in uptake of around 35% and 23%, respectively (Table 2). N3, on the other hand, showed similar C sequestration rates as N0. Expressed as CO_2 equivalents the annual N_2O emission from N0, N1, N2 and N3 corresponded 1.770, 7.582, 4.447 and 0.654 g CO_2 m^{-2} yr^{-1}, respectively (Table 2). N-addition in N1 plots induced an average annual increase in C-sequestration of 222.8 g CO_2 m^{-2} yr^{-1}, as compared to N0 plots, while the increase in N_2O emissions corresponded to 5.7 g CO_2 m^{-2} yr^{-1}. Thus, the mean C sequestration remained markedly higher than the N_2O emission in CO_2-equivalents.

3.2. Rosinedal

The rate of N_2O emission at Rosinedal was significantly increased by N addition (Fig. 3a & 4 a). The average values of the N_2O emissions were 8.7 ± 3.7 (mean ± SE), 16.5 ± 5.2, and 57.1 ± 5.5 μg m^{-2} d^{-1} in control, LN, and HN plots, respectively. The estimated cumulative annual fluxes (mg N_2O m^{-2} yr^{-1}) from C, NL, and HL plots were 3.7 ± 1.5, 6.3 ± 2.2 and 16.9 ± 1.2. The cumulative emission showed a statistically similar N_2O emission in the control and LN, while the enhanced N_2O emission in HN was statistically different (Fig. 4a). When compared with the annual rate of N addition, in LN and HN, the N loss as N_2O corresponded to 0.2% and 0.22%, respectively (Table 3).

The soil in Rosinedal was a net sink for CH_4 (Fig. 3b). The average rates of the CH_4 flux were − 1.08 ± 0.06 (mean ± SE), − 1.25 ± 0.10, and − 1.69 ± 0.11 mg m^{-2} d^{-1} in control, LN, and HN plots, respectively. The annual cumulative CH4 uptake was significantly higher in HN plots, as compared to C and LN plots (Fig. 4b; Table 3).

The N addition in HN significantly reduced the soil CO_2 emission (Fig. 3c), while in LN, the flux was not significantly lower, as compared to the control. The average CO_2 emissions were 15.47 ± 1.37 (mean ± SE) in control, 12.87 ± 0.92 in LN, and 9.60 ± 0.49 g m^{-2} d^{-1} in HN.

When expressed as CO_2-equivalents, the annual flux of N_2O represented only 10% of the absolute value of CH_4 flux in the control (Table 3). The calculation of CO_2 annual flux was not possible because of

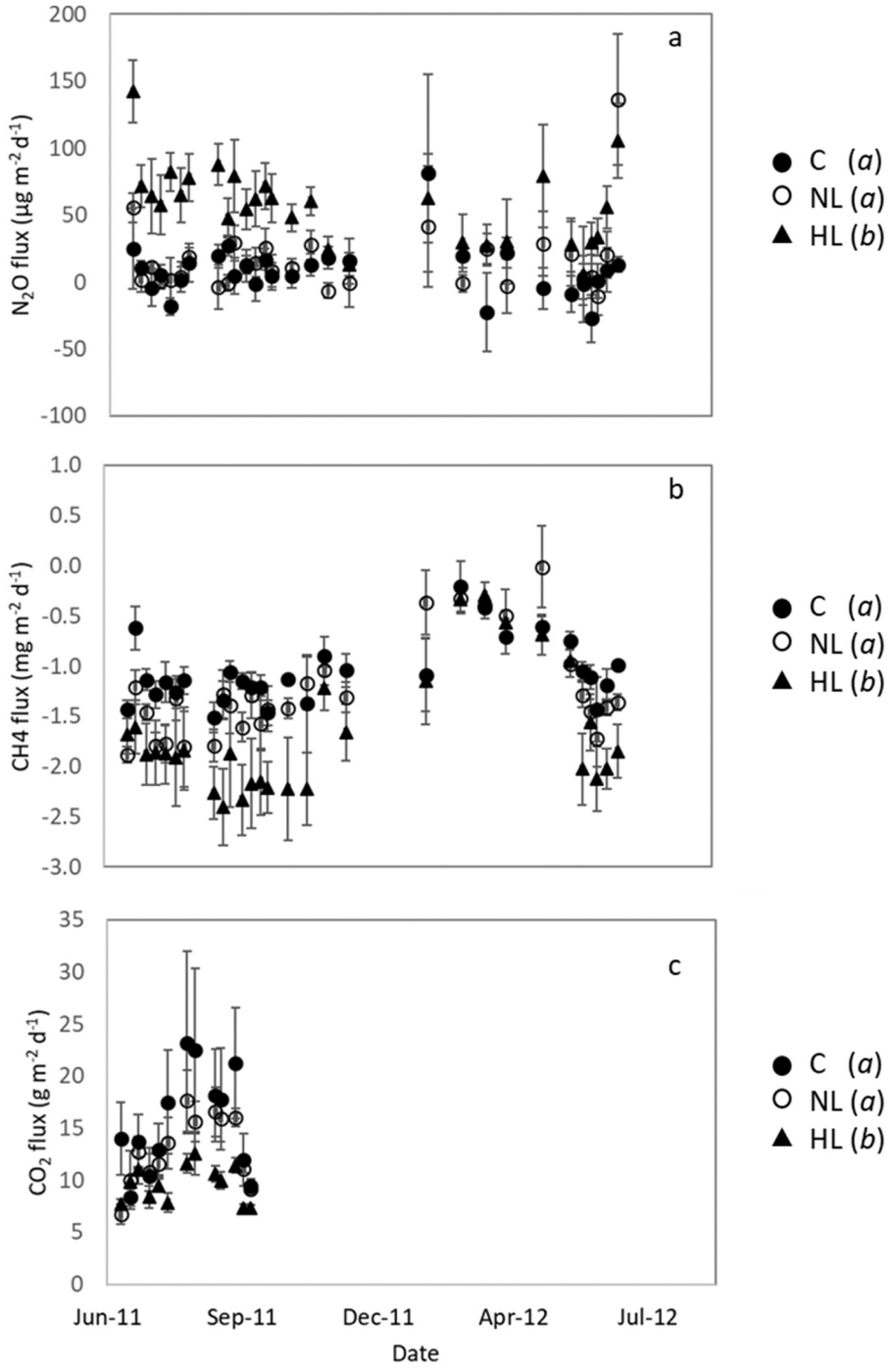

Fig. 3. Fluxes of (a) CO_2, (b) CH_4, and (c) N_2O fluxes in Rosinedal for control, low nitrogen (LN), and high nitrogen (HN) plots. Values are means; bars represent ± SE (n = 6). Significant differences among treatments are indicated by different letters ($p < 0.05$).

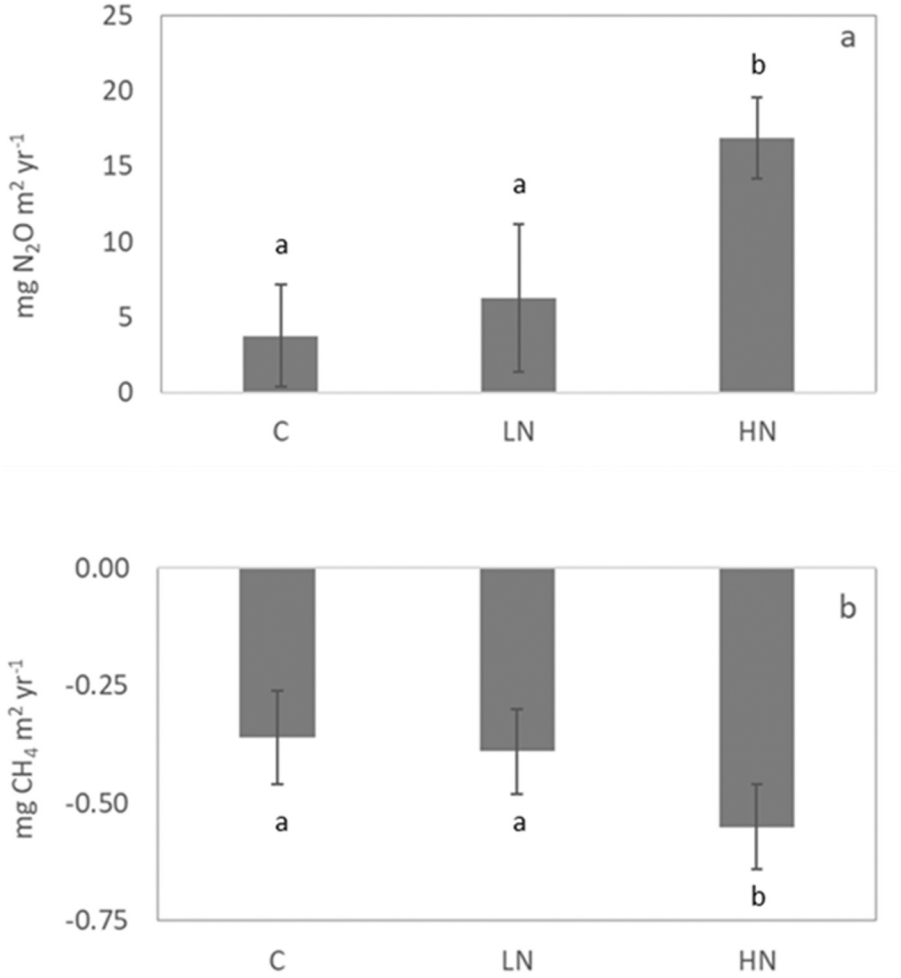

Fig. 4. Cumulative annual flux of (a) N_2O and (b) CH_4 in Rosinedal for control, LN, and HN plots. Significant differences among treatments are indicated by different letters ($p < 0.05$). Bars represent ± SE ($n = 6$).

Table 3
Comparison between annual emission of CH_4 and N_2O in Rosinedal, expressed as mg/μg m^{-2} yr^{-1} and as g CO_2 equivalent m^{-2} yr^{-1}; N_2O emission for each treatment, expressed as kg N ha^{-1} yr^{-1}; annual N addition; N emission factor as the ratio between N_2O emission and N addition; values of tree NPP (data from Lim et al., 2015).

		CONTROL	LN	HN
N_2O	mg m^{-2} yr^{-1}	3.7	6.3	16.9
	CO_2-eq (g m^{-2} yr^{-1})	1.0	1.7	4.6
CH_4	g m^{-2} yr^{-1}	-0.36	-0.39	-0.55
	CO_2-eq (g m^{-2} yr^{-1})	-10.1	-10.8	-15.3
N_2O emission	kg N ha^{-1} yr^{-1}	0.02	0.04	0.11
N addition rate	kg N ha^{-1} yr^{-1}	0	20	50
Emission factor	%		0.20	0.22
Tree NPP (2006-2013)	g CO_2 m^{-2} yr^{-1}	959		1352
ΔTree NPP (2006-2013)	g CO_2 m^{-2} yr^{-1}	-		393

the insufficient data. The effects of N addition were higher for N_2O than for CH_4 fluxes.

The N_2O emission (in CO_2-equivalent) was 0.1% of that of CO_2 uptake by the vegetation as annual NPP (data from Lim et al., 2015) in the control and 0.3% in HN (Table 3). N-addition in HN plots induced an average annual increase in C-sequestration of 286 g CO_2 m^{-2} yr^{-1}, while the concomitant increase in N_2O emissions corresponded to 3.6 g CO_2 m^{-2} yr^{-1} (Table 3).

4. Discussion

This study illustrates the effect of N loading on soil GHG emissions at different addition rates and at different time scales in two boreal Scots pine forest stands. Overall, the size of the induced increase in soil N_2O emission, following annual and long-term N addition, did not compare to the response observed in biomass production nor to the magnitude of soil CO_2 emission. In addition, at Rosinedal where measurements allowed annual atmospheric CH_4 oxidation estimates, the rates of soil CH_4 consumption was on the same order of magnitude as the increase in soil N_2O emission after N-addition, taking into account their respective GWP. Despite the large differences in the duration of the manipulation experiments, as well as the total N load supplied to the two stands, the two sites showed remarkably similar responses to N addition. Based on the obtained results, we may reject the underlying hypothesis that increased CO_2 sequestration and biomass production following long-term N addition to boreal forest stands is counteracted by concomitant changes in the soil GHG exchange.

The magnitude of our N_2O emission rates compare well with a previous study (Rutting et al., 2017) carried out in the same area on a fertilized Norway spruce stand. It is well known that soil GHG fluxes in general, and N_2O fluxes in particular, can be highly variable in both time and in space (e.g. Tidje, 1988). This is evident in our data and both the individual GHG measurements, as well as the annual GHG exchange estimates, are associated with high variability. As we had relatively low number of chamber replicates in our measurements, this may have affected the overall robustness in our data. However, despite this

inherent variability we are able to detect consistent and significant differences among treatments at both experimental sites, giving validity to our conclusions. However, we cannot rule out that more subtle impacts may not have been captured by the experimental design. Soil N_2O emission was significantly enhanced in the HN plot in Rosinedal, as well as in the plots in Norrliden still receiving N (N1), with 3–7-fold increase in emission rates. This is coherent with the literature, which reports that the N_2O emission in forests, in general, would increase under N fertilization (Aronson et al., 2012; Siljanen et al., 2020), although studies about N-induced soil N_2O emission in boreal forests are scarce. Håkansson et al. (2021) observed that repeated N fertilization in a Norway spruce forest in Sweden increased the soil N_2O emission in the fertilization years but not during the subsequent years. Maljanen et al. (2006) conducted a study in a Norway spruce stand in Finland, where a one-time N fertilization equal to 200 kg N ha^{-1} yr^{-1} did not influence the N_2O emission. This is different from our results, probably due to the duration and the design of the experiments (single fertilization vs. repeated annual N additions for several years) and/or the soil environment (e.g., Siljanen et al., 2020). Linked to practical forestry, single applications of larger doses at longer time intervals can be more relevant than annual applications of smaller doses (cf., Nohrstedt, 2001). The ratio between annual soil N_2O emission and N addition differed between the two experimental sites, probably due to both the rate and the length of the experiments. While in Norrliden the annual N_2O emission was 0.5% of the N addition, in Rosinedal, this value was ca. 0.2% for both LN and HN. Similar emission factors in the two treatments at Rosinedal suggests that the N_2O emission factor at short timescale may depend on the background conditions of the site, rather than the fertilization rate itself. In a nearby Norway spruce forest stand subjected to annual N addition (50 kg N ha^{-1} yr^{-1} for 23 years), the emission factor based on two years of chamber measurements was estimated to 0.3% (Rütting et al., 2021), falling in-between our estimates. Values at both our sites were also coherent with the emission factors from IPCC 2006 (De Klein et al., 2006), which averaged 0.1 (range 0.02 – 0.3) for nutrient poor boreal forest stands and 0.6 (range 0.16 – 2.4) for nutrient rich boreal forest stands. Thus, the long and constant N application in Norrliden seems to have completely released the stand from N-limitation and this site can now be classified as nutrient-rich or at least N-rich forest. This is further supported by the soil C/N ratio, which was similar in N1, N2, and N3 (28.7, 28.7, and 27.9, respectively) but significantly lower than N0 (40.2) (Högberg et al., 2014). However, a recent study by Högberg et al. (2024) suggests that this is valid only during the N-application phase and that the site return to N-limitation following termination despite a lower C/N ratio in the upper part of the soil (mor layer).

Soil CH_4 uptake did not show very strong responses to N addition. However, although patterns were mainly insignificant, the trend at both sites indicates that ongoing N addition does not result in reduced CH4 uptake, but rather the opposite as the measured CH4 uptake were significantly higher in the N1 treatment as Norrliden and the HL treatment in Rosinedal. This result is in contrast with several previous studies, reporting that N fertilization suppresses CH_4 uptake (Oertel et al., 2016; Shrestha et al., 2015; Zhang et al., 2017). Noteworthy is the study by Börjesson and Nohrstedt (2000), who studied how soil CH_4 uptake responded to a one-time application and observed that, the first year after fertilization of 150 kg N ha^{-1} yr^{-1}, soil CH_4 uptake was inhibited, while during the second year, it was higher in the fertilized plots than the control plots. Håkansson et al. (2021) reported that repeated N fertilization in a Norway spruce forest decreased the soil CH_4 uptake over time. Uptake of CH_4 can be inhibited by N-addition because of the competition with NH_4 for reduction (Shrestha et al., 2015), while NO_3^- can be inhibitory only at high concentration, because of an osmotic effect (Bodelier and Laanbroek, 2004). However, other studies showed a positive or a negligible effect of N fertilization on soil CH_4 uptake (Maljanen et al., 2006; Saari et al., 2004; Shrestha et al., 2015; Whalen and Reeburgh, 2000; Xing et al., 2014). Xing et al. (2014) reported that a low N fertilization rate (< 20 kg N ha^{-1} yr^{-1}) possibly promoted CH_4 uptake by providing N to the CH_4 oxidizing microbial communities, since they were affected by N-limitation, as well. While our results can be attributed to enhanced growth of methanotrophs, the fertilization rates applied in Rosinedal exceed the threshold set by Xing et al. (2014). Therefore, it is plausible that the current fertilization rates in Rosinedal sustain soil CH_4 uptake, without reaching the threshold that may lead to inhibition.

Soil CO_2 emission was reduced by N addition at both sites, but not at all levels of N-loading. In Rosinedal, the effect was significantly different from control only at the high addition rate. A suppression of soil respiration at high N addition rates is in line with earlier studies (Janssens et al., 2010; Knorr et al., 2010; Olsson et al., 2005). At low N-addition rates, however, the response was found to be minor (Ambus and Robertson, 2006; Maljanen et al., 2006; Allison et al., 2008), or even resulted in enhanced soil respiration (Bowden et al., 2004; Hasselquist et al., 2012). Indeed, a study by Hasselquist et al. (2012), also carried out at the Rosinedal N-addition experiment, reported higher soil CO_2 emission in LN plots than in the other plots, while control and HN plots had similar fluxes. Discrepancy between studies can be explained by the different measurement methods and the different time scales since Hasselquist et al. (2012) collected data only during the growing season. In addition, Hasselquist et al. (2012) underlined that the components of soil respiration responded differently to N addition, where the autotrophic respiration differed significantly between treatments; indeed, autotrophic respiration was higher in LN than in the control and HN, while heterotrophic respiration did not differ among plots. However, this is not generally valid, since in other boreal stands both heterotrophic and autotrophic respiration significantly decreased under N addition (1275 kg N ha^{-1} applied over 14 years; Olsson et al., 2005).

Other studies also reported that N-addition might influence the two main component fluxes (autotrophic respiration and heterotrophic respiration) of soil respiration differently (e.g., Forsmark et al., 2020), further complicating interpretation of response patterns. In addition, response patterns in soil respiration to N-addition can be explained by the fact that CO_2 emission is affected not only by N availability but also by other factors such as soil temperature and moisture, quality of soil organic matter (SOM), and soil C/N ratio (Shrestha et al., 2015). Although N addition may influence some of these factors, for instance, N availability to both plants and microbial communities, it remains a secondary driver of soil CO_2 emission compared to, e.g., soil temperature (Li et al., 2019; Oertel et al., 2016; Shrestha et al., 2015) and SOM decomposition rate (Wang et al., 2000).

In Norrliden, the N application in N2 and N3 plots was terminated in 2009 and 1990, respectively. Although the annual N loads and the duration of N addition differed between N2 and N3, they had received similar total loads of N when treatment stopped; a total of 2520 kg N ha^{-1} in N2, and 2160 kg N ha^{-1} in N3. We may use the time elapsed from the time of termination of N addition to the time of GHG measurements as a proxy of system recovery rate with respect to soil N_2O and CO_2 exchange. The N2 plots, terminated two years before GHG measurements, still showed N_2O emission rates not significantly different from neither N1 nor N0 plots. In N3 plots, terminated twenty years before GHG measurements, N_2O emission rates were similar (or even slightly lower) to those in the control plots. Thus, it appears that the response to N addition is reversible and that the recovery time, with respect to enhanced N_2O emission rates, following long term N addition is up to or less than decadal time scales. Similar rates of recovery on N transformation processes and ectomycorrhizal abundance at the site has been reported from the Norrliden experiment (Högberg et al., 2011). A more recent study (Högberg at al, 2024) show that following termination of the N-addition to N2 and N3, foliar $\delta^{15}N$ decrease, indicating a return of ectomycorrhizal fungi resulting in a tighter N-cycle typical for N-limited boreal forests.

Soil CO_2 efflux in N0, N1, and N2 was measured also in a previous study at three occasions, during the late growing seasons of 1996–1999 (Franklin et al., 2003). At that time, the N2 plots showed a ca. 50%

reduction in soil respiration rates, as compared to the control plots. In the present case, the reduction was ca. 25% in N2 as compared to the control, during the growing season. However, in our measurements, the soil CO_2 flux rates in N3, in which N addition terminated twenty years before GHG measurements, was also around 25% lower than in the control plots. This may suggest a fast partial recovery after N addition was stopped. However, the data set from the late 1990 s is quite limited and available for the late growing season only (August or September) and, therefore, part of the response can also be attributed to year-to-year variability in growing season soil respiration rates, which can be of equal magnitude (Franklin et al., 2003).

According to the estimated annual GHG budget, the N-induced increase in soil N_2O emission, in terms of CO_2 equivalents, has low significance when compared to the other GHG fluxes. Particularly, the stimulation of soil N_2O emission (in CO_2 equivalents) in Norrliden was marginal in comparison with soil CO_2 emission. For this reason, the increased soil N_2O emission due to N addition was far from counteracting the observed reduction of soil CO_2 emission driven by N addition. Similarly, soil N_2O emission in Rosinedal was small also in comparison with soil CH_4 uptake. This is valid despite the highly significant increase of soil N_2O emission under N addition and despite the high global warming potential of this gas. It is worth noting that the climate impacts of soil N_2O emission on the long term was probably overestimated in our calculation since we used the global warming potential approach rather than the radiative forcing (e.g., Frolking et al., 2006). However, we argue that, even in the worst scenario, as in our case, the increase in soil N_2O emission may still not override the increased net sequestration of CO_2. This should also be seen in the light of fertilization practices in Sweden where the recommendations according to the Swedish Forest Agency prescribes a maximum dose of 200 kg N ha^{-1}, with at least 8 years between additions, and a maximum of 450 kg N added over a rotation.

Finally, the N-induced increase in soil N_2O emission appears negligible in comparison with the estimated increased tree NPP in both Norrliden and Rosinedal. However, it is plausible that our tree NPP values are overestimated, as they do not contain e.g. fine root productivity, nor the carbon sequestration by root associated ectomycorrhizal fungi, both which are expected to decline after N addition. In fact, and attempt to account for fine roots and mycorrhizal growth at Rosinedal in response to N addition (Lim et al., 2015) concluded that much of the observed above ground NPP enhancement may be counteracted by a reduction in these below ground components. Nonetheless, overall contribution even to enhanced N_2O emissions are small in comparison to C fluxes including magnitude of soil respiration rates. For N-induced impact on C sequestration it is also highly relevant to evaluate effect on the ecosystem level. A recent study by (Zhao et al., 2022) utilizing Eddy Covariance measurements at the Rosinedal treatments concluded that the net ecosystem productivity (NEP) increased by ca. 16% in the HN plots, relative to the control plots, shifting the system to new steady state.

Overall, the N-induced increase in N_2O emissions counteracted the increased C-sequestration in our NPP estimates by 3.4% and 1.1%, at Norrliden and Rosinedal, respectively. Previous studies reported that the increased N_2O and CH_4 emissions due to N addition counteracted 53%–76% of the enhanced CO_2 uptake at global scale (Liu and Greaver, 2009). Zaehle et al. (2011), using a process-based model considering different land uses and both natural N deposition and N-based fertilization, estimated that the increased N_2O emission offset the CO_2 accumulation by 30% at the global scale. Using a similar approach, Tian et al. (2015) assessed that in North America the increased N_2O and CH_4 emissions counteracted 58–138% of the anthropogenic N-induced CO_2 sequestration. Deng et al. (2020) calculated that the global warming potential of the enhanced GHG emissions was 1.7 times the increased soil C sink and, therefore, the C sequestration was completely offset. However, the studies referred to above report results based on models performed at the global or continental scale, while the present analysis is built on measurements at stand level. Furthermore, the above-mentioned studies cover a wide range of land uses and soil conditions, moving vastly beyond the boreal forest biome. In conclusion, our results derived from the long-term N additions and field GHG measurements suggest that the fertilization with N in the boreal forest may contribute to sequestering C, rather than offsetting soil GHG emissions, in terms of CO_2-equivalents.

Author contributions

Mats G. Öquist.: Conceptualization, Methodology, Writing – original draft, Funding acquisition. Hongxing He: Methodology, Writing – review & editing. Anna Bortolazzi: Formal analysis, Writing – original draft. Mats B. Nilsson: Conceptualization, Writing – review & editing. Mirco Rodeghiero: Writing – review & editing. Roberto Tognetti: Writing – review & editing. Maurizio Ventura: Writing – review & editing. Gustaf Egnell: Investigation, Forma analysis, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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