ECOSYSTEMS

Low and High Nitrogen Deposition Rates in Northern Coniferous Forests Have Different Impacts on Aboveground Litter Production, Soil Respiration, and Soil Carbon Stocks

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ABSTRACT

Nitrogen (N) deposition can change the carbon (C) sink of northern coniferous forests by changing the balance between net primary production and soil respiration. We used a field experiment in an N poor *Pinus sylvestris* forest where five levels of N (0, 3, 6, 12, and 50 kg N ha^{-1} yr^{-1}, $n = 6$) had been added annually for 12–13 years to investigate how litter C inputs and soil respiration, divided into its autotrophic and heterotrophic sources, respond to different rates of N input, and its subsequent effect on soil C storage. The highest N addition rate (50 kg N ha^{-1} yr^{-1}) stimulated soil C accumulation in the organic layer by 22.3 kg C kg^{-1} N added, increased litter inputs by 46%, and decreased soil respiration per mass unit of soil C by 31.2%, mainly by decreasing autotrophic respiration. Lower N addition rates ($\leq$ 12 kg N ha^{-1} yr^{-1}) had no effect on litter inputs or soil respiration. These results support previous studies reporting on increased litter inputs coupled to impeded soil C mineralization, contributing to enhancing the soil C sink when N is supplied at high rates, but add observations for lower N addition rates more realistic for N deposition. In doing so, we show that litter production in N poor northern coniferous forests can be relatively unresponsive to low N deposition levels, that stimulation of microbial activity at low N additions is unlikely to reduce the soil C sink, and that high levels of N deposition enhance the soil C sink by increasing litter inputs and decreasing soil respiration.

HIGHLIGHTS

- The high N treatment increased soil C stocks by 22.3 kg C kg N^{-1} added.
- The high N treatment increased litter production and decreased soil respiration.
- Low N treatments did not impact litter production, soil respiration, or C stocks.

INTRODUCTION

Net primary productivity (NPP) in northern coniferous forests is often limited by the low availability of nitrogen (N) (LeBauer and Treseder 2008; Tamm 1991; Vitousek and others 1997). During the last century, N availability has increased in many ecosystems as a consequence of the extensive use of fossil fuels and industrial N_2 fixation (Galloway and others 2008; Reay and others 2008). Deposition of N in northern coniferous forests has the potential to increase uptake of carbon (C) in vegetation and thus counteract the ongoing rise in atmospheric CO_2 concentration (Houle and Moore 2008), but the importance of this effect for the global C balance has been debated for decades (de Vries 2009; de Vries and others 2008; Holland and others 1997; Magnani and others 2007; Myneni and others 2001). The controversy can be ascribed to two key sources of uncertainty. First, N addition experiments typically apply one order of magnitude more N than current upper N deposition rates in northern coniferous forests, making it uncertain if the NPP responses are applicable to the range of relatively low inputs of N deposition (de Vries and others 2014; From and others 2016; Hyvönen and others 2008; Nohrstedt 2001). Second, most of the C in northern coniferous forests is stored in soils, yet little is known about how different levels of N deposition will impact soil C pools and fluxes (Janssens and others 2010; Olsson and others 2009). Although increasing NPP will likely increase soil C stocks by increasing aboveground litter inputs, it remains highly uncertain how N affects the emissions of C back into the atmosphere via soil respiration and how these fluxes are affected by low versus high levels of N inputs (Bowden and

others 2004; Hasselquist and others 2012; Peng and others 2017). Quantification of the effect of different rates of N addition on the main inputs and outputs of C to soils is therefore needed to better predict changes in the C sink of northern coniferous forests receiving different levels of N deposition.

Differences in N demand between plants and soil microorganisms are likely to play an important role in regulating soil C fluxes at different rates of N inputs (Kaye and Hart 1997; Kuzyakov and Xu 2013). Trees allocate a substantial part of their C belowground to roots and root-associated microbes involved in nutrient acquisition, especially when the availability of N is low (Hermans and others 2006; Litton and others 2007; Mäkelä and others 2008). This C flux is an important driver of soil respiration (that is, autotrophic CO_2 efflux) (Chen and others 2014) and also contributes to decomposition processes (Kuzyakov 2010; Talbot and others 2008). When N-limited trees are supplied with high amounts of N, a reduction in C allocation to N acquisition could enable a higher aboveground growth, leading to an increase in aboveground litter inputs, while the autotrophic respiration could remain the same, or decrease (Chen and others 2014; Litton and others 2007; Vicca and others 2012). At lower N supply, however, soil microbes including root-associated mycorrhizal fungi are also likely to be N limited and stimulated by the increase in N availability, leading to increased autotrophic respiration (Franklin and others 2014; Hasselquist and others 2012; Janssens and others 2010). Despite that most northern coniferous forest is strongly N limited and also receives very low N deposition levels (Gundale and others 2011), few studies have simultaneously assessed the impact of different rates of N input on the major C fluxes (de Vries and others 2014).

In addition to autotrophic soil respiration that is linked to plant C allocation, a substantial portion of C emission from forest soils is derived from saprotrophic activity (that is, heterotrophic respiration) (Hanson and others 2000; Högberg and others 2001) and may respond more directly to changes in N availability (Janssens and others 2010). Most studies evaluating N effects on saprotrophic activity have found decreased heterotrophic respiration (− 15%) (Janssens and others 2010), decomposition (Ågren and others 2013; Fog 1988; Knorr and others 2005), and microbial biomass (Treseder, 2008), all of which typically decrease in response to high (≥ 20 kg N ha^{-1} y^{-1}) N addition rates. In contrast, the impact of lower N addition rates (≤ 12 kg N ha^{-1} y^{-1}), which is more relevant for

Table 1. Summary of Stand Properties in Response to Different Levels of Long-term Nitrogen Additions (0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}).

	Nitrogen treatment				
	0 N	3 N	6 N	12 N	50 N
Annual (cumulative) nitrogen addition (kg ha^{-1} (y^{-1}))	Control (0)	3 (39)	6 (78)	12 (156)	50 (650)
Tree layer properties					
Stem density (n ha^{-1})	472 ($\pm$ 61)	476 ($\pm$ 33)	558 ($\pm$ 104)	483 ($\pm$ 112)	540 ($\pm$ 58)
Basal area (m^2 ha^{-1})	25.2 ($\pm$ 2.5)	22.3 ($\pm$ 1.7)	23.7($\pm$ 1.3)	25.9 ($\pm$ 1.8)	25.8 ($\pm$ 1.4)
Change in basal area 2005–2014 (m^2 ha^{-1})	1.10 ($\pm$ 0.10)	1.05 ($\pm$ 0.04)	1.21 ($\pm$ 0.15)	1.25 ($\pm$ 0.16)	2.23 ($\pm$ 0.14)
Foliage nitrogen concentration 2014 (mass%)	1.16 ($\pm$ 0.04)	1.16($\pm$ 0.05)	1.16 ($\pm$ 0.06)	1.15 ($\pm$ 0.02)	1.4 ($\pm$ 0.09)
Forest floor vegetation					
Ericaceous shrubs	205 ($\pm$ 12)	264 ($\pm$ 20)	256 ($\pm$ 12)	304 ($\pm$ 20)	269 ($\pm$ 22)
Bryophytes	213 ($\pm$ 18)	179 ($\pm$ 22)	201 ($\pm$ 13)	209 ($\pm$ 17)	108 ($\pm$ 15)
Lichens	168 ($\pm$ 6)	174 ($\pm$ 8)	169 ($\pm$ 5)	159 ($\pm$ 11)	139 ($\pm$ 8)
Total	582 ($\pm$ 13)	616 ($\pm$ 19)	626 ($\pm$ 11)	672 ($\pm$ 17)	515 ($\pm$ 17)
Soil chemistry					
Organic layer nitrogen concentration (C:N)	44.0 ($\pm$ 1.27)	41.9 ($\pm$ 1.20)	41.7 ($\pm$ 1.84)	41.2 ($\pm$ 1.14)	35.6 ($\pm$ 1.02)
Organic layer pH	3.3 ($\pm$ 0.06)	3.2 ($\pm$ 0.04)	3.3 ($\pm$ 0.03)	3.2 ($\pm$ 0.03)	3.1 ($\pm$ 0.04)
Mineral 0–10 cm pH	4.0 ($\pm$ 0.05)	4.0 ($\pm$ 0.03)	4.1 ($\pm$ 0.06)	4.0 ($\pm$ 0.05)	3.9 ($\pm$ 0.04)
Mineral 10–20 cm pH	4.4 ($\pm$ 0.07)	4.5 ($\pm$ 0.06)	4.4 ($\pm$ 0.06)	4.5 ($\pm$ 0.07)	4.4 ($\pm$ 0.07)

pH determined in CaCl$_2$.

Treatments consisted of annual addition of ammonium nitrate beginning in 2004. Tree layer properties were measured eight years after the experiment started and are reported in From and others (2016). The forest floor vegetation layer is the average number of pinpoint hits per m², a measure of relative abundance, of eight floral surveys 2004–2015 period using the point intercept method, as described in Strengbom and others (2002). All values are means of six replicate plots (± standard error).

understanding N deposition effects in northern coniferous forests, remains less understood. Lower rates of N input may have the opposite effects to high N addition rates by alleviating N limitations on saprotrophic activity, which could lead to increased emissions of CO_2 via heterotrophic soil respiration (de Vries and others 2014; Knorr and others 2005). If soil microbial communities, including both free-living saprotrophs and root-associated mycorrhizal fungi, are actually N limited, they could immobilize into their biomass the relatively low amounts of N inputs that are typical of the northern coniferous forests (1–12 kg N ha^{-1} y^{-1}; Gundale and others 2011), which could subsequently increase their activity and limit plant access to that N. Enhanced saprotrophic activity in response to low levels of N deposition would result in higher respiration rates and potentially also lower C accumulation rates than demonstrated in high-level N addition experiments (de Vries and others 2014; Knorr and others 2005).

We used a long-term (12–13 years) replicated ($n = 6$) experiment designed to simulate a gradient of N deposition rates (0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}) to better understand how litter C inputs and C outputs via soil respiration respond to different rates of N input and further to investigate its subsequent effect on soil C accumulation. The experiment is located in a region where N deposition is low (ca. 2 kg N ha^{-1} y^{-1}), thereby allowing us to evaluate the effects of lower N addition rates on C dynamics than has been done in most previous studies. We measured the input of C from aboveground litter and the output of C via soil respiration. We used root isolation treatments, that is, trenches, to further isolate the impact of N on the heterotrophic versus autotrophic contribution to soil respiration. First, we hypothesized that a shift in tree C allocation from below to aboveground structures in response to N additions would result in increased litter inputs and decreased autotrophic soil respiration. Further, we anticipated that these changes would occur only at high levels of N addition (50 kg N ha^{-1} y^{-1}) because trees are likely to have greater access to external N inputs when it is applied at high rates (Gundale and others 2011; Kaye and Hart 1997). Second, we hypothesized that high levels of N addition would decrease heterotrophic respiration due to inhibitory effects on soil saprotrophs (Fog 1988), whereas low levels

of N addition (< 12 kg N ha^{-1} y^{-1}) would increase both heterotrophic respiration and autotrophic respiration by alleviating N limitations on saprotrophic and mycorrhizal activity (de Vries and others 2014). Collectively, we anticipated that changes in these major inputs and outputs of C to the soil would help explain the effect of different rates of N addition on soil C storage and that testing these hypotheses together would provide a more mechanistic understanding of the impact of N deposition on the soil C sink of northern coniferous forests.

MATERIALS AND METHODS

Site and Treatments

We used a forest N addition experiment at Åheden in the Svartberget research forest (64° 13′ N, 19° 48′ E) in inland northern Sweden designed to simulate N deposition (Table 1). The experiment is located in a region with very low N deposition (< 2 kg N ha^{-1} y^{-1} (Karlsson and others 2012)). The mean annual temperature is $+ 1°C$ and mean annual precipitation 600 mm. The forest stand at the experimental site is dominated by Scots pine (*Pinus sylvestris* L.), which naturally regenerated after a forest fire, and was by the time of this study c. 140 years old (From and others 2016). The soil at the site is developed on an alluvial sand–silt plain and classified as a Typic Haplocryod (FAO Cambic Podzol). The forest floor is covered with a sparse layer of Ericaceous shrubs, including *Vaccinium vitis-idea* and *Calluna vulgaris*, *Cladina* and *Cladonia* spp. lichens, and the mosses *Pleurozium schreberi* (Brid.) Mitt, and *Dicranum* spp. (Gundale and others 2011). Scots pine is one of the dominant tree species in the northern coniferous forests of Eurasia, and in Sweden, it constitutes 39.2% of the forest tree volume (Fransson 2017). The dry sites characteristics of our experiment site also share similarities with dry pine forests in the boreal region of North America, for example, *P. banksiana* (Bonan 1989).

Nitrogen treatments at five levels were assigned to 0.1 ha plots in a randomized block design ($n = 6$). Treatments commenced in 2004 (Table 1) with N additions at 0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1} (hereafter referred to as 0 N, 3 N, 6 N, 12 N, and 50 N, respectively) in the form of solid ammonium nitrate granules evenly distributed directly applied after snowmelt each year. The low doses (3 N, 6 N, and 12 N) simulate a gradient of N deposition from central to southern Fennoscandia, whereas the high N treatment (50 N) simulates upper N deposition rates in central Europe and also serves as a useful comparison with other N addition experiments.

To separate the effects of N on total soil respiration into its source components, that is, heterotrophic and autotrophic soil respiration (inclusive of tree roots and their associated microorganisms), we established root trenching treatments within each N addition plot (Hanson and others 2000; Maaroufi and others 2019). Each main plot was split into four equal-sized quadrat subplots, and two of the subplots were assigned to the trenching treatment, whereas the other two remained untrenched (Figure S1). Trenching was done by permanently inserting 50×50 cm steel frames reaching 50 cm into the soil in July 2014, 2 years before the first measurements in this study. The C stock in fine-root biomass at Åheden is around 900 kg ha^{-1} (Scarascia-Mugnozza and others 2000), and we assume that the contribution of severed roots to soil respiration inside the frames was short-lived and negligible by the time we began our measurements in 2016. One frame was installed in the middle of each of the two subplots assigned to the trenching treatment. We removed any tree seedlings that were established in the frames to maintain the absence of processes associated with the tree root zone, whereas the very sparse forest floor vegetation at this site was left intact to minimize disturbance to these permanent plots. Studies have shown that the forest floor vegetation has a negligible effect on soil respiration rates (Bond-Lamberty and others 2011). In a parallel study focused on microbial community composition, we confirmed that the trenching treatment was successful at greatly reducing fungal phospholipid fatty acid concentrations, as well as DNA sequences of ectomycorrhizal fungi (Maaroufi and others 2019), indicating that the trenching treatment was successful at excluding soil communities associated with tree roots. We further assume that the 2 years that elapsed since the trenching treatment provided sufficient time to minimize the influence of the disturbance caused by the installation of the steel frames (Hanson and others 2000).

Soil Carbon Stocks

The organic layer and upper 20 cm of the mineral soil were sampled in late July 2017 at ten predefined locations on each main plot, outside the steel frames (Figure S1). We confined our sampling to the organic layer and mineral soil down to 20 cm because previous studies in similar systems have

shown that the majority of roots and C are located within this depth range (Helmisaari and others 2007; Maaroufi and others 2015). The organic layer was sampled by collecting the forest floor down to the mineral soil surface with a 10-cm-diameter PVC tube fitted with a serrated blade. Mineral soil samples were then collected from the exposed mineral soil surface using a sharp steel corer at 0–10 cm and 10–20 cm depth. Parent material was reached in many places at 10–20 cm depth; thus, our sampling covered a majority of the soil C stock in each plot. The ten cores from each depth were pooled to form one composite sample for each depth within each plot. The composite samples were subsequently sieved (2 mm) to remove intact roots and thoroughly homogenized. A subsample of each composite sample was dried at 65°C and ground to a fine powder using a custom-made roller mill (Stuiver and others 2015), and soil C concentrations were then estimated by mass spectrometry (DeltaV IRMS coupled to a Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) with duplicate subsamples. Organic layer C stocks were calculated as the C concentration multiplied with the dry mass of the sample and finally divided with the total area sampled. Mineral soil C stocks were calculated by multiplying the C concentration with the mass of the specific soil volume, based on site-specific bulk density estimates (Persson and others 2000). All masses were expressed on a per hectare basis, without correction for stoniness because stones were completely absent at this site. Annual C accumulation in the organic layer was estimated as the difference in soil C stocks between the average of the control treatments and each plot, divided by the duration of the N treatments (13 years).

Litter Input

Aboveground litter production was estimated using the standard methodology in Ukonmaanaho and others (2016). Five plastic trays (0.36 m^2 with drainage holes) per plot were installed to collect litter continuously from May 2016 to May 2018. Trays were emptied every second month during the growing season and after the winter in May 2017 and 2018. Litter was defined as tree litter, including twigs and branches up to 20 mm thick and annual litter C input calculated as the average of the annual sum of the mass of each collection of litter and then multiplied by a litter C concentration of 50% (Gundale and others 2014; Sandström and others 2007). Coarse woody debris was assumed to be equal across treatments and therefore does not contribute to treatment differences in litter C input. Similarly, the forest floor vegetation biomass at this site is low compared to other forests in this region, for example, Maaroufi and others (2016), and thus was assumed to have contributed a negligible quantity of litter compared to trees.

Soil Respiration

Emissions of C by soil respiration were measured as the efflux of CO_2 at the soil surface using dark and sealed chambers (Pumpanen and others 2004) at permanent measurement locations inside and outside the steel frames (Figure S1). Each of the measurement locations consisted of a cylindrical 12.5-cm-high and 25-cm-wide (0.05 m^2, 6 l) PVC collar installed in a predefined location in May 2016, 6 weeks before the first measurement. The collars were gently installed on top of the moss and the intact litter layer and sealed from the outside with a thin rim of sand. The height of each collar was then recorded in four cardinal directions to estimate the collar-specific volume. All aboveground living biomasses of mosses and shrubs were removed to avoid their contribution to CO_2 fluxes. Five collars were installed systematically, spaced at least four meters apart outside the steel frames, whereas space limitations allowed only one collar inside each of the two steel frames within each main plot. In total, 210 collars were used, which included five outside the steel frames and two inside, in each of the 30 main plots. This design allowed us to estimate the effect of N on total soil respiration (outside steel frames) as well as on soil without tree roots, that is, heterotrophic respiration only (inside steel frames) which further allowed us to estimate the contribution of tree roots and their associated microbiota, which we hereafter refer to as autotrophic respiration, by subtraction (outside–inside steel frames). The separation of the total respiration into component fluxes was validated in a separate study showing that the trenching treatment did not affect humus mass loss (Maaroufi and others 2019). Differences in soil C stocks could potentially confound conclusions on the impact of N on soil respiration. To control for this, we also estimated the total and the heterotrophic respiration per mass unit of soil C by dividing the flux with the sum of C stocks in the upper 20 cm of the soil, including the organic and the mineral layers.

Measurements were started in July 2016 and continued to October (five measurement occasions) and were repeated from June to October 2017 (six occasions) with approximately three-week intervals (11 occasions in total). At each
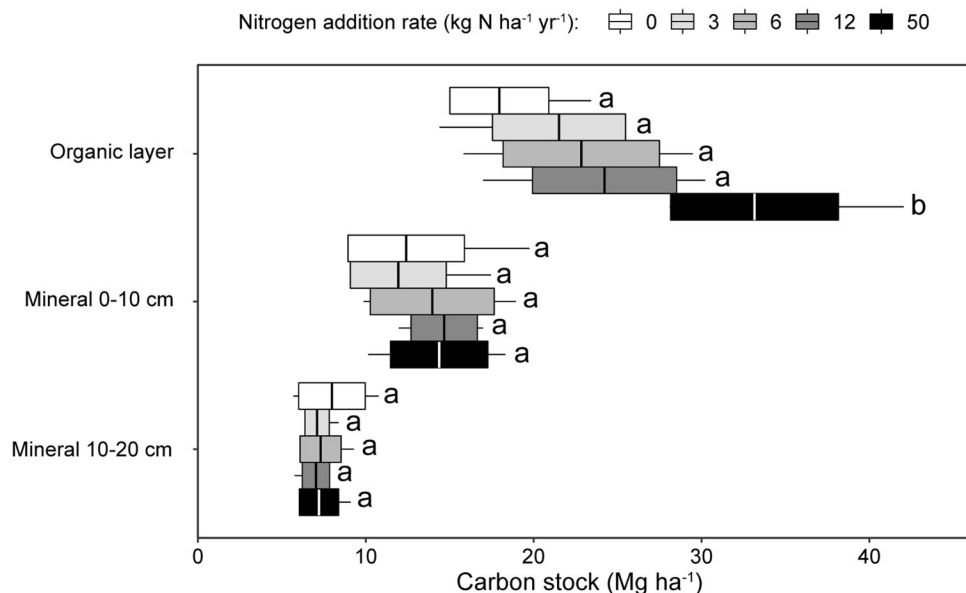
Nitrogen addition rate (kg N ha⁻¹ yr⁻¹):   0   3   6   12   50

Figure 1. The soil carbon stock in the organic layer, 0–10, and 10–20 cm mineral layers after 13 years of annual nitrogen additions (0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}) in a Scots pine forest. Boxes display the median and one standard deviation above and below the median, and whiskers are the maximum and the minimum ($n = 6$). Boxes sharing letters within each soil layer are not significantly different at $p < 0.05$ according to Tukey's test.

measurement occasion, all collars were measured at least twice within 3 days with the different subsample measurements at the same collar made at a different time of the day, such that collars measured early in the day during the first sub-sampling were measured later in the day during the second subsampling. We measured CO_2 concentrations with Vaisala CARBOCAP GMP343 infrared gas analyzers mounted to portable plastic lids. Each lid had a small fan mounted on the underside to mix the chamber headspace gases during the measurement. After the lid was attached, the buildup of CO_2 in the headspace was monitored for 180 s, and the CO_2 efflux was subsequently estimated using linear regression ($R^2 > 0.9$).

Statistical Analyses

The effect of N on soil C stocks in each of the soil layers, annual soil C accumulation, litter inputs, and soil respiration per mass unit soil C was tested using analysis of variance, with N treatment (five levels) defined as a fixed factor and block ($n = 6$) as a random factor. Significant differences were then further explored using Tukey's honestly significant difference test. Before statistical testing, data were found to meet the assumptions of normality using the Shapiro–Wilk test and homoscedasticity using

Levene's test. The effect of N, time (11 levels), and their interaction on areal soil respiration variables was analyzed using linear mixed effects models with block defined as random factor and using a first-order autoregressive covariance structure where the covariance decreases as the time between measurement increases (Legendre and Legendre 2012). Analyses were done in SPSS (Version 22, IBM Corp., Armonk, New York, USA), and effects were considered significant when $p < 0.05$.

Results

Soil Carbon Stocks

Nitrogen additions increased soil C stocks in the organic layer, but not in the mineral soil during the study period (Figure 1). The C stock in the organic layer of the 50 N treatment was 14.5 Mg ha^{-1}, or 75% higher than in the control ($p < 0.001$), whereas the C stock in the 3 N, 6 N, and 12 N treatments was not significantly different from the control. The annual C accumulation rate in the 50 N treatment during the 13 years of N addition was 1117 kg C ha^{-1} yr^{-1} higher than in the control treatment and had accumulated 22.3 kg C kg^{-1} N added (Figure 2A).
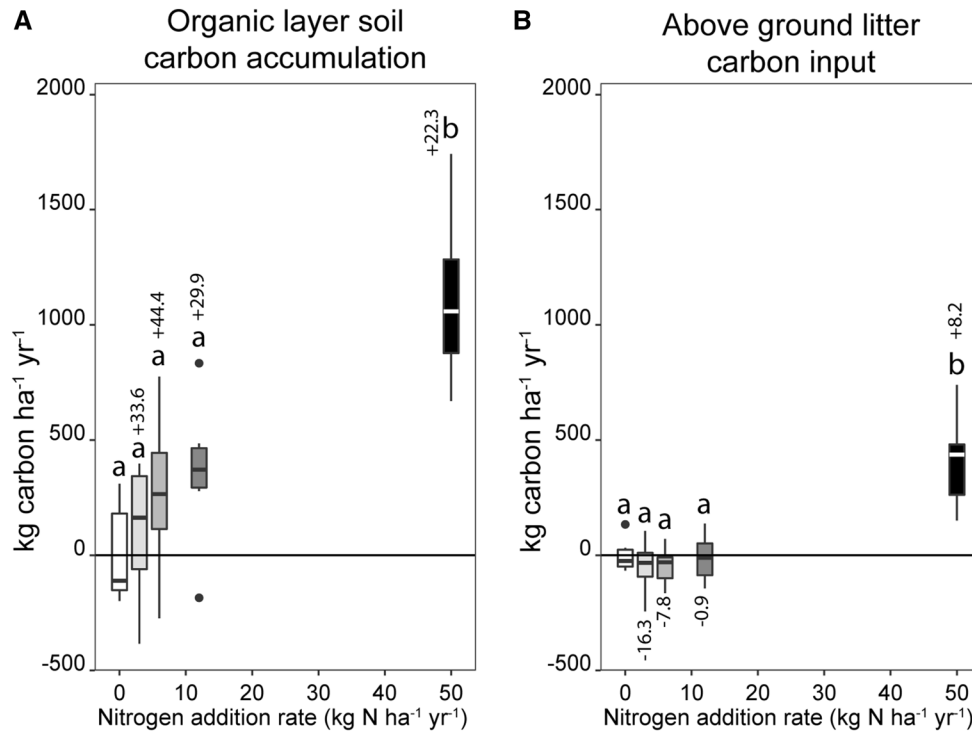
Figure 2. Annual carbon accumulation rate in the organic layer averaged over a 13-year period (**A**) and annual aboveground litter carbon input averaged over the 12th and 13th year of annual nitrogen additions (0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}). Values are relativized to the control treatment average, denoted with horizontal lines. The input of aboveground litter carbon was 890 kg ha^{-1} y^{-1} in the control treatment. Values above or below boxes represent average carbon flux per unit mass of nitrogen added (kg C kg^{-1} N added). Boxes display the median and the quartile above and below the median and whiskers are the maximum and the minimum ($n = 6$). Outliers (> 1.5 interquartile outside the box) are plotted individually. Boxes sharing letters within each panel are not significantly different at $p < 0.05$ according to Tukey's test.

Litter Input

The litter input was 890 kg C ha^{-1} y^{-1} in the control treatment, which significantly ($p < 0.001$) increased by 410 kg C ha^{-1} y^{-1} in the 50 N treatment, which is equivalent to a 46% increase and an additional C input rate of 8.2 kg C kg^{-1} N added (Figure 2B). The lower N addition treatments did not affect litter input rates ($p > 0.1$).

Soil Respiration

Due to differences in soil C stocks between N addition treatments, the effect of N on soil respiration differed depending on whether it was expressed on a per unit area or per unit mass soil C (Figure 3A–D). Nitrogen had no significant effect on areal total respiration (Figure 3A, Table 2). However, when the total soil respiration was expressed on a per unit mass soil C basis, we found a significant decrease by 31.2% between the control and the 50 N treatment (Figure 3B). Nitrogen addition increased heterotrophic respiration sig-

nificantly by 22.7, 15.7, and 22.0% in the 3 N, 12 N, and 50 N treatments relative to the control, respectively (Figure 3C), while the 6 N treatment did not differ significantly from any of the other treatments. Expressed on a per mass unit soil C, the heterotrophic respiration was unaffected (Figure 3D). The autotrophic component was unresponsive to the 3 N, 6 N, and 12 N treatments, whereas the 50 N treatment significantly decreased by 29.3% compared to the control (Figure 3E).

Total soil respiration varied seasonally with the highest recorded flux in August around day 220 both years (Figure 4A, B, $p < 0.001$, Table 2). This seasonal variability was driven primarily by the autotrophic component that contributed 62% of the total soil respiration in the control treatment in August and then decreased to 48% in October (Figure 4C–F). In the 50 N treatment, the autotrophic component contributed 44% in August and decreased to 39% in October. The effect of N did, however, not change significantly over the season

(Figure 4, Table 2, $p > 0.05$ for the interaction terms).

DISCUSSION

This study explores the effect of a gradient of simulated N deposition rates on soil C stocks in an N poor coniferous forest and evaluates three factors that may contribute to C stock changes, including litter input, autotrophic, and heterotrophic respiration. We found a significant increase in soil C stocks in the organic layer when N was added at a rate of 50 kg N ha^{-1} y^{-1} annually for 13 years, which is equivalent to a C accumulation rate of 22.3 kg C kg^{-1} N added. This response is twice as high as was reported in a nearby Norway spruce forest with similar N addition rates (10 kg C kg^{-1} N) (Maaroufi and others 2015) and three times higher than the average for *P. sylvestris* forests in Sweden and Finland (7 kg C kg^{-1} N) (Hyvönen and others 2008), but similar to the average in a global meta-analysis of temperate forests (Janssens and others 2010) (19 kg C kg^{-1} N). Our site is a N poor (Table 1) and well-drained pine forest that experiences seasonal water limitation on net primary production (Palmroth and others 2014; Lim and others 2017). Despite this water limitation, the forest achieved a relatively high rate of soil C accumulation when enriched with high levels of N (Hyvönen and others 2008).

In agreement with our first hypothesis that a shift in C allocation between growth and autotrophic respiration would contribute to this response, we observed an increase in litter C inputs by 46% and a 29% decrease in autotrophic soil respiration in response to the 50 N treatment. Notably, this response only occurred at the highest N addition rate, whereas lower N addition rates had no effect on litter C inputs or on autotrophic respiration, which is consistent with the high potential for mosses (Gundale and others 2011), soil microbes (Kaye and Hart 1997), and abiotic sinks in the soil to immobilize N that reduces its availability to trees when input rates are relatively low (Templer and others 2012). The decrease in autotrophic respiration was highest in August when it contributed 62% of the total CO_2 efflux in the control treatment, whereas it only contributed 44% of total respiration at the highest N addition rate (Figure 4). The peak in autotrophic respiration in August (Figure 4E, F) is consistent with studies that have shown trees exhibit a high rate of belowground C allocation late in the growing season (Pumpanen and others 2015) and the N-induced reduction in autotrophic respiration is consistent

with a reduction in the input of C via roots due to shift in allocation (Litton and others 2007). In addition to decreasing inputs of C via roots, autotrophic respiration could also decrease if N influences the relation between biomass production and respiration in the root zone (Chen and others 2014; Högberg and others 2002). We speculate that in addition to shifts in allocation between above and belowground structures, the decrease in autotrophic respiration could also be caused by changes in the allocation of C between root functions such as exudation and export into microbial communities in the root zone. These shifts could potentially impact priming effects (Kuzyakov, 2010) and the production of root biomass (Rasse and others 2005; Yuan and Chen 2012), which both are likely to impact soil C storage and could help explain the decrease in microbial biomass and decomposition observed in this (Maaroufi and others 2019) and many other N enrichment experiments (Knorr and others 2005; Treseder 2008).

We secondly hypothesized that soil microbial communities in this type of nutrient-poor coniferous forest are N limited and because of this that both heterotrophic and autotrophic respiration will increase at low N addition rates, whereas we hypothesized that high N addition rates would suppress their activities, as has been shown in many other studies (Janssens and others 2010; Knorr and others 2005; Treseder 2008). Our results revealed a generally positive effect of N additions on areal heterotrophic soil respiration (Figure 3C) at both high and low N addition rates, but not on autotrophic respiration (Figure 3E). The stimulating effect of N only appeared in the areal fluxes, and no significant effects appeared when we expressed the heterotrophic soil respiration per mass unit soil C (Figure 3D), indicating that most of the increase in areal heterotrophic respiration in this study was driven by the larger soil C stocks in the N-treated plots, rather than alleviation of N limitation on saprotrophic communities. In contrast, some studies have observed increased decomposition (Knorr and others 2005) or respiration (Bowden and others 2004) after short term or low levels of N additions, which potentially could decrease soil C stocks (de Vries and others 2014). As one example from an experiment in a Scots pine forest 7 km from our experiment, Hasselquist and others (2012) detected a 50% increase in total areal soil respiration after five years of annual N additions of 20 kg ha^{-1} y^{-1}, whereas a much higher N addition rate of 100 kg N ha^{-1} y^{-1} over the same period had no effect. They attributed the increase to root and mycorrhizal respiration, that is, what we define
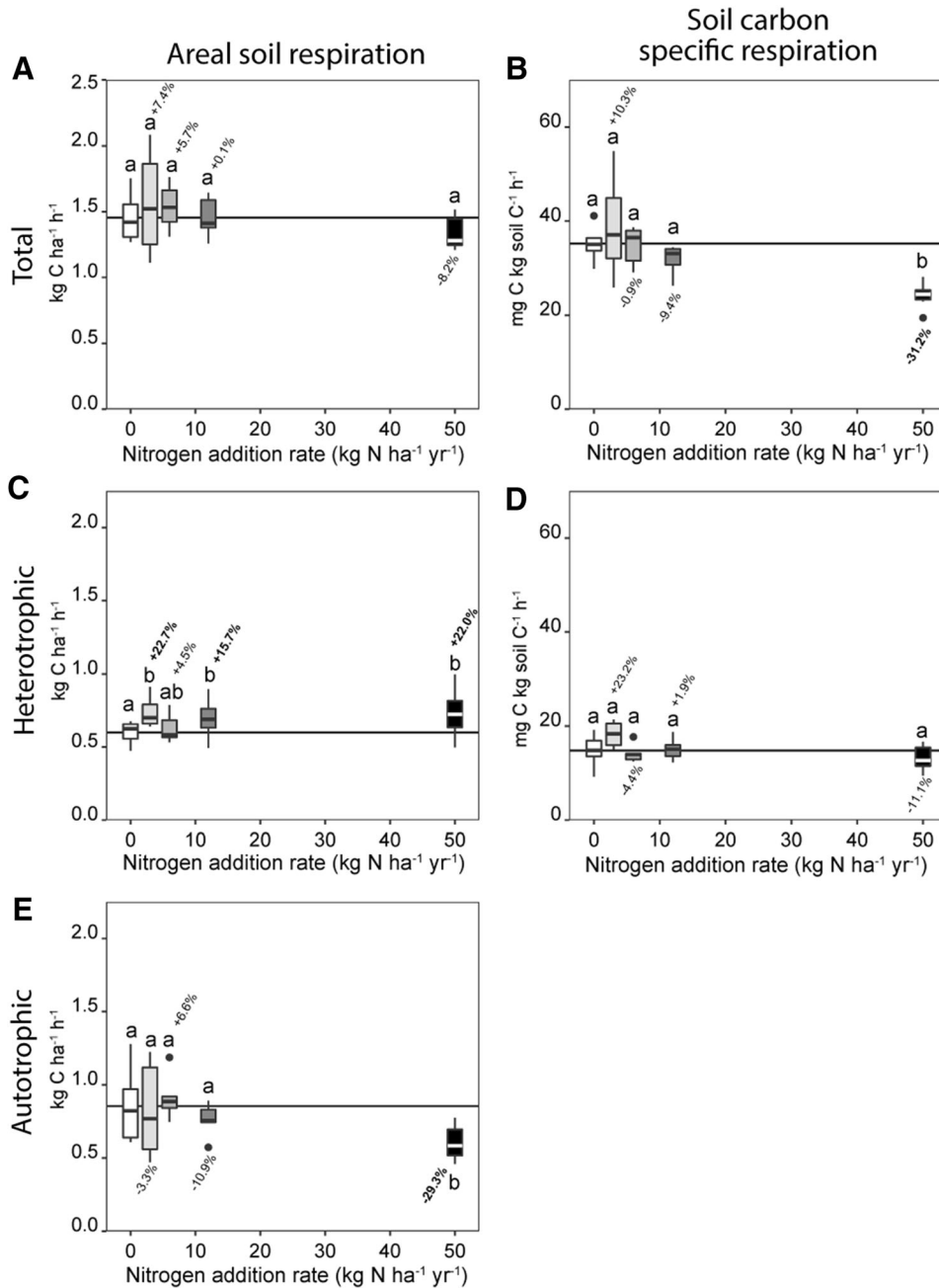
Figure 3. Soil respiration averaged for 11 measurement occasions during the growing season after 12–13 years of annual nitrogen additions (0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}). The total (**A**, **B**) and heterotrophic (**C**, **D**) respiration expressed on an area (left) and per mass unit carbon down to 20 cm in the soil (Figure 1) (right), and the difference in areal respiration between total (**A**) and heterotrophic (**C**) respiration, that is, autotrophic (**E**). Boxes display the median and the quartile above and below the median and whiskers are the maximum and the minimum (*n* = 6). Outliers (> 1.5 interquartile outside the box) are plotted individually. Boxes sharing letters within each panel are not significantly different at $p < 0.05$ according to mixed effect models (areal respiration) or Tukey's test (soil carbon-specific respiration). Horizontal lines denote mean respiration in the control treatment, and values above or below boxes represent percentage difference in mean respiration compared to the control treatments.

Table 2. The Effect of 12 to 13 Years of Annual Nitrogen Addition at 0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}, Time (Repeated 11 Times Over Two Consecutive Growing Seasons), and their Interaction on Total Soil Respiration, and Divided into its Heterotrophic and Autotrophic Components as Analyzed by Mixed Effect Models, and the Effect of Nitrogen on Total and Heterotrophic Soil Respiration Expressed on a Per Unit Mass Soil Carbon, Analyzed with ANOVA.

Source	Denominator DF	F-ratio	p value
Total areal respiration (LMM)			
Nitrogen	30.5	1.5	0.232
Time	197	195.0	**< 0.001**
Nitrogen × time	197	1.2	0.249
Heterotrophic areal respiration (LMM)			
Nitrogen	41.4	4.0	**0.007**
Time	178	93.9	**< 0.001**
Nitrogen × time	178	1.4	0.078
Autotrophic areal respiration (LMM)			
Nitrogen	38.3	3.8	**0.011**
Time	182	48.6	**< 0.001**
Nitrogen × time	182	1.2	0.173
Total soil carbon-specific respiration (ANOVA)			
Nitrogen	25	5.590	**0.002**
Heterotrophic soil carbon-specific respiration ANOVA)			
Nitrogen	25	1.936	0.07

p values < 0.05 are in bold font.
LMM: numerator degrees of freedom (DF) were 4, 10, and 40 for the nitrogen, time, and the interaction term, respectively, for the areal respiration.
ANOVA: numerator degrees of freedom were 4 for the nitrogen effect on soil carbon-specific respiration.

as autotrophic soil respiration. Similarly, Bidartondo and others (2001) detected an instant and strong increase in respiration in some species of mycorrhizal fungi but not others when N was added to conifer tree seedlings grown in mesocosms.

In contrast to these studies, our data do not show an increase in autotrophic respiration. It is possible that such effects occurred during earlier phases of our experiment, but have now diminished. However, we do not find any lasting signs of soil C losses in these treatments (Figure 2A), which higher respiration rates would likely have caused (de Vries and others 2014). The general decrease in soil respiration expressed on a per mass unit soil C we observed supports the result of a parallel study in the same system focused on decomposition that found that N enrichment decreases the abundance of both fungal and most bacterial biomarkers, which corresponds with a reduced rate of litter decomposition (Maaroufi and others 2019). Taken together, the general decrease in soil respiration, markedly driven by reduced autotrophic respiration, along with reduction in microbial biomass, fungal community composition shifts, and reduced decomposition rates that we have shown in a previous studies (Maaroufi and others 2019), likely

plays a major role in the accumulation of C observed in this and other N addition experiments.

Weighing the relative contribution of changes in C inputs via litter and output via soil respiration to the accumulation of soil C is challenging due to differences in temporal resolution, but can also provide mechanistic insights. The organic layer C stock in the 50 N treatment was 14.5 Mg C ha^{-1} higher than the control treatment (Figure 1), and aboveground tree litter input at the forest floor was elevated by on average 410 kg C ha^{-1} y^{-1} in this treatment, possibly adding 5330 kg C ha^{-1} in total during the experiment (Figure 2 B). This value is likely to be an accurate estimate of tree litter C input of the entire duration of the experiment, as the maximum growth response was attained at first in the seventh year of the experiment, and has since then declined (From and others 2016). However, it is possibly an overestimate of the total C inputs, as litter from plant groups that often decline in response to N, such as understory vegetation, is not included in our estimate (Maaroufi and others 2016). As an example, the moss layer in this experiment, dominated by *Pleurozium schreberi* (Brid.) Mitt., decreased by 795 kg C ha^{-1} during the first four years in the 50 N treatment, leading to
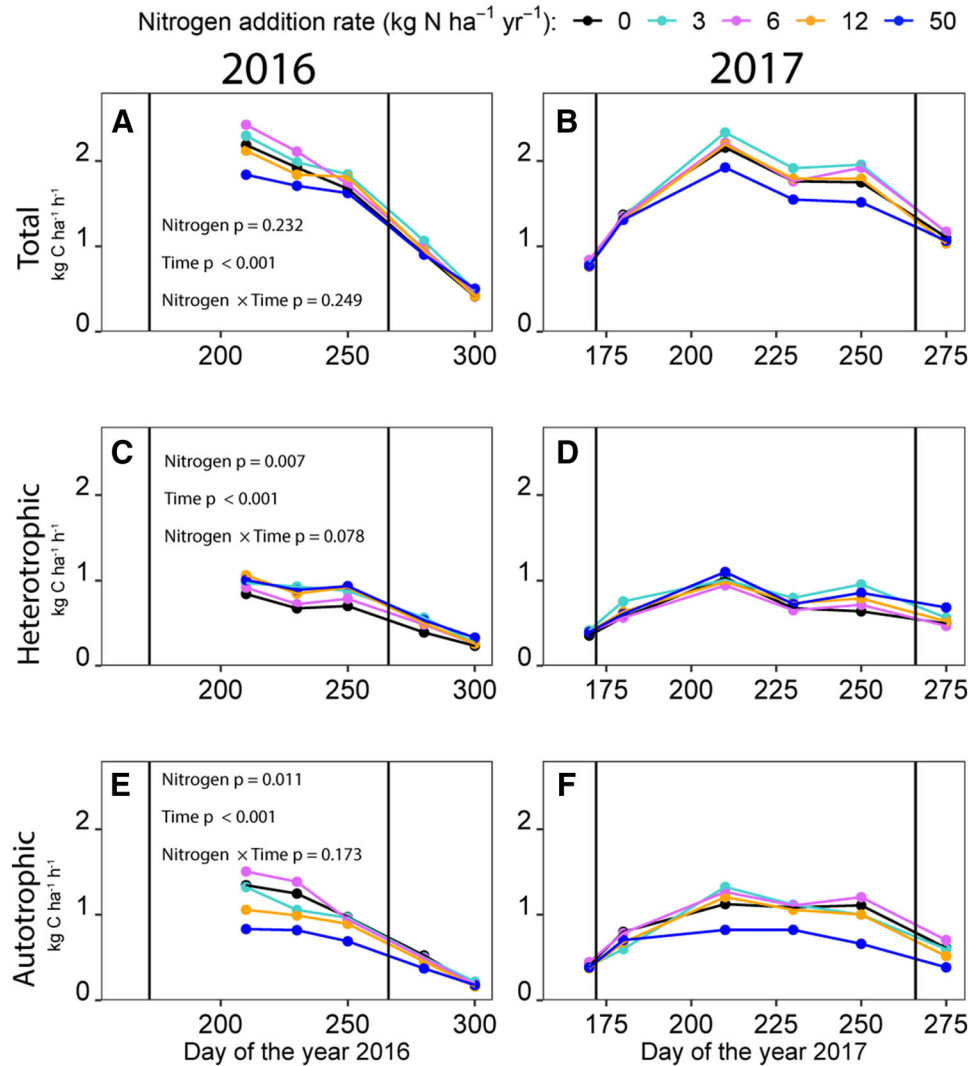
Figure 4. Total areal soil respiration (**A**, **B**) partitioned into its heterotrophic (**C**, **D**) and autotrophic components (**E**, **F**) over the growing season for five levels of nitrogen additions (0, 3, 6, 12, and 50 kg N ha^{-1} y^{-1}) for 12 and 13 years in 2016 (left) and 2017 (right), respectively. Soil respiration was partitioned by removing tree roots (that is, trenching) in 2014 on permanent subplots. Vertical lines indicate the summer solstice and autumn equinox, respectively. p values are the direct and interactive effects of nitrogen addition and time evaluated with mixed effect models (Table 2).

a small short-term flush of C inputs, at the beginning of the experiment, but a loss of moss litter production during the rest of the experiment (Gundale and others 2011). Increased litter C inputs have therefore clearly contributed to the increase in organic layer C stocks in the 50 N treatment, but it is insufficient by itself to explain more than one-third of the difference in C stocks (Figure 2). This indicates that the various mechanisms that cause soil respiration (Figure 3) and decomposition (Maaroufi and others 2019) to decline are likely to be of relatively greater impor-

tance in explaining why soil C stocks increase in response to high levels of N addition.

CONCLUSIONS

Our study provides rare experimental data describing how low versus high levels of N enrichment simulating atmospheric N deposition rates in northern coniferous forests impact soil C inputs and outputs, and changes to soil C stocks. One key finding of our study was that C accumulated in the organic layer, by an average of 22.3 kg

C kg N^{-1} in the high N treatment (50 kg ha^{-1} y^{-1}) averaged over the 13 years the experiment has run. This increase appears to be driven by both an increase in litter input and a decrease in autotrophic soil respiration. In contrast, lower N addition rates ($\leq$ 12 kg ha^{-1} y^{-1}) that are more representative of the range of atmospheric N deposition in northern coniferous forests did not stimulate aboveground litter production during the study period. Further, in contrast to our expectation that low levels of N would stimulate microbial activity, we found that neither heterotrophic nor autotrophic respiration was stimulated by our three lowest N treatments (3, 6, and 12 kg N ha^{-1} y^{-1}) relative to the control. Our findings have substantial implications for potentially improving the way N deposition is represented in Earth systems models, which most commonly emphasize that N enhances soil C stocks by enhancing NPP and litter inputs (Parton and others 1993; Smith and others 2014; Thomas and others 2013, 2015). In contrast, our work clearly shows that litter inputs in northern forests can be relatively unresponsive to N at low addition rates and that changes in aboveground litter input only explain approximately one-third of the C that accumulates in the soil. Instead, decreased respiration is clearly a major factor contributing to the accumulation of C in the soil, and we suggest that shifts in C allocation between different plant functions above- and belowground, and within the root zone, play a pivotal role in the soil C dynamics in N-enriched forest soils, where further research is needed.

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OPEN ACCESS

DATA AVAILABILITY

Data from this article can be found at: https://www.safedeposit.se/projects/199.

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