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The carbon sequestration response of aboveground biomass and soils to nutrient enrichment in boreal forests depends on baseline site productivity

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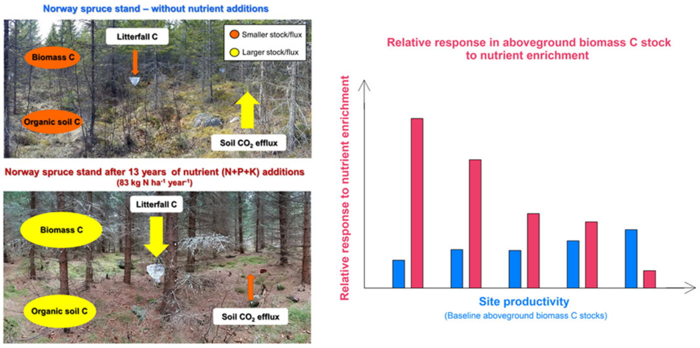
HIGHLIGHTS

- We studied forest productivity impacts on ecosystem responses to nutrient additions.
- Site productivity modulates ecosystem C–N responses to nutrient enrichment.
- Lower baseline site productivity coincided with higher C uptake in tree biomass.
- Higher biomass C uptake was balanced by weaker C uptake in the organic soil horizon.
- Increase in biomass and soil C stocks associated with lower soil CO_2 efflux.

GRAPHICAL ABSTRACT



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ABSTRACT

Nutrient enrichment can alleviate productivity limitations and thus substantially increase carbon (C) uptake in northern coniferous forests. Yet, factors controlling stand-to-stand variation of forest ecosystem responses to nutrient enrichment remain unclear. We used five long-term (13 years) nutrient-enrichment experiments across Sweden, where nitrogen (N), phosphorus, and potassium were applied annually to young Norway spruce forests that varied in their baseline ecosystem properties. We measured tree biomass and soil C and N stocks, litterfall C inputs, soil CO_2 efflux, and shifts in composition and biomass of soil microbial communities to understand the links between above and belowground responses to nutrient enrichment. We found that the strongest responses in tree biomass occurred when baseline site productivity was lowest. High increases in tree biomass C stocks were generally balanced by weaker responses in organic soil C stocks. The average ecosystem C–N response rate was 35 kg C kg^{-1} N added, with a nearly five-fold greater response rate in tree biomass than in soil. The positive nutrient enrichment effects on ecosystem C sinks were driven by a 95% increase in tree biomass C stocks, 150% increase in litter production, 67% increase in organic layer C stocks, and a 46% reduction in soil CO_2 efflux accompanied by compositional changes in soil microbial communities. Our results show that ecosystem C uptake in spruce forests in northern Europe can be substantially enhanced by nutrient enrichment; however, the strength of the responses and whether the enhancement occurs mainly in tree biomass or soils are dependent on baseline forest productivity.

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1. Introduction

Forests play a major role in climate change mitigation by acting as carbon (C) sinks and providing a variety of other ecosystem services (Aggestam et al., 2020; Lundmark et al., 2014). The quest for enhanced terrestrial C sinks and the ongoing transition towards a circular bioeconomy has increased demand for biomass production in forests worldwide (Aggestam et al., 2020). Forest productivity is often constrained by nutrient availability globally (Lu et al., 2011b; Schulte-Uebbing et al., 2022; Yue et al., 2016), and in northern European coniferous forests the primary growth limitation is nitrogen (N) availability (Binkley and Högberg, 2016; Högberg et al., 2017; Nohrstedt, 2001). When N availability is high, tree growth is frequently co-limited by other nutrients (Harpole et al., 2011; Hyvönen et al., 2008; Tamm, 1991), and these limitations may become progressively aggravated in forests in the future due to rising atmospheric CO_2 concentrations (Craine et al., 2018; Johnson, 2006; Luo et al., 2004), or alternatively shift towards other limiting factors due to, e.g., anthropogenic N deposition or shifts in N mineralization induced by soil warming. Nutrient availability is therefore likely going to be dynamic and dependent on the complex interactions between the local site conditions and global change drivers. The exact magnitude to which boreal forest ecosystems take up C in response to nutrient enrichment remains uncertain, particularly for other less studied biomass pools beside stem wood. Less is also known about the impacts of nutrient enrichment on C uptake in young coniferous forests (Hyvönen et al., 2008). Soils globally store more C than vegetation and the atmosphere combined, and the fraction of ecosystem C stored in soils is particularly high in northern coniferous forests. The uncertainty in soil C responses to nutrient enrichment in this globally large pool is of particular concern, as any shifts in soil C stocks could potentially have major consequences for the global C cycle and climate change aggravation (Friedlingstein et al., 2020; Lu et al., 2011b; Xu et al., 2021; Yue et al., 2016).

Soil organic carbon (SOC) originates essentially in photosynthesis through processes mediated by soil microorganisms, while soil C stock is determined by the balance between inputs of above and belowground plant litter, root exudation, and C outputs, mainly via soil CO_2 efflux (Bahn et al., 2010; Clemmensen et al., 2013; De Vries et al., 2014; Kyaschenko et al., 2017). Nitrogen additions have been shown to have no effect (Janssens et al., 2010), stimulate (Yue et al., 2016), or substantially increase litterfall production in forest ecosystems (Forsmark et al., 2020). Soil CO_2 efflux, on the other hand, is frequently down-regulated by N addition, although effect sizes ranges from slightly positive to strongly negative (Bowden et al., 2004; Hasselquist et al., 2012; Janssens et al., 2010; Olsson et al., 2005). Intuitively, higher aboveground litterfall C input should stimulate the heterotrophic microbial community, which contradicts the evidence of negatively impacted soil organic matter decomposition rates, and heterotrophic soil respiration in response to N addition (Berg and Matzner, 1997; Fog, 1988; Janssens et al., 2010; Knorr et al., 2005). Northern coniferous forests typically sequester 10–50 kg C ha^{-1} yr^{-1} in soils, whereas fertilization studies have shown highly diverging responses, from losses of soil C, to annual accumulation rates of more than 1000 kg C ha^{-1} yr^{-1} (Blaško et al., 2013; Forsmark et al., 2020; Maaroufi et al., 2015), while less is known about nutrient enrichment impacts in the deeper mineral soil layers in which SOC is less susceptible to disturbance (Lu et al., 2011b; Mayer et al., 2020; Xu et al., 2021). Understanding these uncertainties and mechanisms is crucial for predicting the ecosystem C–N responses to nutrient enrichment, i.e. how much C is stored per unit N added in particular ecosystem pools.

When biomass production is limited by nutrients, a large portion of photosynthate is transferred belowground as various C compounds to root growth and root-colonizing ectomycorrhizal (ECM) fungi ECM to ensure nutrients acquisition for plants (Gill and Finzi, 2016; Högberg et al., 2010; Litton et al., 2007; Prescott et al., 2020). The ECM fungal network thus acts as a significant sink for plant photosynthates, while the ECM fungal litter is a source for stable SOC (Clemmensen et al., 2013; Wallander et al., 2011). Ectomycorrhizal fungi are also a source of CO_2 through decomposition of the soil organic matter (i.e. priming and nutrient mining), which drives autotrophic respiration that typically represents 40–60% of the soil CO_2 efflux (Forsmark et al., 2020; Hasselquist et al., 2012, 2016; Högberg et al., 2001). Many observational and modeling studies suggest that when nutrient limitation is removed, the belowground C allocation is reduced and a larger portion of photosynthate is allocated to aboveground biomass production (Chen et al., 2014; Fernández-Martínez et al., 2014; Litton et al., 2007; Vicca et al., 2012), which frequently serves as an explanation for reduction in ECM fungal biomass (Högberg et al., 2010; Näsholm et al., 2013; Prescott et al., 2020; Wallander et al., 2011), and consequently decreases in autotrophic respiration in northern coniferous forests in response to nutrient enrichment (Forsmark et al., 2020; Hasselquist et al., 2016, 2012; Olsson et al., 2005). While these specific mechanisms of nutrient enrichment on soil C accumulation have been examined in specific forest ecosystems, few studies have attempted to study the relative contribution of these mechanisms towards C accumulation in response to nutrient enrichment across a gradient of forest productivity. Moreover, few studies have attempted to link plant and soil C responses to nutrient enrichment, for example by showing how key biomass pools such as fine roots and fungi, and key C fluxes such as litterfall and respiration, respond to changes in nutrient enrichment across forests that differ in their baseline site productivity.

We studied the impacts of intensive nutrient enrichment on ecosystem C stocks in five young Norway spruce stands in Sweden varying greatly in their baseline productivity, soil C:N ratio, and soil N stocks (Table 1). Nitrogen (83 kg N ha^{-1} yr^{-1} added on average during this period) and other nutrients (phosphorus, potassium) were added annually for 13 years (Table 1) in ratios ensuring optimal nutrient supply (Bergh et al., 2008; Linder, 1995). We estimated responses of aboveground biomass (AGB) C, belowground biomass (BGB) C, and SOC stocks to nutrient enrichment, and investigated the C–N response of different ecosystem compartments in relation to site productivity across the five experimental sites (Hyvönen et al., 2008). We investigated whether litterfall C inputs, soil CO_2 efflux, and soil microbial biomass and composition could provide a mechanistic explanation for the nutrient enrichment impacts on plant and soil C stocks at different site-productivity levels. We hypothesized that (H1a) nutrient enrichment would enhance total ecosystem C stocks, and that stronger C–N responses would be elicited in AGB relative to BGB and soil due to shifts in C allocation to AGB (Chen et al., 2014; Litton et al., 2007). We also hypothesized (H1b) positive responses in soil C stocks due to a combination of an increase in litterfall inputs and decrease in soil CO_2 efflux. We expected the latter to occur due to combined effects of reduced decomposition and C allocation belowground to roots and root-associated microbes (Gill and Finzi, 2016; Janssens et al., 2010; Prescott et al., 2020; Vicca et al., 2012), which would be accompanied by a decrease in fine-root biomass, and shifts in the composition of soil microbial communities, in particular reduction in fungal biomarkers in tree fine roots and soil (Högberg et al., 2010; Janssens et al., 2010; Litton et al., 2007; Prescott et al., 2020). Our second hypothesis (H2) explored how baseline site productivity, defined here as AGB C stocks, soil C:N, and soil N stocks in the control plots, influenced the ecosystem responses to nutrient enrichment. More specifically, we hypothesized that (H2) the strongest responses in AGB, BGB, and soil C stocks to nutrient enrichment would occur in the least productive stands, which should also be reflected in higher C–N responses at these sites (Hyvönen et al., 2008; Vicca et al., 2012).

2. Materials and methods

2.1. Sites description and experimental design

The nutrient enrichment experiments commenced in 2002 in young (13–20 years old) Norway spruce (*Picea abies* (L.) Karst) stands at five locations in Sweden (Fig. 1; Table 1) along a latitudinal and baseline productivity gradient (Bergh et al., 2008). The spruce seedlings of different provenance were planted between 1983 and 1991 on a former forest land (Bergh et al., 2008) following mechanical soil preparation. Thus, the

Table 1
Description and basic properties of the nutrient enrichment experiments in young spruce forests at five different locations across Sweden. Stand properties (avg. ± SE, n = 3) in the control vs. nutrient enrichment plots measured in 2001 before the nutrient additions commenced are shown for comparison of the initial conditions.

		Location				
		Bräcke	Gävle	Grängshammar	Mölnbacka	Ebbegärde
Latitude		62°43′N	60°34′N	60°21′N	59°36′N	56°53′N
Longitude		15°51′E	17°11′E	15°31′E	13°34′E	16°15′E
Altitude (m a.s.l.)		390	40	200	90	35
MAP[a]		664	573	648	714	591
MAT[a]		2.2	5.7	5.7	6.2	6.4
Soil C:N[b]		39.8 ± 5.1	31.7 ± 1.6	25.0 ± 1.8	25.3 ± 0.5	30.5 ± 2.5
pH[b]		3.48 ± 0.19	3.30 ± 0.07	3.37 ± 0.08	3.54 ± 0.04	3.32 ± 0.06
Site index (Si H100)		22	24	24	26	29
Stand age[c]		37	37	37	37	30
Diameter[d] (cm)	Control	4.6 ± 1.0	2.5 ± 0.3	4.2 ± 0.3	2.6 ± 0.3	2.3 ± 0.4
	Nutrients	4.0 ± 0.8	2.4 ± 0.3	3.8 ± 0.1	2.2 ± 0.3	2.1 ± 0.5
Stand density[e] (n ha^{-1})	Control	2091 ± 65	1853 ± 429	1913 ± 184	1650 ± 268	2235 ± 406
	Nutrients	2144 ± 73	1740 ± 135	2063 ± 238	2203 ± 271	2208 ± 293
Basal area (m^2 ha^{-1})	Control	3.5 ± 0.1	0.9 ± 0.2	2.6 ± 0.2	0.9 ± 0.1	0.9 ± 0.2
	Nutrients	2.7 ± 0.1	0.8 ± 0.1	2.3 ± 0.3	0.8 ± 0.1	0.8 ± 0.1
Background N deposition (kg ha^{-1} yr^{-1})[a,f]		1.6	3.8	3.2	5.8	5.0
Average N addition rate (kg ha^{-1} yr^{-1})		86	83	83	83	82
Total N added (kg ha^{-1})		1119	1077	1077	1077	1070
Total P added (kg ha^{-1})		260	247	247	247	379
Total K added (kg ha^{-1})		603	579	579	579	1183

[a] Swedish Hydrological and Meteorological Institute; MAP, MAT − mean annual precipitation (mm), respectively temperature (°C), more details about the meteorological stations can be found in Table S1 in Supporting information.
[b] Mean values ± SE, n = 3, in the organic soil layer of the control plots.
[c] As of 2018.
[d] Mean diameter at 1.3 m height.
[e] n − number of trees.
[f] Swedish Environment Institute IVL

stand age ranged 30–37 years at the time of this study (Table 1). Mean annual temperature (MAT) ranged from 2.2 °C at the northernmost site (Bräcke) to 6.4 °C at the southernmost site (Ebbegärde) (Table 1). Background N deposition was lowest at the northernmost site Bräcke (1.6 kg N ha^{-1} yr^{-1}) and increased to 5.8 and 5.0 kg N ha^{-1} yr^{-1} at the southernmost sites (Mölnbacka and Ebbegärde, respectively; Table 1). The soil C:N ratio was highest at the northernmost site (40), and ranged between 25 and 32 among the southernmost sites corresponding to the average N deposition rates across sites ($R^2_{adj} = 0.27, p < 0.05$; Table 1). Mean annual precipitation ranged between 591 and 714 mm (Table 1).

At all locations, podzolic soils were developed on glacial till or sediments. Bräcke and Grängshammar sites are located above the highest historical sea-level, while Gävle, Mölnbacka, and Ebbegärde are located below (Table 1). At Bräcke, a mor type of humus developed on a till with about 10% clay and mesic soil moisture conditions. Grängshammar was dominated mostly by a mull type of humus developed on sandy moraine and mesic-moist conditions. Below the highest historical sea-level, the soil types at Gävle were more variable. The humus types ranged from mor to moder to mull on a complex and mosaic moraine with clay sediments, while the soil moisture ranged from mesic to moist and occasionally moist with water-logged patches. At Mölnbacka, mor or mull developed on glacial clay sediments and mesic to moist conditions, while mor on a sandy moraine and mesic-moist to water-logged conditions prevailed at Ebbegärde.

Each experiment at each of the five sites included three blocks and each of the blocks comprised a control and a nutrient enrichment plot. The plots were 50 × 50 m at Gävle, Grängsammar, Mölnbacka sites, or 40 × 40 m at Bräcke and Ebbegärde, which included a buffer zone surrounding net plots that were 31.6 × 31.6 m (Gävle, Grängsammar, Mölnbacka), or 25 × 25 m (Bräcke, Ebbegärde). All measurements were made within the net plot area to avoid edge effects. In total, summed across all sites, there were 15 control and 15 plots enriched with nutrients.

Nutrients were added as commercially available solid NPK fertilizers (Bergh et al., 2008) including nitrogen, phosphorus (P), and potassium (K) (Yara International, Norway) annually in May between 2002 and 2014. Depending on the nutrient concentrations in needles, fertilizers with different ratios of the elements were used to match the target ratio of N:P:K and ensure thus optimal balance of nutrients (Bergh et al., 2008; Linder, 1995; Wallander et al., 2011). On average, 82–86 kg N ha^{-1} was added annually at each site, with a total of 1070–1119 kg N ha^{-1}, while 247–379 kg ha^{-1} of P, and 579–1183 kg ha^{-1} of K were added along in total (Table 1).

2.2. Tree biomass

During 2016 − 2017, three spruce trees from each plot and block were sampled for biomass estimates. Thus, 9 trees per treatment and location covering a range of diameters at 1.3 m (DBH) were sampled in total to obtain allometric functions for stem, bark, branches, and foliage, while the DBH of all trees within each experimental plot was measured (Blaško et al., 2020). We then applied the location- and treatment-specific allometric functions to all the trees in the experimental plots, which enabled us to first calculate the biomass at plot level and then scale it up to 1 ha. For biomass of coarse-roots and stumps, and biomass of other tree species (*Pinus sylvestris* and *Betula* spp., which accounted for only ~7% of aboveground C stocks), we used national allometric equations (Marklund, 1988; Petersson and Ståhl, 2006). Grey alder (*Alnus incana*) was present in one case, in which the function for birch was used to estimate the stem biomass. The biomass estimates include both, spruce and other tree species, and all the trees in the plot.

To estimate the fine-root biomass (≤ 2 mm in diameter) in the organic soil horizon, we randomly collected 20 soil cores (48 mm diameter) of the F and H sub-layers (FH) of the organic soil horizon within each plot in September 2017. We used a 4 mm sieve to separate the fine roots from the organic soil directly in the field. Sieved organic soil and fine-root samples were then immediately put in a cooler box during sampling and transport before they were kept in the freezer at −20 °C. In the laboratory, fine-root samples were washed and placed in a water-filled tray, from which the fine roots (≤ 2 mm) were hand-picked by tweezers (Blaško et al., 2020; Forsmark et al., 2021). Sorted fine roots were freeze-dried and used for subsequent microbial analysis (see Section 2.9). The dry mass of the

Fig. 1. Locations of the five nutrient enrichment experiments in young Norway spruce forests along a latitudinal and productivity gradient in Sweden. At each site, control and nutrient enrichment plots were replicated three times. Sources: Esri, Gebco, DeLorme, NaturalVue, Eurostat, Gisco.

fine roots was related to the area sampled and scaled up to one hectare (Blaško et al., 2020).

2.3. Tree biomass C and N stocks

Stem, bark, needles, and fine roots of the spruce trees were analyzed for C and N concentrations, while the concentrations in branches were estimated from the average C and N concentrations in stem and bark. The concentrations of C and N for coarse roots were approximated from the concentrations in stem wood. For other tree species than spruce, C concentration was assumed to be 50% for all parts of the biomass. Nitrogen stocks were estimated only for the spruce trees, which was the focal dominant species at every site (93% of the aboveground biomass C stocks), and because N% data for biomass of other tree species were not available.

2.4. Soil sampling for SOC and N stock estimates

Soil samples for SOC and N stock estimates were taken between autumn 2016 and summer 2017 following the sampling and processing methods described in detail previously (Blaško et al., 2020, Supporting information), except that in the current study, sampling points were pre-determined by evenly distributed intersections in a grid that covered the whole plot. Briefly, the F and H sub-layers of the organic soil horizon were sampled with a 10 cm diameter corer from every second point in the grid with an aim to collect 10 soil cores in total per plot, which were sieved on a 4 mm sieve and pooled to one bulk sample per plot (Blaško et al., 2020). A sampling point was skipped if a sample could not be taken due to, e.g., the presence of a coarse root or rock. In such cases, additional cores were collected from other grid points if there was an insufficient amount of sample for analyses. The soil dry mass was related to the area of the forest floor sampled, which enabled upscaling of C and N mass per unit area (Blaško et al., 2020).

The mineral soil was sampled with an open-side auger (2.54 cm diameter) at 0–10 and 10–20 cm depths (Blaško et al., 2020). Samples were collected from 20 pre-determined points in the grid (see above), sieved on a 2 mm sieve, and pooled into one sample per plot and depth (Blaško et al., 2020; Dane et al., 2002). Sampling points that could not be sampled due to a rock or tree root were not repeated. To account for boulders and stones in the SOC and N stock estimates, we employed the rod penetration method to estimate the stoniness index first, and thus, the percentage volume of stones and boulders (SB%; diameter > 2 cm) occupying the upper 30 cm of the mineral soil (Blaško et al., 2020; Eriksson and Holmgren, 1996; Stendahl et al., 2009; Viro, 1952). We then used pedotransfer functions with C% and organic matter (o.m.) content (loss on ignition at 550 °C, 6 h) of the soil samples as input parameters to estimate the dry bulk density of the soil in the remaining volume after correcting for SB% (Blaško et al., 2020; Nilsson and Lundin, 2006). Finally, the C and N stocks were calculated following (Blaško et al., 2020; Eq. S1, Supporting information).

2.5. Analysis of soil and biomass C, N, organic matter content, and moisture

Oven-dried and homogenized samples of the tree biomass and sieved soil were analyzed for C and N content on an elemental analyzer coupled to an isotope ratio mass spectrometer (Flash EA 2000, respectively DeltaV, Thermo Fisher Scientific, Bremen, Germany) (Werner et al., 1999).

2.6. Carbon-nitrogen response to nutrient enrichment

The C–N response for the different ecosystem compartments was calculated as the difference between the fertilized and control plots within each block divided by the total mass of N added during the entire treatment period (Forsmark et al., 2020; Hyvönen et al., 2008; Maaroufi et al., 2015). Carbon–nitrogen responses were estimated for the aboveground tree biomass (AGB), belowground tree biomass (BGB), organic soil layer, mineral soil layers (0–20 cm), sum of the organic and mineral soil C stocks, and for the whole ecosystem including all measured plant and soil pools. Aboveground biomass pools included stem wood, bark, branches, and needles, while the BGB pools included allometric estimates of stumps and coarse roots (> 2 mm) (Petersson and Ståhl, 2006), and the measured fine-root biomass (≤ 2 mm).

2.7. Soil CO_2 efflux

The soil CO_2 efflux was measured following Blaško et al. (2020) at three occasions during summer 2017: at the end of June, and in the beginning of August and September. At each plot, ten permanent collars were inserted into the soil 1 cm deep to prevent severing of roots and the green parts of the vegetation inside the collars were removed (Blaško et al., 2020; Forsmark et al., 2020; Maaroufi et al., 2015). Two identical portable infra-red CO_2 gas analyzers (CARBOCAP, model GMP 343, Vaisala, Finland) were used simultaneously to estimate the mean soil CO_2 efflux rate at the plot level. The efflux rate was estimated from the linear build-up of CO_2 inside the headspace at 15-s intervals for 3 min to minimize the CO_2 saturation in the headspace and prevent the associated decline in respiration rate (Blaško et al., 2020; Davidson et al., 2002).

2.8. Litterfall

Litterfall was collected continuously for one year. Five nylon-mesh litter traps 60 cm in diameter were systematically placed in each plot and emptied at three occasions between May 2017 and June 2018 (Blaško et al., 2020). Collapsed or damaged litterfall traps were excluded from the calculations. Litter was dried at 70 °C for 48 h before weighing and included different fractions: foliage litter, cones, twigs and branches up to 10 mm thickness together with other minor litterfall as bark, lichens, etc. (Blaško et al., 2020). The C% of the litterfall was assumed to be 50% (Blaško et al., 2020; Forsmark et al., 2020).

2.9. Soil microbial community

2.9.1. Phospholipid fatty acids biomarkers

Subsamples of the sieved (4 mm) soil samples collected in September 2017 (for the estimate of fine-root biomass, see details above) were analyzed for the abundance of the phospholipid fatty acids (PLFA) to assess the general composition of microbial communities and to approximate microbial biomass (Frostegård et al., 2011; Frostegård and Bååth, 1996). We followed modified Bligh and Dyer (1959) method (White et al., 1979). In total, 28 PLFA biomarkers were identified in the soil samples (Fanin et al., 2019; Pluchon et al., 2016), while some PLFAs were used as biomarkers for specific groups of soil microbes or microbial indices (Frostegård et al., 2011; Zelles, 1999). We used 12 different PLFA biomarkers (Supporting information) indicative of bacteria including, gram negative (G−), gram positive (G+) and G+ actinobacteria (Frostegård and Bååth, 1996; Ruess and Chamberlain, 2010; Zelles, 1999). The PLFA 18:2ω6,9 was used as a fungal biomarker (Frostegård et al., 2011; Frostegård and Bååth, 1996; Wallander et al., 2013; Zelles, 1999). The abundance of PLFAs was expressed in nmol g^{-1} of organic matter. The relative contribution (mol%) of a particular PLFA biomarker to the sum of the 28 PLFA biomarkers was used in multivariate analysis to explore the shifts in microbial community composition. We employed an unconstrained ordination (PCA) on log-transformed and double-centered data (Kenkel, 2006; Šmilauer and Lepš, 2014).

2.9.2. Ergosterol analysis

In addition to PLFA biomarkers, the ergosterol concentrations, a fungi-specific component of the cell membrane, was measured in fine roots as a proxy for ECM fungal biomass (Salmanowicz and Nylund, 1988). Ergosterol was extracted as described by (Clemmensen et al., 2013; Forsmark et al., 2021). Briefly, 15 mg of the ground fine roots were suspended in 500 μl MeOH (99.8%) by vigorous shaking and incubation at 20 °C for 30 min. Heavy and coarse particles were removed by centrifugation (10,000 x Gravitational Forces) and the MeOH supernatant finally filtered (45 μm) into auto sampler vials. We injected 100 μl of the root extract into MeOH (isocratic) at a flow rate of 1.5 ml min^{-1} on a Shimadzu prominence HPLC and separated on a reverse-phase column (Ascentis® Express C18, 2.7 μm). Ergosterol was detected with an optical-ultraviolet detector (SPD-20A UV/VIS) after 3 min.

2.10. Ecosystem responses to nutrients enrichment

To evaluate the effect size of the suite of ecosystem responses to nutrient enrichment measured across a wide environmental gradient and facilitate cross-site comparisons, we employed a response ratio (RR) approach and calculated the natural logarithm of the RR frequently used in meta-analyses (Hedges et al., 1999; Lu et al., 2011b, 2011a; Luo et al., 2006):

$$\begin{aligned} \mathrm{RR} &= \ln\ \frac{\text{Mean fertilized plot}}{\text{Mean control plot}} \\ &= \ln\ (\text{Mean fertilized plot}) - \ln\ (\text{Mean control plot}) \end{aligned} \tag{1}$$

Therefore, to explore the relationships between the site conditions and the effect size of the ecosystem responses to nutrients enrichment across the locations, the estimated RR of each variable was further correlated (Pearson-product moment) with the baseline site productivity indicators, i.e. levels of response variables in control plots. We used the plot values for baseline levels or response ratios ($n = 3$) at every location in correlation analysis, thus, the total $n = 15$. Correlations with $p < 0.1$ but ≥ 0.05 were considered weak (light color shade, Table 3), correlations with $p < 0.05$ but ≥ 0.01 were considered moderate (medium dark color shade, Table 3), while correlations with $p < 0.01$ were considered strong (darkest color shade, Table 3).

2.11. Data and statistical analyses

We used ANOVA and a mixed effects model to evaluate the nutrient enrichment effects on the response variables. The location of the site (df = 4, 10) and nutrient enrichment (df = 1, 10) were used as fixed, whereas blocks nested within the location as random (df = 4, 10) factors in the model. We also included the interaction of location and treatment term in the model. If the location or interaction of treatment and location terms were significant ($p < 0.05$), post hoc Tukey's pairwise comparisons followed to identify where the differences occurred. In case of C–N responses, we used general linear model (GLM) model with location as fixed factor ($n = 5$) and blocks ($n = 3$) included within the error term. To explore the effect ($p < 0.05$) of nutrient enrichment on soil CO_2 efflux and litterfall, average values for the whole season (soil CO_2, n = 3) or sum of all litterfall collections (n = 3) were used. In those cases, we used a mixed effects model with location and fertilization as fixed factors and blocks nested within locations as random factors. We used Minitab (Minitab, version 18.1, PA, USA), Canoco (v. 5.0, Biometris, Wageningen University, Netherlands), and Sigmaplot (Sigmaplot 10, Systat Software Inc., CA, USA) to perform statistical analyses and create figures.

3. Results

3.1. Nutrient enrichment impacts on ecosystem C stocks and C-N responses

Nutrient enrichment generally increased biomass and ecosystem C stocks, whereas the size of the responses was dependent on the baseline site productivity. The AGB, BGB, and total ecosystem C stocks increased at all locations (Fig. 2a, c; Table S2a, Supporting information). The ecosystem C stocks increased by 47% on average, with higher than average increases at Bräcke (85%), Ebbegärde (50%), Gävle (48%), and lower than average increases at Grängshammar (32%) and Mölnbacka (21%) (Fig. 2c). Tree AGB C stocks, except for needle biomass, increased with nutrient enrichment (Table S2a, Supporting information), whereas the highest relative increases occurred at the sites with lower baseline AGB C stocks (Fig. 2a). Belowground biomass C stocks, in particular C stocks in the stumps and coarse roots (> 2 mm), increased following nutrient enrichment (Fig. 2a), while the C stock in the fine-root biomass remained unchanged (Fig. 6b; Table S2a, Supporting information).

Soil responses were dependent on soil horizon and site productivity. Nutrient enrichment enhanced C accumulation in the organic, but not in the mineral soil (0–20 cm), neither overall in the total SOC stocks, i.e. sum of the organic and mineral soil horizons (Fig. 2b; Table S2a, Supporting information). The largest relative increases in the organic soil C stocks occurred at the sites with the lowest SOC stocks in the control plots: Mölnbacka (159%), Bräcke (128%), and Grängshammar (66%). In contrast, SOC stocks at the two sites with higher baseline SOC levels (Gävle and Ebbegärde) led instead to a loss of a small fraction of SOC stocks (−11% and −5%, respectively), which tended to be partly balanced by increases in the mineral soil SOC stocks (Fig. 2b).

The C–N response in the tree biomass was on average five times larger than in the soil (Table 2). The average C–N response in the AGB was 17.2 kg C kg^{-1} N added and the highest C–N response rates tended to occur at the locations with the lower AGB C stocks in the control plots: Bräcke, Gävle, Ebbegärde, and also Grängshammar (Fig. 2a, Table 2). In contrast, the lowest AGB C–N response at Mölnbacka coincided with the highest AGB C stocks in the control plots at this site (Fig. 2a, Table 2).

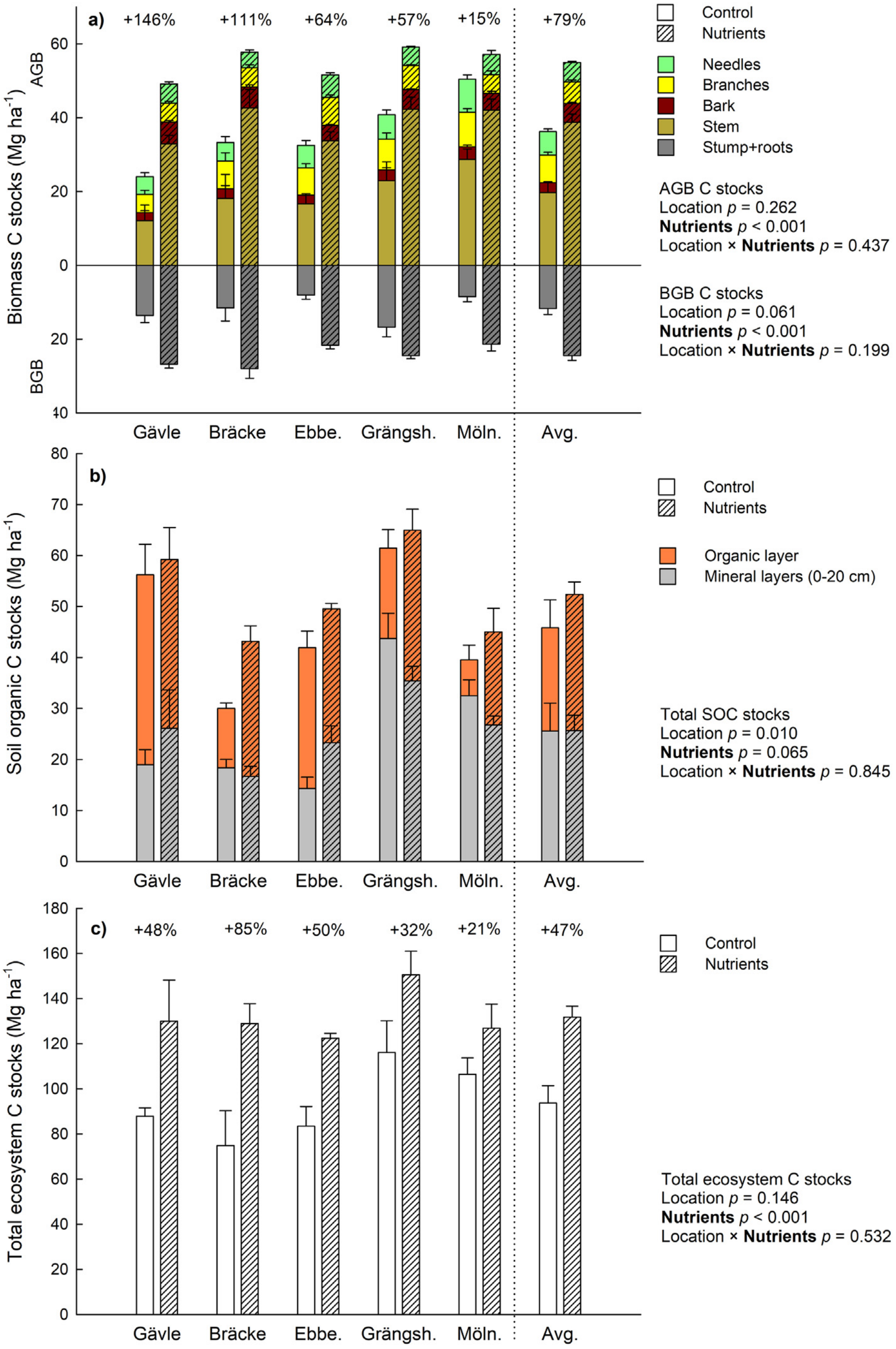

Fig. 2. Nutrient enrichment impacts on a) above (AGB) and belowground biomass (BGB) C stocks (mean ± SE, $n = 3$); b) C stocks in the organic and mineral soil layers; and c) total ecosystem C stocks in five young spruce stands along a site-productivity gradient in Sweden. The sites are ordered according to the strength of the responses in the AGB C stocks to nutrient enrichment from the strongest response on the left to the weakest response on the right. The average (Avg. ± SE, $n = 5$) nutrient enrichment effect over all the locations is separated from the site-specific responses with a dotted line. See Sections 2.2–2.4 and Supporting information for details on C stocks` estimates and 2.11 for details on statistical analyses. Roots include both coarse (> 2 mm) and fine (≤ 2 mm) fractions. The bars on the left represent the control plots, while the hashed bars on the right represent the plots that received 1070–1119 kg N ha^{-1} during 13 years along with phosphorus and potassium (see Table 1).

The average C–N response in the AGB was 1.5 times higher than in the BGB and 2.9 times higher than in the organic soil layer solely (Table 2). The slight reductions in the organic soil C stocks following nutrient enrichment at Gävle and Ebbegärde led to negative C–N responses that were balanced by higher C accumulation in the mineral soil at these locations (Table 2), which resulted in similar average C–N responses in the organic soil vs. the whole soil profile (Table 2). The ecosystem C–N responses thus ranged between 19 and 48 kg C kg^{-1} N added, while the average rate was 35 kg C kg^{-1} N (Table 2).

3.2. Ecosystem N stocks

Total ecosystem N stocks increased following nutrient enrichment with larger relative increases in tree biomass than in soil. Ecosystem N stocks increased on average by 28% with higher than average increases at Bräcke (49%), Ebbegärde (40%), and Gävle (32%), and lower than average increases at Mölnbacka (12%) and Grängshammar (8%) (Fig. 3c; Table S3a, Supporting information). The largest relative increases occurred in the AGB N stocks in the stem wood and bark, while no significant change in

Table 2

Average C–N response rates (n = 3, ± SE, kg C accumulated per kg of N added, see Section 2.6) to nutrient enrichment in the different ecosystem pools of the five young spruce forests differing in baseline productivity. Negative values indicate loss of C in the organic layer following nutrient enrichment. Capital letters represent differences among locations (Tukey's pairwise comparisons if $*p < 0.05$, df = 4, 10, see Section 2.11).

Location	C–N response rate					
	AGB	BGB	Total biomass	Organic soil	Total SOC	Total ecosystem
Gävle	23.3 ± 9.3	13.1 ± 2.6	36.4 ± 11.8	−3.9 ± 2.5 B*	2.8 ± 9.4	39.1 ± 17.0
Bräcke	21.9 ± 7.8	14.7 ± 2.5	36.6 ± 10.3	13.3 ± 2.3 A*	11.8 ± 1.7	48.4 ± 11.9
Ebbegärde	17.8 ± 4.5	11.5 ± 0.6	29.3 ± 5.0	−1.2 ± 2.7 AB*	7.1 ± 6.3	36.5 ± 6.2
Grängshammar	17.0 ± 4.5	11.7 ± 1.6	28.7 ± 6.1	10.9 ± 4.2 AB*	3.3 ± 6.5	32.0 ± 8.5
Mölnbacka	6.2 ± 6.1	7.7 ± 2.1	13.9 ± 8.2	10.4 ± 4.0 AB*	5.1 ± 6.5	19.0 ± 14.7
Average	17.2 ± 3.0	11.7 ± 1.2	29.0 ± 4.1	5.9 ± 3.5	6.0 ± 1.6	35.0 ± 4.8

AGB – aboveground tree biomass, BGB – belowground tree biomass, Total SOC – sum of carbon in organic and mineral soil.

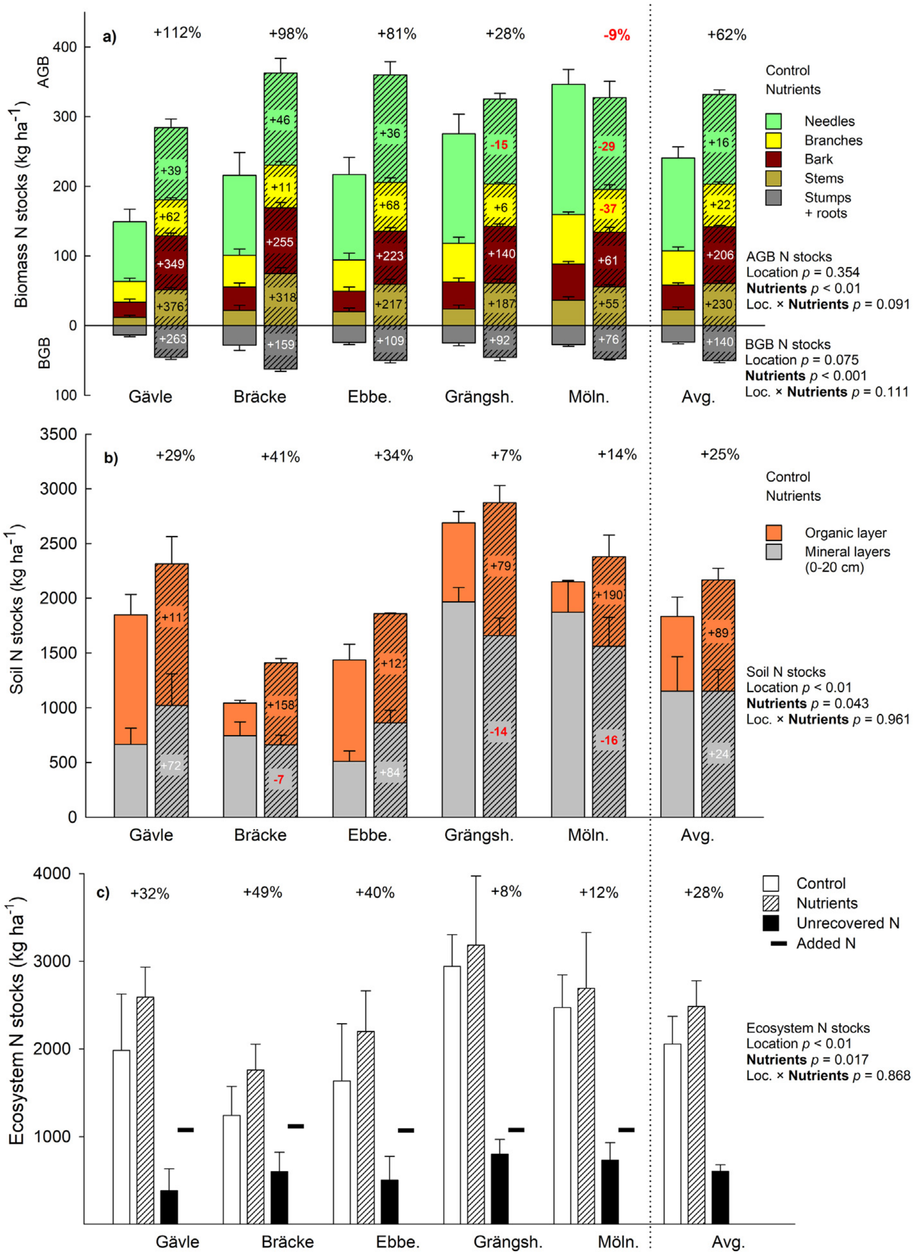

Fig. 3. Nutrient enrichment impacts on the a) above- (AGB) and belowground biomass (BGB) N stocks (mean ± SE, $n = 3$); b) soil N stocks in the organic and mineral soil layers; and c) ecosystem N stocks in five young spruce stands along a site-productivity gradient in Sweden. See Sections 2.2–2.4 for details on stock calculation and 2.11 for details on statistical analyses. The bars to the left are control plots, and the hashed bars to the right represent the plots that received 1070–1119 kg N ha^{-1} during 13 years along with phosphorus and potassium (see Table 1). The values above the bars represent the relative change (%) of the a) AGB, b) total soil N, and c) ecosystem N stocks. The average percentage change of N stocks in response to nutrient enrichment in the respective pool relative to the control are shown in the hashed bars. The amount of unrecovered N from the N added (c) represents the amount of N that could not be accounted for in the budget, and equals the total N added − (ecosystem N pools fertilized − ecosystem pools control plots).

needle and branch N stocks occurred. Large relative increases occurred also in the BGB due to increased N stocks in the stumps and coarse roots (Fig. 3a; Table S3a, Supporting information). Responses in soil N stocks were more variable and layer-dependent (Fig. 3b). For instance, the highest increases in organic soil layer N stocks at Mölnbacka, Bräcke, and Grängshammar were associated with decreased N stocks in the mineral soil layers at these sites (Fig. 3b).

The largest fraction of the recovered N was found in the soil, however, large part of the fertilizer-N could not be recovered in the measured pools. On average, one third of the added N was recovered in soil, 6.3% in the AGB, and 2.4% in BGB pools (Fig. 3a, b). The amount of N that we could not account for in the budget was calculated from the change in ecosystem N stocks in the nutrient enrichment compared to control plots, and by subtracting the calculated difference from the total amount of N added. On average, half of the added N, i.e. 605 kg ha^{-1}, was not recovered in the measured pools (Fig. 3c). The highest average values for this unrecovered N tended to be at Grängshammar and Mölnbacka, although there was a large variation in fertilizer-N recovery across the sites.

3.3. Litterfall C inputs and soil CO_2 efflux

Litterfall C inputs varied significantly among the sites and in response to nutrient enrichment (740–2230 kg ha^{-1}). The litterfall C inputs increased by 150% to around 3000 kg ha^{-1} yr^{-1} on average following nutrients enrichment (Fig. 4a). Consequently, this led to a variation in the nutrient enrichment effect, which ranged from only 32% at Mölnbacka to a threefold increase at Gävle (Fig. 4a). The largest increases observed at Gävle, followed by Bräcke, and Ebbegärde, and these site-specific responses corresponded to the responses in the AGB C stocks (Fig. 2a, Fig. 4a).

Nutrient enrichment reduced soil CO_2 efflux by nearly half ($p < 0.001$) (Fig. 4b). The strongest reductions occurred at Grängshammar, followed by Gävle, and Bräcke, where CO_2 efflux rates declined by more than a half. However, the soil CO_2 efflux was reduced by about a third even at the least affected sites (Fig. 4b). Although there were no differences among the sites, baseline soil CO_2 efflux rates in the control plots tended to be lower at Grängshammar and Mölnbacka compared to the other sites (Fig. 4b).

3.4. Response of soil microbial communities to nutrient enrichment

Unconstrained PCA ordination of all the 28 identified PLFAs indicated a clear separation between the soil microbial communities in the control and fertilized plots (Fig. 5a). The first principal component (PC1) explained 49.1% and the second (PC2) 20.3% of variation in the soil microbial communities, respectively (Fig. 5). The separation along the PC1 was mainly driven by dominance of G− bacteria and 16:1ω5 PLFA (commonly used as a biomarker for arbuscular mycorrhiza) in the control plots, and dominance of G+ bacteria including actinobacteria in the fertilized plots

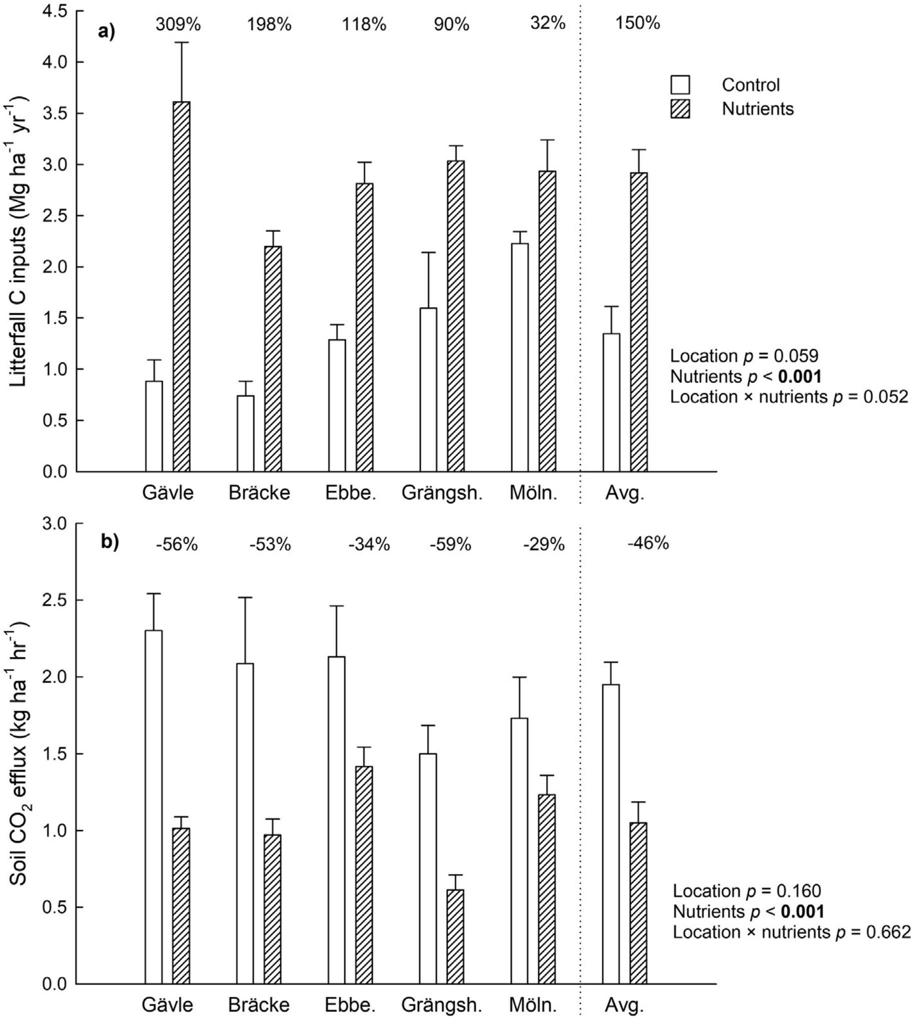

Fig. 4. Nutrient enrichment impacts on a) litterfall C inputs and b) soil CO_2 efflux rates during a growing season in young spruce stands at five different locations along a site-productivity gradient in Sweden. Litterfall C inputs were collected during one year (2017–2018) and the soil CO_2 efflux is an average rate ($n = 3$, ± SE) measured in 2017 (see Sections 2.7 and 2.8, respectively, for details). Averages over all five locations (n = 5, ± SE) for both parameters are separated from the site-specific responses with a dashed line. See Section 2.11 for details on statistical analyses.

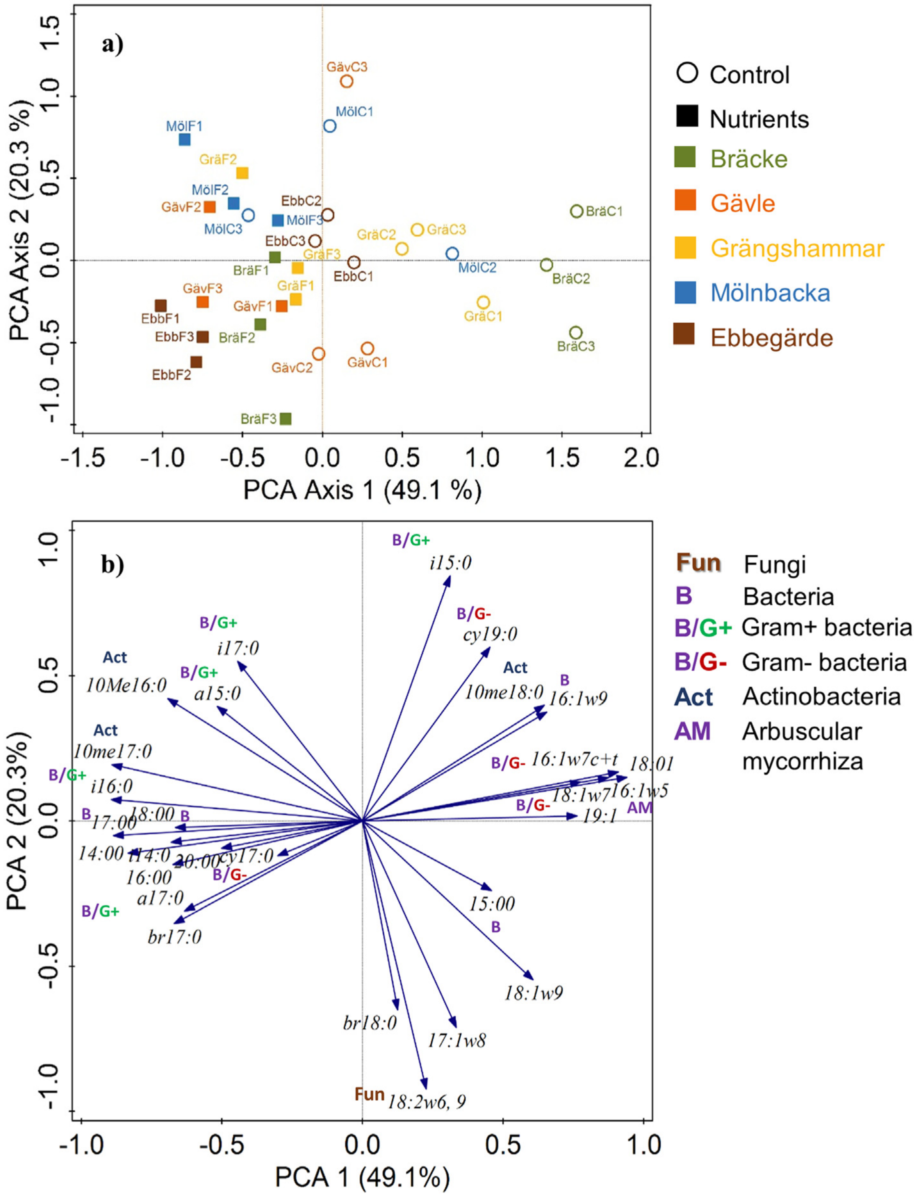

Fig. 5. Unconstrained ordination of the 28 identified PLFA biomarkers using principal component analysis (PCA) indicates a) clear separation and b) shifts in the soil microbial community composition following nutrient enrichment. The first two components explained together 69.4% of the variation in the PLFA biomarkers.

(Fig. 5b). The separation of the microbial communities along PC2 was driven mainly by the fungal 18:2ω6,9 and G+ bacterial i-15:0 PLFAs (Fig. 5b).

Despite the clear separation of the microbial communities in the control vs. nutrient-enriched plots, only a few shifts in the abundance of individual or functional group-specific microbial PLFA biomarkers were detected (Fig. S7a, Table S4, Supporting information). Neither the abundance of total PLFA biomarkers (Fig. S7a, Table S4, Supporting information), nor fungal PLFA biomarker 18:2ω6,9 (Fig. 6a) or fungi:bacteria ratio (Fig. S7b, Table S4, Supporting information) were affected by nutrient enrichment. However, the bacterial PLFAs abundance decreased following nutrients enrichment due to a decrease in G− bacteria (Fig. S7a–c, Table S4, Supporting information). While the abundance of G+ bacteria biomarkers remained unchanged (Fig. S7c, Table S4, Supporting information), the proportions (mol%) of G− bacteria in the microbial community decreased and mol% of G+ increased, respectively, rendering an increased G+:G− ratio (Fig. S7b, d; Table S4, Supporting information). In addition, we detected other shifts in the composition of soil microbial communities in response to nutrient enrichment indicated by changes in the ratios of saturated:mono-unsaturated, cyclopropyl:precursor PLFAs (Fig. S7e, f; Table S4, Supporting information).

Tree fine-root biomass and ergosterol concentrations in roots varied greatly among the locations, however, both tree fine-root biomass and root ergosterol concentrations remained unchanged in response to nutrient enrichment (Fig. 6b–d).

3.5. The role of baseline site productivity in ecosystem responses to nutrient enrichment

Ecosystem responses to nutrient enrichment depended on the baseline levels in the control stands, but none of the RRs were related to site index (Si H100) (Table 3). The response ratios (RRs) of tree biomass, including fine roots, and also ecosystem C stocks were strongly negatively correlated with their respective baseline levels in the control plots (Table 3). The AGB and BGB C stock RRs were positively correlated with organic soil C:N and C stocks, but negatively related to pH (Table 3). There was a strong positive correlation between ecosystem C stocks RR and baseline soil C:N ratio, and negative correlation with baseline soil N stocks and MAT (Table 3). The RR

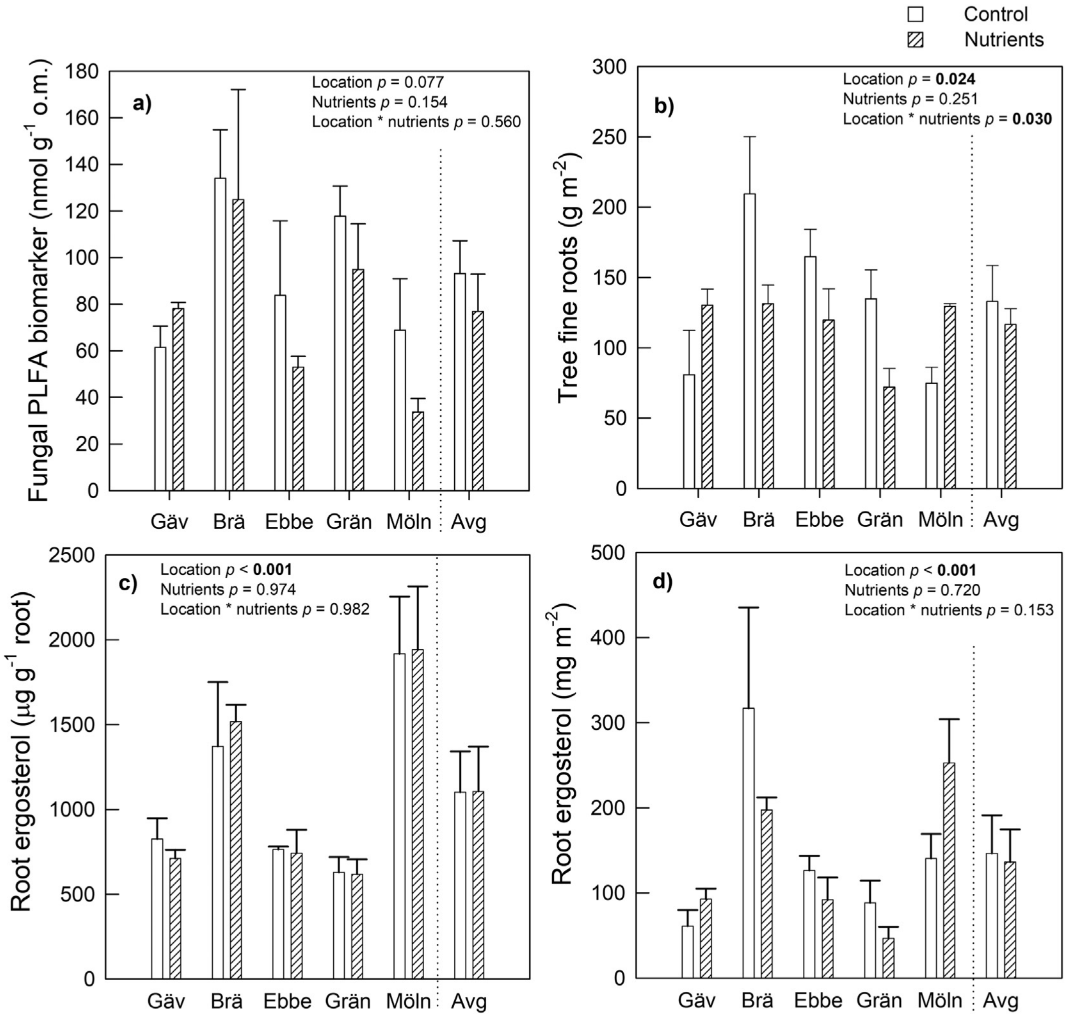

Fig. 6. Nutrient enrichment impacts on a) fungal 18:2ω6,9 PLFA biomarker concentrations (nmol g^{-1} o.m.) in organic soil layer, b) tree fine-root biomass in the organic soil layer (g m^{-2}), and c) ergosterol concentrations (μg g^{-1} root dry-weight) in fine roots representing the root-associated fungi in five young spruce forests along a site-productivity gradient in Sweden. Root ergosterol contents were also expressed per unit area (mg m^{-2}), d), accounting for the fine-root biomass. See Section 2.11 for details on statistical analyses.

of litterfall C was positively correlated to baseline C stock levels in the organic soil (Table 3), but negatively related to its baseline levels, MAP, baseline levels of tree biomass and ecosystem C stocks, and fine-root ergosterol concentration, i.e. root fungi (Table 3; Table C1, Supporting information).

The soil responses including soil CO_2 efflux were negatively related to the baseline levels with most of the responses confined within the organic soil (Table 3). The RR of the organic soil C stocks was correlated positively with MAP and baseline levels of foliage N%, litterfall C, tree biomass C stocks, and soil and root fungi (Table 3; Table C1, Supporting information). The RR of the fungal 18:2ω6,9 PLFA biomarker in the organic soil was positively related to baseline organic soil C stocks, and negatively to MAP and baseline litterfall C levels. The RR of root fungi, was positively related to baseline soil CO_2 efflux rate.

4. Discussion

4.1. Tree biomass and soil responses to nutrient enrichment

In line with our first hypothesis (H1a), AGB, BGB, and organic soil layer C stocks were significantly enhanced by nutrient enrichment (Fig. 2). On average, total ecosystem C stocks increased by 47%, or 38 t ha^{-1}, after 13 years of annual nutrient additions, most of which occurred in tree biomass above and belowground. The C–N response rate was 17 kg C kg^{-1} N in the AGB and 5.9 kg C kg^{-1} N in the organic soil layer, which was in line with global modeling estimates for boreal forests (De Vries et al., 2014; Schulte-Uebbing et al., 2022). Some authors suggested even higher C–N response rates (44 and 13 kg C kg^{-1} N in the AGB and soil respectively) for spruce forests of various age within northern Europe when P and K were added along with N, whereas the highest C–N response rates were achieved at low enrichment rates (30–35 kg N ha^{-1} yr^{-1}) (Hyvönen et al., 2008). The lack of nutrient enrichment impact on SOC stocks in the mineral soil layers (0–20 cm) in our study is consistent with several other studies (Mayer et al., 2020; Xu et al., 2021), whereas the changes in SOC stocks in the deeper mineral soil horizons may require longer time to be detected than was the duration of the experiments in our study.

4.2. Nutrient enrichment impacts on soil CO_2 efflux

Consistent with our first hypothesis (H1b), we found that the accumulation of C in the organic layer following nutrient enrichment was associated with, on average, a 150% increase in litterfall C inputs, but also with a reduction of soil CO_2 emissions by 46% (Fig. 4). Our soil CO_2 efflux estimates encompassed both heterotrophic respiration of free-living saprotrophic microbes and the autotrophic respiration of roots and ECM fungi. The latter flux constitutes a large share of the soil CO_2 efflux in boreal forests and decreases typically by 10–30% in response to N additions, which is usually explained as a reduction of C allocation to roots and associated ECM fungi

Table 3

Correlation coefficients ($n = 15$) of the relationships between baseline ecosystem properties and ecosystem responses to nutrient enrichment (response ratios – RR, see Eq. (1) in Section 2.10) in five young spruce forests across Sweden. Red and blue colors indicate positive and negative relationships, respectively, whereas unshaded cells indicate no significant relationship. Light to dark color-shading indicates weak to strong correlation, respectively (see Section 2.10 for details).

	Nutrient enrichment–response ratios (RR)										
Baseline levels ↓	Litterfall C	AGB C	BGB C	Eco. C	Soil N	Soil C	Humus C	Soil fungi [ac]	Fine-root C	Root fungi [d]	Soil CO_2
MAT	-0.31	-0.26	-0.26	**-0.48**	-0.22	-0.42	-0.41	-0.11	0.26	-0.27	0.18
MAP	**-0.55**	-0.42	**-0.50**	-0.20	-0.14	0.18	**0.88**	**-0.51**	0.03	0.18	0.15
Si H100	-0.37	-0.25	-0.19	-0.24	0.00	-0.13	-0.38	-0.25	0.06	-0.16	0.39
pH [a]	-0.11	**-0.56**	**-0.56**	-0.43	-0.26	0.04	0.36	0.08	0.07	0.15	0.18
C:N [a]	0.32	**0.50**	**0.54**	**0.71**	**0.56**	**0.44**	0.13	-0.07	-0.05	0.17	-0.08
N% fol	-0.44	-0.32	-0.35	-0.08	0.03	0.24	**0.55**	-0.38	**-0.50**	-0.06	0.08
Soil N [b]	-0.23	-0.24	-0.32	**-0.57**	**-0.57**	**-0.54**	-0.06	0.12	0.02	-0.29	-0.20
Litterfall C	**-0.91**	**-0.71**	**-0.75**	**-0.60**	-0.11	-0.09	**0.48**	**-0.61**	-0.01	-0.24	0.24
AGB C	**-0.72**	**-0.92**	**-0.94**	**-0.74**	-0.28	-0.10	**0.47**	-0.21	-0.23	-0.11	0.31
BGB C	**-0.70**	**-0.87**	**-0.92**	**-0.71**	-0.32	-0.10	**0.58**	-0.20	-0.26	-0.12	0.24
Ecosystem C	**-0.55**	**-0.70**	**-0.76**	**-0.81**	**-0.47**	-0.40	0.18	0.00	-0.19	-0.30	0.05
Soil C [b]	0.12	0.13	0.08	-0.30	-0.36	**-0.54**	-0.43	0.32	0.02	-0.34	-0.34
Humus C [a]	**0.56**	**0.51**	**0.57**	0.23	0.16	-0.15	**-0.85**	**0.52**	0.27	-0.23	-0.10
Fine-root C [a]	-0.05	-0.08	-0.04	0.14	-0.01	0.14	0.03	0.05	**-0.81**	0.03	0.04
Soil CO_2 efflux	0.36	0.30	0.35	0.20	-0.12	-0.10	-0.19	-0.02	0.13	**0.47**	**-0.62**

MAT, MAP – mean annual temperature, respectively precipitation; Si H100 – Site index representing the height of a sample tree at the age of 100 years; N% fol – N% in needles; Soil N, SOC – N and C stocks in organic and mineral soil layers summed; humus C – organic soil-layer C stocks; AGB C and BGB C – aboveground and belowground tree-biomass C stocks; Ecosystem C – includes C stocks in tree biomass and soil, fine roots (see Sections 2–2.4); [a] Organic soil-layer levels, [b] Levels in the organic and mineral soil horizons combined, [c] Fungal 18:2ω6,9 PLFA biomarker, [d] Fine-root ergosterol levels.

(Forsmark et al., 2020; Hasselquist et al., 2016, 2012; Olsson et al., 2005). The reduction of soil CO_2 efflux by nutrient enrichment in our study was, however, higher compared to the declines suggested by these studies (Forsmark et al., 2020; Hasselquist et al., 2012, 2016; Olsson et al., 2005), and also higher than the declines observed in other ecosystems (Janssens et al., 2010). This implies that not only autotrophic, but likely also the heterotrophic respiration, decreased as a result of reduced heterotrophic microbial activity, which is consistent with the increases in SOC stocks in the organic layer (Berg and Matzner, 1997; Janssens et al., 2010; Maaroufi et al., 2019; Xu et al., 2021).

Besides litterfall, fine-root biomass is an important source of plant litter inputs in soils, and an alternative sink for C not allocated to autotrophic respiration (Fernández-Martínez et al., 2014; Forsmark et al., 2021). Contrary to our hypothesis of reduced C allocation to roots, we found that standing biomass of fine roots remained constant, which is particularly interesting considering the soil CO_2 efflux declined by almost a half. One possible explanation for this discrepancy could be enhanced efficiency of root production and reduction of C allocation to root exudation and root-associated microbial respiration in nutrient enriched soils, which in turn coincided with increased organic layer C stocks (Fernández-Martínez et al., 2014; Forsmark et al., 2021; Vicca et al., 2012). These results could also imply that nutrient enrichment-induced shift in microbial community composition (Fig. 5) represents a shift in the composition of soil microbial communities towards taxa more efficient in using C for biomass production instead of respiration (Fernández-Martínez et al., 2014; Janssens et al., 2010; Manzoni et al., 2010), which would contribute to reconcile the soil C accumulation (Fig. 2b) and drastic reduction in soil CO_2 efflux (Fig. 4b).

4.3. Impacts of nutrient enrichment on soil microbial communities

Further in line with our first hypothesis, we found nutrient enrichment-induced shifts in soil microbial community's composition (Fig. 5), which may be a result of direct effects of nutrient enrichment, or indirect effects such a shifts in tree belowground C allocation to root exudation and nutrient acquisition. Composition shifts included a greater prevalence of G− bacteria in the control plots, and G+ bacteria, including actinobacteria, in the fertilized plots (Fig. S7b–d, Table S4, Supporting information), which is consistent with several other studies (Blaško et al., 2013; Zhang et al., 2018). Gram negative bacteria are more common in the rhizosphere and more sensitive to nutrient enrichment-induced reduction in belowground allocation of labile C (Demoling et al., 2008), while G+ bacteria have a higher demand for N to build the double-layered phospholipid cell walls (Paul, 2006).

Contrary to our first hypothesis, however, we found no evidence for nutrient enrichment impact on concentrations of the fungal biomarker ergosterol in ECM roots (Fig. 6c, d), nor the fungal PLFA biomarker (18:2ω6,9) in soils (Fig. 6a). Nutrient enrichment may have caused shifts in production and turnover of fine roots, and thus also mycorrhizal fungi (Forsmark et al., 2021), which we were unable to detect in our standing biomass estimates. Moreover, the fungal PLFA does not discriminate between saprotrophic or ECM fungi, hence, nutrient enrichment may also have induced species shifts within and between these functional guilds (Forsmark et al., 2021; Maaroufi et al., 2019). However, our finding that fungal PLFA and fungi:bacteria ratio were unresponsive to nutrient enrichment is consistent with other studies in boreal spruce stands enriched with N (Blaško et al., 2013).

4.4. The role of site productivity in ecosystem responses to nutrient enrichment

In support of our second hypothesis, we found that the strength of the C–N responses was related to baseline site properties, such as AGB C stocks, soil C:N, and soil N stocks in the control plots (Fig. 2, Table 3). More specifically, we found that the highest positive responses to nutrient enrichment in AGB, BGB, and ecosystem C stocks occurred at sites with lower baseline levels of AGB C, higher soil C:N ratios, and soil N stocks (Table 3, Fig. 2). Partly in disagreement with our first hypothesis, however, only RRs of the ecosystem C and soil C stocks were negatively correlated to baseline levels of soil N (Table 3). At the sites with higher soil C:N ratios (>30, Bräcke, Gävle, Ebbegärde), the tendency for higher C–N responses in AGB, BGB, and ecosystem C pools was associated with higher increases in litterfall C inputs and fertilizer-N recoveries. In contrast, the weaker responses in AGB, BGB, and ecosystem C pools to nutrient enrichment tended to occur at sites with the higher baseline AGB C stocks, lower soil C:N (≈ 25) and higher soil N stocks, i.e. sites which we described as more productive (Mölnbacka and Grängshammar, Fig. 2a, c, Fig. 3b). At these sites, a tendency for lower ecosystem C–N response was also associated with a higher amount of unrecovered fertilizer-N (Fig. 3c) indicating a higher loss of added nutrient from initially N-rich ecosystems.

Soil responses to nutrient enrichment were negatively linked to the baseline soil C stocks (Table 3), however, partly in disagreement with our second hypothesis, the weaker C–N responses in the AGB, BGB, and mineral soil at the more productive Grängshammar and Mölnbacka sites tended to be balanced by stronger positive C–N responses in the organic soil layer (Fig. 2), which was corroborated by the positive correlation between the RR of the organic soil-layer C stocks and the baseline tree biomass C stocks. The higher C–N responses in the organic soil layer at the more productive sites coincided also with a tendency for higher recovery of added N (50% and 46% respectively). On the contrary, the SOC RR was not correlated with the baseline AGB C stock, instead, the SOC RR was positively related to baseline soil C:N and negatively to soil N stocks (Table 3). Further, the strong correlation between RRs of the organic layer C stocks with MAP and a moderate correlation to foliage N%, could indicate that the microbial decomposition of the high-lignin spruce litter was impeded more by nutrient enrichment at the sites with higher foliar N concentrations and wetter conditions (Berg and McClaugherty, 2014; Fog, 1988; Janssens et al., 2010; Knorr et al., 2005). The C–N responses at the northernmost site Bräcke with the highest soil C:N differed from the other locations, because C stocks increased substantially in tree biomass but also in the organic soil in response to nutrient enrichment. The ecosystem C gains (88%) and C–N response rate (48 kg C kg^{-1} N added) at Bräcke tended to be the highest, which implies the strongest plant and microbial nutrient limitation in comparison to other locations.

The larger increases in the organic layer C stocks at Mölnbacka and Grängshammar (154% and 67%, respectively) did not correspond well with the tendency of lower increases in the litterfall C inputs at these more productive compared to less productive sites, which was reflected also in the moderately negative correlation between the RRs of organic layer C stocks and litterfall C (Table C1, Supporting information). The baseline litterfall C inputs at Mölnbacka and Grängshammar tended to be higher than at the other three, less productive, sites where we recorded higher increases in the litterfall C inputs. One explanation for the lack of a strong positive correlation could be the difference in temporal resolution between litterfall and SOC stocks measurements. For instance, the different response in litterfall C inputs and litter inputs from the concomitant shifts in species composition and biomass of forest floor vegetation (Fig. S1, Supporting information) in response to nutrient enrichment (Gundale et al., 2014; Palmroth et al., 2014; Bobbink et al., 2010) likely promoted C accumulation in the litter layer, which had not been fully humified yet. Given that needle-biomass N represented a substantial proportion of the ecosystem N budget, and that needle-biomass N stocks decreased in the fertilized plots at these two sites, this unaccounted pool could also explain a portion of added N that could not be recovered in the measured pools, and that tended to be highest at the two more productive sites (Fig. 3c). The C and N accumulation differences in the organic soil layer between the control and nutrient enrichment plots may therefore be expected to gradually increase over time as more of the litter decomposes. Another explanation for the lack of a strong positive correlation between litterfall C and organic layer C stocks may be the cross-site variations in proportions between above and belowground litter inputs from the canopy, understory vegetation, root litter (Forsmark et al., 2021), and litter from root-associated ECM fungal mycelia (Clemmensen et al., 2013; Wallander et al., 2011). For instance, a previous study found that the ECM fungal mycelium growth was negatively impacted by nutrient enrichment at Ebbegärde but not at Grängshammar (Wallander et al., 2011). Thus, a difference in the nutrient enrichment impacts on the ECM fungal biomass among the sites may have also contributed to the larger increase in the organic layer C stocks at the latter site.

5. Conclusions

Coniferous forests of the northern latitude cover roughly one third of the world's forests and shifts in C balance in this biome can have major consequences for the global C cycle. Boreal forests exhibit the strongest C–N responses to atmospheric N deposition or N enrichment compared to other biome types globally. Therefore, establishment of such C–N relationships may help improve global estimates and our understanding of the impacts of anthropogenic N deposition, or nutrient enrichment as a forest management tool, on terrestrial C uptake (Schulte-Uebbing et al., 2022). Our study thus contributes with crucial insights about the range and variation of ecosystem C–N responses to nutrient enrichment in relation to site productivity within boreal forests, which may help to refine land surface models used to project global C dynamics. Specifically, our results help establish how basic forest parameters such as baseline tree biomass productivity, soil C:N, or soil N stocks, modulate C–N responses to nutrient additions and whether stronger C–N responses can be expected in the tree biomass or soil. Further, our study reveals for the first time an inverse relationship between aboveground biomass and organic soil C sequestration in response to nutrient enrichment in relationship to site productivity. This relationships will not only inform and improve land surface modeling, but will also inform forest-policy makers and forest managers regarding the potential impacts and benefits of nutrient enrichment in different types of forests for maximizing ecosystem service related to the provision of wood products versus long-term C sequestration in soils.

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CRediT authorship contribution statement

Róbert Blaško: Conceptualization, Project administration, Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. Benjamin Forsmark: Conceptualization, Methodology, Writing original draft, Writing – review & editing, Validation. Michael J. Gundale: Conceptualization, Formal analysis, Writing original draft, Writing – review & editing, Supervision. Hyungwoo Lim: Data curation, Formal analysis, Writing original draft, Writing – review & editing, Validation. Tomas Lundmark: Funding acquisition, Conceptualization, Methodology, Supervision. Annika Nordin: Funding acquisition, Conceptualization, Methodology, Writing – review & editing, Supervision.

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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