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What happens to trees and soils during five decades of experimental nitrogen loading?

P. Högberg [a,*,1], R.W. Lucas [a,b,2], M.N. Högberg [a,3], U. Skyllberg [a,4], G. Egnell [a,5], J. Larson [a,6], D. Binkley [c,7]

[a] *Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*
[b] *Southwest Environmental Institute, Phoenix, AZ 85087, USA*
[c] *Northern Arizona University, 200 E Pine Knoll Drive, Flagstaff, AZ 86011, USA*

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ABSTRACT

High deposition of nitrogen was postulated to drive losses of NO_3^- and nutrient base cations, causing soil acidification, nutrient deficiencies reducing tree growth and ultimately tree mortality. We tested these predictions in a uniquely long-term study involving three NH_4NO_3 addition treatments (N1-N3) in a boreal *Pinus sylvestris* forest. The lowest level (N1), 30 kg N ha^{-1} yr^{-1} was applied during 50 years. Twice this rate (N2) was added 38 years, followed by 12 years of recovery, while thrice this rate (N3) was added 20 years followed by 30 years of recovery. We compared tree growth, changes in foliar and soil chemistry among treatments including control plots without N additions. As predicted, the N treatments lowered soil pH and reduced soil base saturation by around 50 %. They also lowered foliar levels of Ca, Mg, K, P and B initially, but after 50 years only Ca and Mg remained lower than in the control. Lack of B motivated a single addition of 2.5 kg ha^{-1} after ten years of N treatment. Tree stem growth became and then remained higher in N1 than in the other treatments through the 50 years of treatments. In N2 and N3, foliar $\delta^{15}N$ increased during the N-loading phase, but declined during the recovery phase, indicating a return of ectomycorrhizal fungi and their role in tightening the N cycle in N-limited forests. In the terminated, initially highest N treatments, N2 and N3, the trees even show signs of returning to N-limitation. In these treatments, the soil base saturation remains lower, while the pH was only lower at 0–10 depth in the mineral soil, but not in the 10–20 cm depth horizon or in the superficial organic mor-layer. Accurately documenting the effect of N additions on forest growth required a long-term approach, where reasonable rates of application could be compared with extreme rates. Such long-term experiments are necessary to support forest management in achieving goals for developing future forests as they shift in response to major, global-scale changes.

1. Introduction

Nitrogen additions to terrestrial ecosystems from agricultural activities, combustion in industrial processes and in vehicles may exceed natural biological N_2-fixation (Fowler et al., 2013; Penuelas et al., 2013). Terrestrial surfaces receive an estimated deposition of 5 kg N ha^{-1} yr^{-1} on average, but levels more than ten times higher occur locally (Penuelas et al., 2013). High N deposition on forests can cause leaching of NO_3^- and acidification of soils due to loss of base cations (Johnson and Lindberg, 1992; Aber et al., 1998; Lucas et al., 2011; de Vries et al., 2014). Nitrogen deposition also changes understory plant species composition (Bobbink et al., 2010). Very high experimental N additions

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* Corresponding author.
E-mail address: Peter.Hogberg@slu.se (P. Högberg).

[1] ORCID: 0000-0002-2849-2719.
[2] ORCID: 0009-0001-1493-1653.
[3] ORCID: 0000-0003-1258-7630.
[4] ORCID: 0000-0001-6939-8799.
[5] ORCID: 0000-0001-8744-4613.
[6] ORCID: 0000-0003-2576-5731.
[7] ORCID: 0000-0002-0992-8425.

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have led in a few cases to tree mortality (Nohrstedt, 2001; Magill et al., 2004), but the exact cause of death was not identified.

Nitrogen deposition is increasing in densely populated areas in Asia (Tian et al., 2018; Ackerman et al., 2019). Efforts to reduce N deposition in Europe and North America have resulted in moderate decreases in deposition since the 1990́s (Lajtha and Jones, 2013; Gilliam et al., 2019; Schmitz et al., 2019). Even small decreases may be important, since experimental reductions of N load have resulted in rapid declines in the leaching of NO_3^- (Boxman et al., 1998; Bredemeier et al., 1998; Johannisson et al., 1999; Corre and Lamersdorf, 2004). However, the relations among N supply, plant N demand and leaching of NO_3^- may be complex, as shown by the unexpected cessation of leaching after clear-felling of a forest subject to high N deposition (Göttlein et al., 2023).

Aber et al. (1989) hypothesized that N deposition on N-limited forests would first increase their growth. When the combined plant and microbial demand for N was saturated, nitrification and the subsequent leaching of NO_3^- would accelerate causing losses of accompanying nutrient cations like Ca^{2+}, Mg^{2+} and K^+. This should ultimately result in soil acidification, nutrient imbalances in trees and a decline in tree growth. These authors considered soil microbiota to be carbon-limited and, hence, weak sinks for N. Thus, nitrification triggered by reduced plant demand for N was suggested to play a pivotal role in controlling losses of N and nutrient cations from forest ecosystems.

However, experiments showed that forests subject to high experimental N loads maintained a high soil N retention capacity delaying the onset of nitrate leaching. Aber et al. (1998) recognized the need to explain the N sink in soils. The fact that soil microorganisms remained strong sinks called the attention to mycorrhizal fungi, which receive fresh carbohydrates from the trees. Attention was thus paid to trenching experiments, which disrupt the flow of C from trees outside trenches to mycorrhizal fungi inside trenched areas. This caused a quick increase in nitrification (Vitousek et al., 1979) in the same type of temperate forests as those studied by Aber et al. (1989). In their 1998 paper, Aber et al. concluded that high additions of N cause less response in terms of increased nitrification and leaching of NO_3^- than does the disruption of the flow of C to mycorrhizal fungi. It is of particular interest in this context that the proportion of C trees allocate below-ground to roots, mycorrhizal fungi and associated soil biota decreases as the availability of N increases (Mäkelä et al., 2008; Högberg et al., 2010; Vicca et al., 2012). Hence, mycorrhizal fungi play a dynamic role in N retention (Högberg et al., 2011; Näsholm et al., 2013).

A classic view has been that the leaching of nitrate, a mobile anion, drives losses from soils of so-called nutrient base cations such as Ca^{2+}, Mg^{2+} and K^+. They are removed from the exchange sites, on which they are replaced by H^+ and Al^{3+}, rendering the soil more acid. A critical assumption is that the rate of loss of base cations from the exchange complexes exceeds the rate of their release from minerals through weathering. An additional, influential hypothesis stated that Al^{3+} would bind to sites on root surfaces thereby restricting the uptake of nutrient base cations (Sverdrup et al., 1992; Cronan and Grigal, 1995). These ideas motivated an interest in using liming to mitigate soil acidification (e.g., Sverdrup et al., 1994).

Soil scientists have traditionally measured the amount of exchangeable base cations using a salt extraction with a pH of 7.0 or at ambient pH (neutral salt). Isotope dilution methods show that this may underestimate the real quantity of available base cations (e.g., Bel et al., 2020), likely due to incomplete exchange of the more strongly, organically complexed multivalent cations (Ca^{2+} and Mg^{2+}). Moreover, estimates of rates of weathering are highly uncertain and variable (e.g., Klaminder et al., 2011). Interest in weathering by mycorrhizal hyphae rose after the finding of tunnels allegedly formed by fungi through mineral grains (Jongmans et al., 1997). There is evidence of a link between such tunnels and the presence of mycelium of ectomycorrhizal hyphae, but not clear if the quantities of nutrient base cations so released are of significance in the term of years and decades (Smith and Read, 2008).

Our knowledge of the magnitude of effects of N deposition on forest trees and soils is largely based on short-term experiments with high N addition rates (Bebber, 2021) or derived from correlations among N deposition and its presumed effects across regions experiencing variations in N deposition (e.g., de Vries et al., 2014; Flechard et al., 2020a; b). For example, based on a meta-analysis, Tian and Niu (2015) suggested that boreal forest soils were less likely to acidify from N additions than other soils. Moreover, Tian et al. (2016) found that additions of N increased plant growth globally below a threshold of 50–60 kg N ha^{-1} yr^{-1}. However, there is a lack of long-term experimental studies, which is a critical deficiency since experimental manipulations may show strong initial responses that diminish over time (Leuzinger et al., 2011). The past decades of research on effects of N deposition on forests have identified some mechanisms and broader quantitative relations between N load and ecosystem responses, but there are still important gaps in our knowledge.

We have previously reported effects on trees and soil of 30 years of annual N additions and effects of 20 years of high N loading followed by 10 years of recovery in a boreal pine forest (Högberg et al., 2006). Here, we add observations from another 20 years of treatments and recovery. To our knowledge, this is the longest continuous test of effects of N-loading to forests. We focus on six major questions related to the effects on N deposition:

1) Did multi-decadal N additions at levels from 30 to 100 kg N ha^{-1} yr^{-1} (total additions of 1600 – 2500 kg N ha^{-1}) cause a decline in tree growth?
2) Did N additions at these levels cause soil acidification?
3) Did any change in tree growth relate to soil acidification?
4) Did N additions at these levels cause lower concentrations of nutrients other than N in tree foliage?
5) Did any change in tree growth relate to changes in the concentrations of nutrients other than N?
6) Did soils and trees recover when the increased N load is terminated?

2. Methods

We studied the experiment E55 (now numbered 1555 in the Silvaboreal data base https://www.silvaboreal.com) established by Carl Olof Tamm (Tamm et al., 1999) located at Norrliden, 64°21 ′N, 19°45 ′E, 260–275 m a.s.l., in northern Sweden. This is above the highest coast-line after the latest glaciation, which ended around 9200 years ago exposing a chemically un-weathered till soil. The soil is a typical Haplic Podzol (FAO) with a c. 10 cm deep bleached E horizon underlain by a spodic Bs horizon. The organic mor-layer (O horizon) on top of the mineral soil has a depth of 5–10 cm and has a C/N ratio of 40 and a pH measured in water of 4.3 (Högberg et al., 2014). Mean annual precipitation is around 600 mm and the mean annual temperature is 1.8 °C.

The previous tree stand was harvested in 1951. Prescribed burning was carried out in 1952, followed by planting in 1953 of two-year-old seedlings of *Pinus sylvestris* (L.). Thus, the age of the present stand is counted from 1951. The forest was thinned in 1985 and again in 2000.

Experimental plots were laid out in 1970 (Tamm et al., 1999). Treatments began in 1971 with a total 40 plots. There are three 30 m by 30 m replicate plots of each treatment. Measurements of tree growth, sampling of foliage and soil were done on the central 400 m^2 of the plots (see Supplement). We have focused on 12 plots comprising an N addition trial with controls plots and plots with N added as NH_4NO_3 at three levels in early June each year (Fig. 1). The N doses have varied over time; high initial doses in each treatment were reduced in two steps (Fig. 1). After 1976, the doses were 30, 60 and 90 kg N ha^{-1} yr^{-1} in N1, N2, and N3, respectively. Through the course of the experiment the background deposition has been on average 2 kg N ha^{-1} yr^{-1} (Laudon et al., 2021), but is declining, which is a general trend across Sweden (Karlsson et al., 2022).

In 1990, N3 was terminated, while N1 and N2 were re-started with

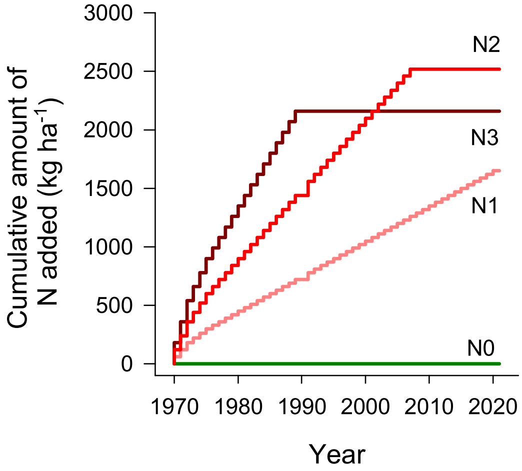

Fig. 1. Nitrogen treatments in experiment E55 at Norrliden. Note that the N-load was terminated in N3 in 1990 and in N2 in 2008. Double doses of N were added in N1 and N2 in 1992 to compensate for zero additions in 1991. N0 is the control. As of 2020, the N additions totaled 1620 kg ha^{-1} for N1, 2520 kg ha^{-1} for N2, and 2160 kg ha^{-1} for N3.

double doses in 1992 to compensate for the lack of N addition in 1991 (Fig. 1). The treatment N2 was terminated in 2008. In 2021, we were thus able to study the effects of 51 years of N additions in the treatment N1 at an average rate of 32 kg ha^{-1} yr^{-1}. In N2, we could study the results of 38 years of treatment at 68 kg N ha^{-1} yr^{-1}, followed by 13 years of recovery. Finally, in N3 we could study the effects of 31 years of recovery from 20 years of N additions at an average rate of 108 kg ha^{-1} yr^{-1}. We will refer to the N-loads as 30 (N1), 60 (N2) and 90 (N3) kg ha^{-1} yr^{-1}, as these were used for most of the treatment periods (Fig. 1). Note also that the last samplings of foliage chemistry, soil chemistry and tree growth were made in 2020, 2021 and 2022, respectively; the periods of treatment in N1 and on recovery in N2 and N3 given above relate to the mean year of these three samplings.

The stem volume and growth of trees were measured at intervals up until 2010, and we made a final assessment of stem volume in 2022. All trees in the inner 400 m^2 were calipered at breast height, and height of a random sample of trees was measured. Secondary functions were used to estimate the height of trees that were not measured. The data were used in volume functions for Scots pine in northern Sweden to estimate the total volume as described in Högberg et al. (2014).

Foliar samples have been collected annually from top whorls of 10 trees per plot to make one bulk sample of current-year needles per plot starting the year before the treatments begun. Here, we add data for 2016–2020. Needle samples were dried for 48 h at 70 °C and milled in a ball mill before chemical analysis. For analyses of B, Ca, Mg, K and P in foliage, samples were digested in a mixture of nitric and perchloric acids before determination on an inductively coupled plasma atomic emission spectrophotometer, ICP-AES (Perkin-Elmer Avio 200, USA). The concentration of N and the natural abundance of ^{15}N were determined on an elemental analyser, EA (Carlo Erba CHN 1110, Italy) coupled to an isotope ratio mass spectrometer, IRMS (Delta V, Thermo Fischer, USA). We express data on ^{15}N abundance as deviations from the standard atmospheric N_2:

$$\delta^{15}N_{sample} = ((R_{sample}/R_{standard}) - 1) * 1000,$$

where R is the ratio $^{15}N/^{14}N$. The interest in $\delta^{15}N$ in this context is based on the fact that the foliage becomes depleted in ^{15}N in N-limited forests where ectomycorrhizal fungi retain relatively more of this heavier N isotope (Högberg et al., 2011; Hobbie and Högberg, 2012).

In September 2021, we sampled soils from three random positions in each plot. For the organic mor-layer, we used a 10 cm diameter auger, and for the 0–10 and 10–20 cm soil depth horizons in the mineral soil, we used a 4.2 cm diameter auger. Living feather mosses, litter etc. on top of the organic layer (the so called S-layer) were removed and not included in the mor-layer. Mor-layer samples were sieved through a 2 mm mesh to remove roots. Mineral soil samples were sieved through a 2 mm sieve to remove roots and larger mineral particles.

We determined pH on fresh soil suspended in water 24 h after soil sampling. We used a mass of soil to volume of water ratio of 1:3 for the organic mor-layer and 1:2 for mineral soil. A 1.0 *M* ammonium acetate solution was used to extract exchangeable base cations (Ca^{2+}. K^+, Mg^{2+}, Mn^{2+}, Na^+) at pH 7.0; extracts were analysed on ICP-AES. The cation exchange capacity, $CEC_{pH7.0}$, was calculated as the sum of exchangeable “base cations” and acidity titrated by NaOH to pH 7.0 and expressed in mmol charge per kg of dry soil. The % base saturation, BS, was calculated based on mol charge as:

$$BS = 100 * \Sigma(Ca^{2+} + Mg^{2+} + Mn^{2+} + K^+ + Na^+)/CEC_{pH7.0}.$$

We also measured the concentrations of C and N on dried soil samples (using the EA-IRMS described above) to determine the C/N ratio.

For statistical comparisons we used the three replicate plots for each treatment ($N = 3$) using mean values of the subsamples of soil and foliage from each plot. We compared treatments by using a one-way analysis of variance (ANOVA). If that indicated significant differences among treatments at $p < 0.05$, and the data passed a test of equal variances, we used pair-wise Holm-Sidak test to see which treatments differed from one another while adjusting for multiple comparisons. The new data reported are available in the Supplement.

Tamm (1988), Tamm et al. (1999) and Högberg et al. (2006) described the effects of N-loading on trees and soils during the first 20–30 years. Högberg et al., (2011, 2014) focused on interactions among plants, microbes and soils, and N cycling in relation to recovery from previous N load. Here, we report the effects of 51 years of the N1 treatment, and 13 years of recovery of the N2 treatment and 31 years of recovery after the termination of the N3 treatment. Statistical tests were only conducted on the new data in search of significant long-term effects of the treatments; results of other tests have been reported in the previous papers mentioned above and can be found in the Supplement.

3. Results

3.1. Questions 1 and 4: Tree growth and foliar chemical data

When treatments started in 1971, the stem volume did not differ among the future treatment plots (the range was from 31 to 37 m^3 ha^{-1}). The N treatments increased growth above that in the control plots at an early stage, but the trend flattened and then decreased slightly in the N3 treatment (Fig. 2a,b). Note that Fig. 2 displays the total (gross) stem production, thus including removals by thinnings and natural mortality. The cumulative increase in stem volume depended on the rate of N addition rather than the total dose of N (Fig. 2b). At the end of the experiment, the N1 treatment had 40 % greater stem volume than the other treatments (Table 1), but the experimental design was not strong enough to detect any long-term differences among the other treatments. During the last period of measurements of growth (2009–2022), the rate of growth in N1 remained higher than in all other treatments.

After 50 years of treatments, needle N % was similar across treatments (Table 2), in contrast to large differences earlier in the experiment (Fig. 3a). The control showed no trend during the 50 years, while a few years into the experiment, the highest N treatment (N3) had almost twice as high N % in the needles as the control. Both N3 and N2 showed declines in foliar N % without any abrupt change as a result of removal of the N load (Fig. 4b).

The $\delta^{15}N$ of needles declined in the control during the 50 years from 0.4 to − 3.8 ‰ (Fig. 3b). In contrast, the two higher N treatments, N2 and N3, both showed increases in $\delta^{15}N$ during the periods of N-loading, followed by clear declines after the treatments were terminated in 2007

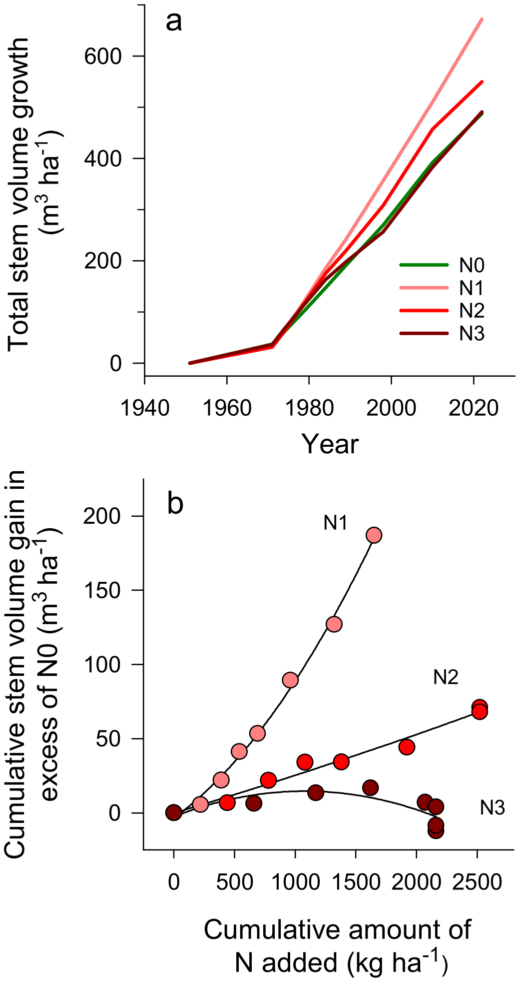

Fig. 2. a. Total (gross) stem volume growth (including removals in thinnings and mortality) in the experiment. Differences in the volume of living trees at the end of the experiment are shown in Table 1. b. Cumulative stem volume gain in excess of that in the control (N0) in relation to the cumulative amount of N added. Additional data on changes in volume and growth during the course of the experiment can be found in a Supplement.

Table 1
Stem volume (excluding dead wood) in 2022 (at the end of the experiment) and average annual growth 2009 – 2022. Treatments in columns N0, N1, N2, and N3 are described in Fig. 1. Column by column, data followed by the same letter are not statistically different (ANOVA, followed by Holm-Sidak test, $p < 0.05$). Data are means ± 1 S.E. ($N = 3$). Additional data on volume and growth can be found in the Supplement.

Treatment	Stem volume (m^3 ha^{-1})	Growth 2009–2022 (m^3 ha^{-1} yr^{-1})
N0	358 ± 15^{a}	6.8 ± 0.9^{a}
N1	500 ± 20^{b}	12.3 ± 0.9^{b}
N2	355 ± 12^{a}	8.0 ± 0.6^{a}
N3	325 ± 45^{a}	8.5 ± 0.8^{a}

and 1990, respectively (Fig. 4c,d). There were significant differences among treatments at the end of the experiment (one-way ANOVA, $p < 0.001$, Table 2). Treatments N0 and N3 were not different, but both differed from N1 and N2 (Holm-Sidak test, $p < 0.05$). When we analyzed the two terminated N treatments, N3 and N2 (Fig. 4d, e), during and after treatments, we found that the difference in δ^{15}N to the control, N0, increased linearly during the period of N-loading, but decreased linearly after termination of the N load.

Table 2
Concentrations of elements in foliage of *P. sylvestris* in the experiment E55 at Norrliden in 2020 (i.e. after 50 years of treatments). Treatments in columns N0, N1, N2, and N3 are described in Fig. 1. Row by row, data followed by the same letter are not statistically different (ANOVA, followed by Holm-Sidak test, $p < 0.05$). Where no significant treatment effects occurred at all according to the ANOVA it was considered unnecessary to use letters to show that. Data are means ± 1 S.E. ($N = 3$).

	Treatment			
Element	N0	N1	N2	N3
N (%)	1.17 ± 0.06	1.32 ± 0.07	1.27 ± 0.07	1.16 ± 0.03
K (%)	0.59 ± 0.02	0.60 ± 0.02	0.57 ± 0.01	0.55 ± 0.03
Mg (%)	0.09 ± 0.00^{a}	$0.08 \pm 0.00^{a,b}$	0.07 ± 0.00^{b}	0.09 ± 0.00^{a}
Ca (%)	0.18 ± 0.00^{a}	0.13 ± 0.00^{b}	0.10 ± 0.01^{c}	0.14 ± 0.01^{b}
P (%)	0.14 ± 0.00	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.00
B (ppm)	19.9 ± 1.7	18.3 ± 1.5	17.1 ± 1.6	16.9 ± 1.2

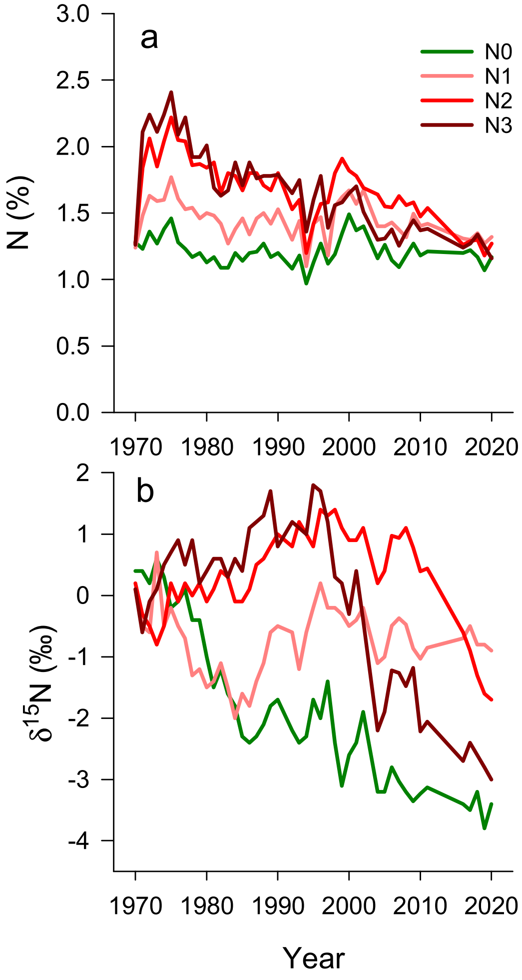

Fig. 3. a. The % N (of dry weight) in needles during the course of the experiment. N-loading was terminated in 1990 in N3 and in 2008 in N2. b. The development of δ^{15}N in needles during the course of the experiment. Data are treatment means based on three replicate plots. For a statistical analysis of changes in % N, see Supplement.

Except for K, elements other than N also displayed significant differences among treatments during the course of the experiment (Fig. 5a–e). The concentration of Mg was significantly lower in N1, N2 and N3 than in the control during the first 30 years of the experiment (Fig. 5b, Högberg et al., 2006), but at its end it was only lower in N2 (Table 2). Calcium remained lower in N1, N2 and N3 than in the control, despite a small increase in N3 at the end of the experiment (Fig. 5c, Table 2, Högberg et al., 2006). The changes in the concentrations of Ca

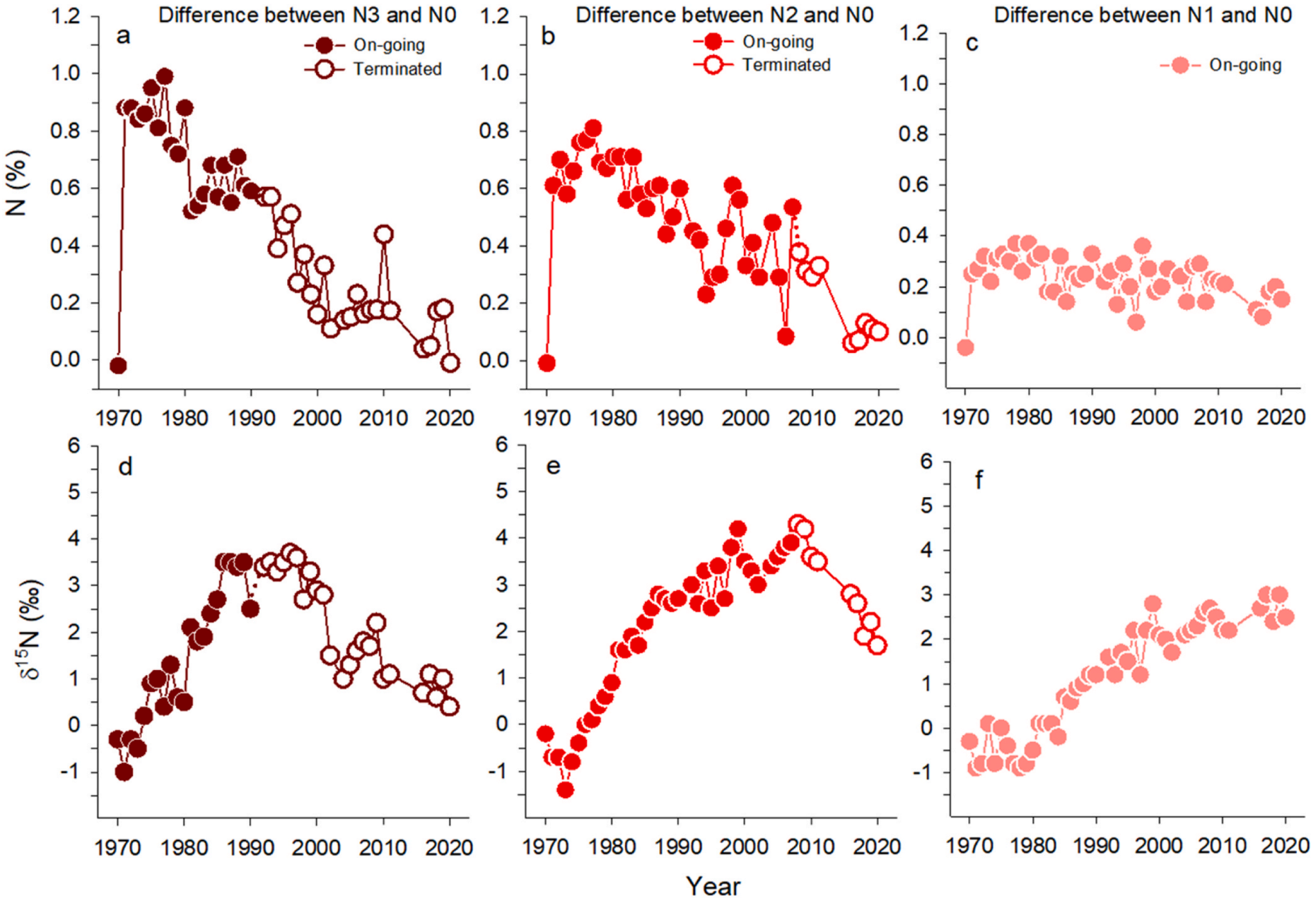

Fig. 4. a–c. The differences in % N (of dry weight) between needles from the treatments N3 and N2, respectively, and needles from the control (N0) plots. The loading of N was terminated in 1990 and 2008 in N3 and N2, respectively. d–f. The changes in $\delta^{15}N$ in needles during the course of the experiment in N3 and N2, respectively, are shown as deviations from needles in control plots. The difference in $\delta^{15}N$ to the control, N0, increased linearly during the period of N-loading ($r^2_{adj} = 0.872$, $p < 0.001$ and $r^2_{adj} = 0.862$, $p < 0.001$, for N3 and N2, respectively), but decreased linearly after termination of the N load ($r^2_{adj} = 0.805$, $p < 0.001$ and $r^2_{adj} = 0.952$, $p < 0.001$, for N3 and N2, respectively). Data are treatment means based on three replicate plots.

and Mg were remarkably similar. The concentration of P was lower in N1, N2 and N3 than in the control during the first 10 years, but since then differences were negligible among treatments (Fig. 5d, Table 2, Högberg et al., 2006). Finally, boron showed a dramatic initial decline in N1, N2 and N3 (Fig. 5e, Högberg et al., 2006), which lead to deficiency symptoms, especially death of treetops. This motivated a B addition of 2.5 kg ha^{-1} to all plots, including control plots in 1980 (Fig. 5e). At the end of the experiment, there were no differences in ppm B among the plots (Table 2). In summary, patterns of foliar nutrients other than N did not relate to the patterns in tree growth (Fig. 2a, Fig. 5), indicating no simple influences were likely.

3.2. *Question 2: soil acidity*

At the end of the experiment, the pH of the mor-layer appeared to decline slightly with N level (from 4.3 in N0 to 4.1 in N3), but the trend was not significant. Soil pH in the 10–20 cm depth mineral soil horizon showed no trend (Fig. 6a, b). However, in the 0–10 cm depth mineral soil horizon, all three N-loading treatments had a lower pH (4.2) than the control (4.8, Fig. 6b).

The base saturation was higher in the mor-layer and at 10–20 cm depth in the mineral soil in the control than in all three N-loading treatments, which did not differ from one another (Fig. 6d-f). In N treatments, the base saturation was on average 46 % and 48 % lower in the mor-layer and the 10–20 cm depth mineral soil horizon, respectively. Nitrogen loading appeared to drop base saturation by about half in the 0–10 cm soil depth horizon (Fig. 6e), but the difference was not statistically significant. There was no overall correlation between soil pH and base saturation across treatments and soil horizons (Fig. 7b). Exchangeable Ca^{2+} was the dominant base cation contributing 33–72 % to the base saturation across treatments and horizons.

3.3. *Questions 3 and 5: Tree growth and soil acidification*

There was no negative relation between measures of soil acidification, such as pH and base saturation (BS), and tree growth. Low rates of N additions increased tree growth above that of the control (Fig. 2, Table 1) in concert with a decline in soil base saturation in two out of three horizons (Fig. 6d–f). Not even the highest total N dose (N2) led to a lower tree growth than in the control over the full course of the experiment. Nor was there an apparent direct relation between tree growth (Fig. 2) and soil pH (Fig. 6a–c). The lower concentrations of Ca and Mg in tree foliage (Fig. 5b-c) in N-loaded plots had no negative effect on tree growth. On the contrary, higher tree growth in the treatment N1 was associated with a decline in Ca concentrations (Tables 1, 2).

3.4. *Question 6: changes after N loading stopped*

There were no apparent sudden changes in tree growth upon termination of the N-loads in N2 and N3 (Fig. 2). The same is true for % N in tree foliage (Figs. 3 and 4), although the terminations of the treatments marked the start of a gradual decline. In addition, the

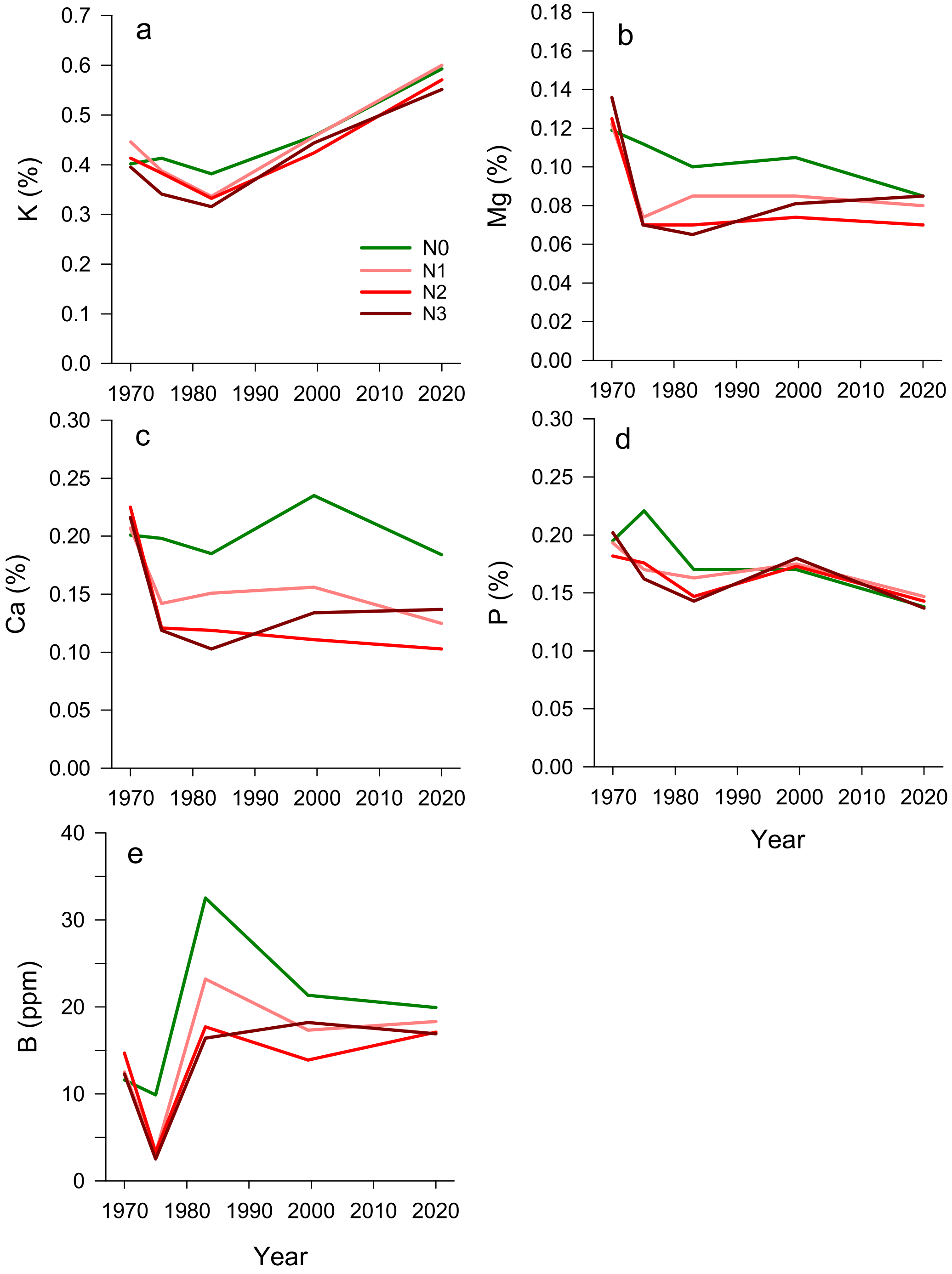

Fig. 5. Changes in the concentrations in tree foliage of K (a), Mg (b), Ca (c), P (d) and B (e) during the course of the experiment. The N loads in N3 and N2 were terminated in 1990 and 2008, respectively. An addition of 2.5 kg B was made to all plots in 1980. Data are treatment means based on three replicate plots. Statistical analysis of variations among treatments are found in the Supplement.

differences in % Mg between N3 and the control decreased (Fig. 5b). Regarding soils, there were no apparent changes in soil acidification measures of the mor-layer, except for a more positive trend in pH in N3 than in the control and the on-going N1 (Table 3). Base saturation in 10–20 cm of the mineral soil of the terminated N2 and N3 did not show the same decline as in the control and N1 (Table 3). The most apparent change was the change in foliage δ^{15}N from increasing to decreasing upon termination of the N-loads in N2 and N3 (Fig. 4d, e).

4. Discussion

Several of the questions we asked in the introduction were based on the presumptions that long-term N-loading would result in rates of NO_3^- leaching that were large enough to deplete base cations from the soil, which would lead to reduced tree growth due to nutrient deficiencies. This expectation was refuted. In this northerly, initially strongly N-limited forest, N-loading did not decrease growth, even at the very high doses of N2 and N3. A long-term dose of 30 kg N ha^{-1} yr^{-1} added over 1600 kg N ha^{-1} over five decades substantially increased tree growth (Fig. 2, Table 1). Regarding our first question, we can conclude that

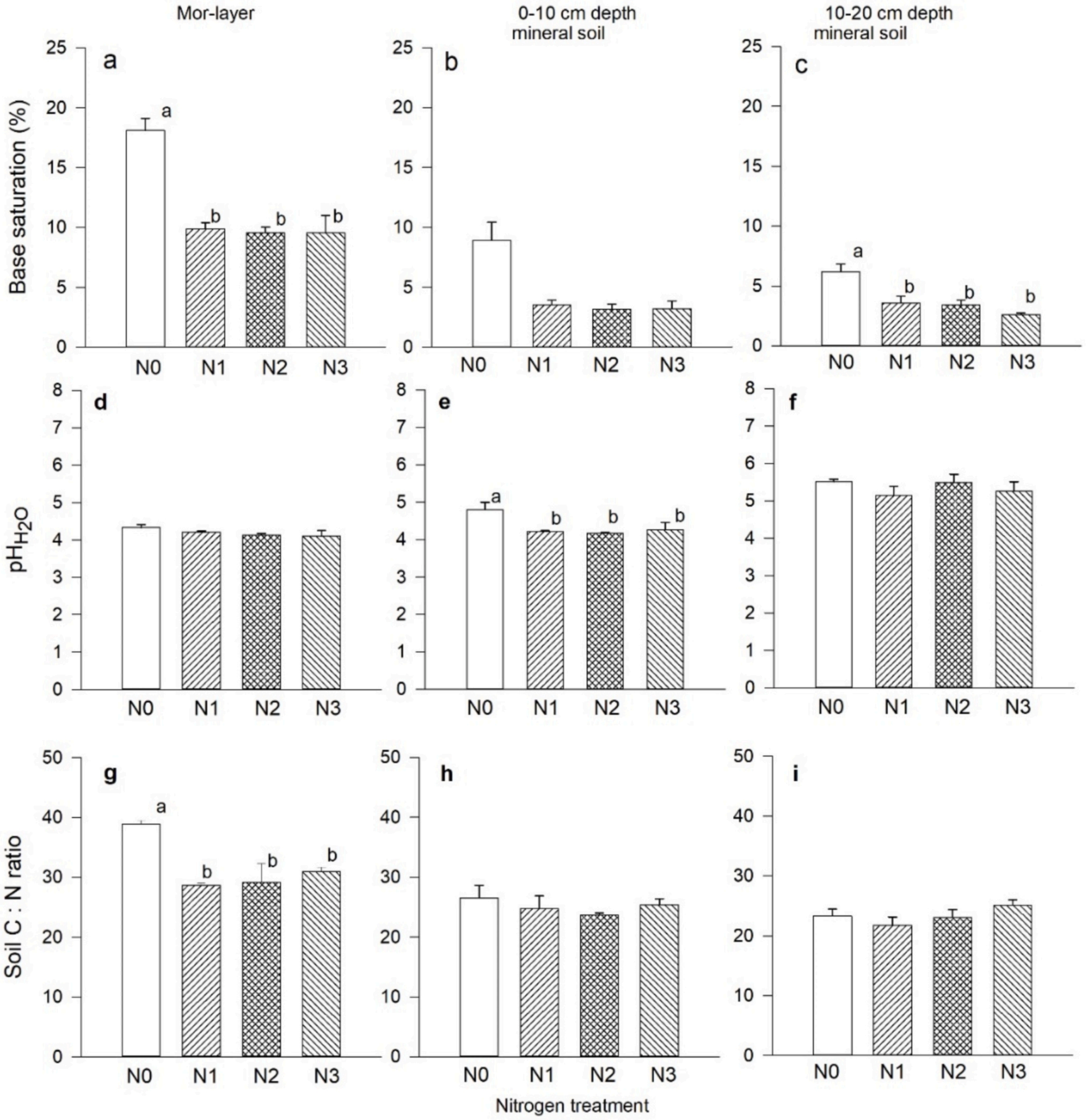

Fig. 6. a–i. Soil pH, base saturation and C/N ratio in the organic mor-layer, and the 0–10 cm and 10–20 cm horizons of the mineral soil at Norrliden in 2021. Data are treatment means based on three replicate plots. Letters above bars denote when there are differences significant at $p < 0.05$. Data are means ± 1 S.E. ($N = 3$).

decades of N additions at rates from 30 to 100 kg N ha^{-1} yr^{-1} had no negative effect on tree growth; on the contrary, the lowest N addition rate increased tree growth.

How representative would this long-term experiment at a single site be for other forests? We note that the site is usually free from snow-cover only from May to October, which means that the period when trees and other biota can metabolize the extra N is short relative to at lower latitudes. Other experiments with single additions of N, or chronic additions of N, show similar growth responses, also refuting the expectation that increased in N inputs would harm trees. Two similar chronic N addition experiments were conducted at locations about 450 km SSW of Norrliden (summarized in Binkley and Högberg, 2016). A Scots pine site at Lisselbo showed a pattern similar to Norrliden, with the N1 treatment increasing growth by about 50 %, and the N3 treatment showing weak initial increases in growth but overall having no long-term growth effect (Tamm et al., 1999). A Norway spruce site at Stråsan showed a somewhat different pattern: N1 more than doubled growth, and N3 increased growth by more than 50 % (after addition of 2500 kg N ha^{-1}; Blaško et al., 2013). All three long-term studies of N loading found that the rate of N addition had more influence on tree growth than the total amount of N added, with rates of 30 kg ha^{-1} increasing growth much more than higher rates. Higher doses of N had little effect on growth at the two pine sites, but greatly increased growth at the Norway spruce site. All three experiments (Norrliden, Stråsan and Lisselbo) support the report by Tian

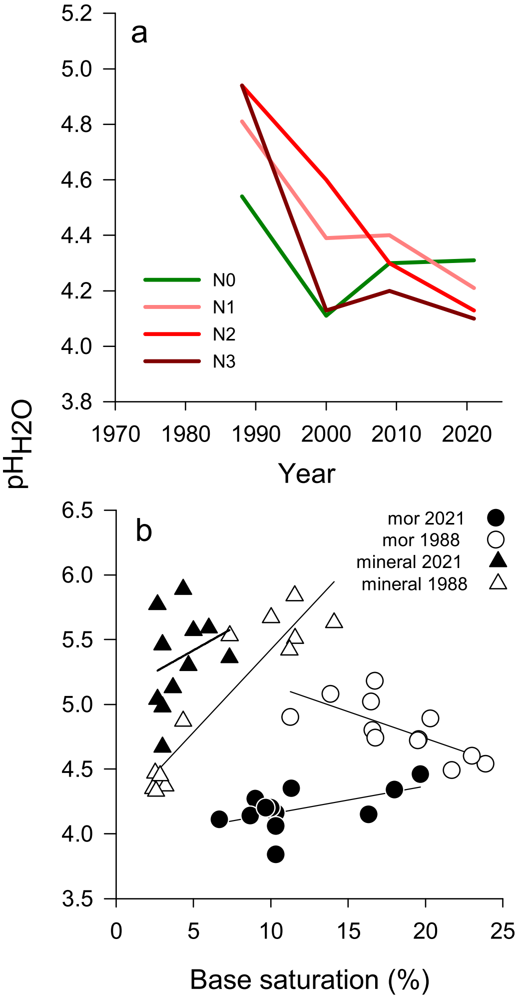

Fig. 7. a. Changes in soil pH in the mor-layer over time (Tamm et al., 1999; Högberg et al., 2006, 2014 and this study). b. Relations between soil pH and base saturation in the mor.layer and the 10–20 cm depth mineral soil in 1988 (Tamm et al., 1999) as compared to in 2021. Data are treatment means based on three replicate plots, N = 3. The regression line for pH versus base saturation in the mineral soil in 2021 is not significant, while the three others are (at $p < 0.05$). Statistical analysis of treatment effects on soil pH are found in the Supplemental material.

Table 3

Changes, denoted by Δ, in soil pH and base saturation in mor-layer and mineral soil from 1988 (Tamm et al., 1999) to 2021. The N2 and N3 treatments were terminated in 2008 and 1990, respectively. Row by row, data not followed by the same letter are statistically different ($p < 0.05$). Data are means ± 1 S.E. ($N = 3$).

Soil horizon Parameter	Treatment			
	N0	N1	N2	N3
Mor-layer				
Δ pH (H_2O)	-0.23 ± 0.08	-0.60 ± 0.07	-0.81 ± 0.14	-0.89 ± 0.28
Δ Base saturation (%)	-4.86 ± 0.34	-9.04 ± 0.75	-7.06 ± 1.42	-5.28 ± 0.70
Mineral soil, 10–20 cm				
Δ pH (H_2O)	-0.10 ± 0.03^{ab}	-0.45 ± 0.20^{b}	0.92 ± 0.08^{ab}	0.89 ± 0.22^{a}
Δ Base saturation (%)	-5.45 ± 1.83^{a}	-6.14 ± 0.90^{a}	0.32 ± 0.16^{b}	0.17 ± 0.18^{b}

et al. (2016) of plant growth increasing at lower doses, but give no clear indication of the exact N addition rates above which tree growth declines.

Immediate decade-length increases in growth are common and well documented from operational-levels of N fertilization of forests across Norway, Sweden and Finland (Nilsen, 2001; Nohrstedt, 2001; Saarsalmi and Mälkönen, 2001). Very high additions of N have not resulted in tree death, with one exception of high mortality after a single dose of 840 kg N ha^{-1} (Nohrstedt, 2001). These results from Fennoscandia clearly show that the rate of N addition is crucial for responses of forest growth. Consistent with the point stressed by Bebber (2021), very high rates of N addition cannot be relied upon for understanding likely effects of atmospheric N deposition.

Much remains to be clarified regarding the physiological responses of forest trees to continuous N additions. Studies at Norrliden have shown that trees subject to high N load store considerable amounts of arginine, an N-rich amino acid (C/N = 1.5), in their needles, and that this storage declines after termination of the N load (Quist et al., 1999). The trees also appear to down-regulate their high-affinity uptake system for NH_4^+ under conditions of high N supply (Bassirirad, 2000), as shown in root bioassays at low N concentration (70 μM) where response was reversed in N3 after termination of the N-load (Högberg et al., 2014). We would like to point out that the % N in needles declined rather continuously in N2 under a constant addition rate of N during the period from 1976 to 2007, and without a major change in % N after termination of the N load (Figs. 3a, 4b), a response consistent with active regulation of high-affinity uptake of NH_4^+.

Changes of nutrient concentration of an element in the foliage may be caused by i) an increased supply, ii) a dilution effect if the addition of another nutrient increases the foliar biomass, iii) a loss of nutrients from the soil through leaching, or iv) a negative interaction with another element, e.g. an antagonistic effect of high availability of K^+ on plant uptake of Mg^{2+}. The initial increases in the concentration of N in the foliage of the trees were remarkable in the N-loading treatments, especially in N2 and N3 (Fig. 4). Foliar biomass was twice as high in N1-N3 as in the control in 1985 (Tamm et al., 1999). This expansion of the canopy caused a dilution of nutrients other than N, as observed for B, Ca, Mg and P, but not for K (Fig. 5, statistical analysis provided in Högberg et al., 2006, see Supplement). In the cases of Ca and Mg, the lower concentrations in tree foliage persisted through the experiment, while the differences in P disappeared, as did the differences in B after the addition of this micronutrient (Fig. 5e, Table 2).

The addition of B in 1980 was motivated by deficiency symptoms (death of top shoots) in N-loaded plots. Foliar B had fallen to around 3 ppm in the N-loaded plots as compared to c. 10 ppm in the control; Aronsson (1983) suggested a threshold for B deficiency of 4–5 ppm. After the addition of B, another shoot took over the apical dominance in damaged trees resulting in a bend on the stem or in a double top. Height and volume growth continued. Boron deficiency has been observed in several N addition experiments in the Nordic countries, especially in regions not receiving B with sea salts (Nilsen, 2001; Nohrstedt, 2001; Saarsalmi and Mälkönen, 2001). We do not know if N-loaded trees would have died without the addition of B, but B deficiency was not a response predicted as an effect of N load based on the idea of loss of base cations. Regarding our initial questions 4 and 5, we conclude that the concentrations in tree foliage of several elements other than N decreased substantially without any negative effect on tree growth.

Sverdrup et al. (1992) and Cronan and Grigal (1995) proposed soil Ca/Al ratios as indicators of the risk of forest decline in areas of high acid or N deposition. Their idea was that Al^{3+} would occupy exchange sites on tree roots, thereby hindering the uptake of base cations from the soil. We previously reported that, due to the higher amount of Mg in the mor-layer of N-loaded plots (evidence of tree uptake from the soil of Mg and its re-deposition on top of the mineral soil) this does not seem to be the case (Högberg et al., 2006). Furthermore, Aronsson et al. (1999) reported that in 1988 the exchangeable Ca^{2+}, Mg^{2+} and K^+ were all higher in the mor of N1 plots than in control plots, while the amounts of Ca^{2+} and K^+, but not Mg^{2+}, were lower in N2 and N3 plots as compared to control plots. In 1988 they found lower amounts of all these cations in the 0–20 cm soil depth mineral soil in N-loaded plots than in control plots, except for K^+ in N1 plots. The fact that tree growth continues at the same level in N2 and N3 plots or even higher in N1 as compared to in the control shows that the lower base saturation of the mineral soil in N-loaded plots is not associated with a negative effect on tree growth, which answers our initial Question 2 and 3.

In contrast to the absence of the predicted decline in tree growth, the N-loading treatments did lead to the expected decrease in soil base saturation (Figs. 6d, 6 f). The decline of around 50 % of exchangeable base cations in the mineral soil and a similar decline in the concentrations of Ca and Mg in foliage had no clear implications for tree growth. We note that the concentrations of these elements in the foliage are not indicative of deficiencies (e.g., Ingestad, 1979; Knecht and Göransson, 2004). Classic assays for plant-available base cations in the soil may underestimate actual supplies, as shown in isotope dilution experiments (Bel et al., 2020). In addition, trees obtain cation nutrients not only directly by ion exchange from surfaces but also from other sources, including through the turnover of organic matter and microbial biomass (Rosenstock et al., 2019), and potentially through weathering of minerals by mycorrhizal fungi (Jongmans et al., 1997; Wallander, 2000; Blum et al., 2002).

Soil pH and base saturation are not static properties, but change as forests grow (Hallbäcken and Tamm, 1986; Tamm and Hallbäcken, 1988) and in the case of pH even seasonally (Skyllberg, 1991). Apart from a rapid initial increase after clear-felling (Nykvist and Rosén, 1985), pH decreases as forests age (Hesselman, 1937; Hallbäcken and Tamm, 1986; Tamm and Hallbäcken, 1988). Soil pH is affected by plant nutrient uptake, loss of base cations, changing properties and amounts of organic matter, exudation of low molecular weight organic acids by roots, mycorrhizal fungi and other soil organisms (e.g. Skyllberg et al., 2001; Van Hees et al., 2005; Löfgren et al., 2015), and by the release of base cations by decomposition of organic matter and weathering of minerals.

While base cation uptake and cycling influence pH in the top of the mor-layer, weathering of Al-silicates and the mineral gibbsite, $Al(OH)_3(s)$, exerts a major control of the pH-value in lower part of the mor-layer and in the top mineral soils (E and Bs horizons) of Podzols (Skyllberg, 1999; Ross et al., 2008). In these horizons, the exchange of H^+ for Al^{3+} (complexed by organic matter) is a better predictor of pH than % base saturation (Ross and Skyllberg 2022). In this experiment, base cation uptake and exchange for H^+, as well as loss of base cations, are the expected major process by which soils were acidified after N treatments. Data provided by Aronsson et al. (1999) do not show any variations in the Al/H ratio among the different N treatments and the control, thus not giving an indication that the process of Al-H exchange was affected by N additions and thereby contributing to differences in pH among treatments in the mineral soil.

Many of these processes are poorly quantified, as is the regulation of tree uptake of base cations in the field and physiological adjustments to low and high supplies of these elements. Curiously, a strong relationship across sites in Fennoscandia of higher tree growth in sites with higher pH and base saturation (Högberg et al., 2017) does not seem to apply to changes over time within individual sites, highlighting that major drivers of biogeochemistry across landscapes may not provide simple local insights.

When we compared our data with those from Tamm et al. (1999), plot by plot ($N = 12$) and by treatments, we saw that N addition increased the pH of the mor-layer in 1988 (Tamm et al., 1999). This was also seen in N1 and N2 in 2000 (Högberg et al., 2006), but the effect did not persist to 2021 (Fig. 7), due to declines in the terminated N3 and N2 treatments. The initial negative relation between pH and base saturation of the mor was replaced by a typical positive relation, as can be expected when soils are acidified by uptake or losses of base cations (Fig. 7). In parallel, the even more positive relation between pH and base saturation in the mineral soil horizon 10–20 cm soil depth was lost (Tamm & Popovic 1995 and Tamm et al., 1999, Fig. 7) as the pH increased in the terminated N3 and N2, while the control and N1 lost base saturation (Table 3). Tamm and Popovic found that N-loaded plots in E55 acidified the mineral soil as much as treatments with sulphuric acid in the companion experiment E57 at Norrliden. As alluded to above, Aronsson et al. (1999) reported a minor, but not significant increase in extractable Al with higher additions of N, which obviously did not cause a decrease in soil pH. Years later, in 2000, Högberg et al. (2006) found an increase in exchangeable Al^{3+} with increasing additions of N at 5–15 cm and 45–55 cm depth in the mineral soil suggesting that acidity from the mor layer/top mineral soil is exported deep into the mineral soil as a consequence of the N additions.

We explain the initial increase in pH and decrease in BS of the mor-layer, and an associated decrease in pH and BS in the mineral soil of the N2 and N3 treatments as an effect of NH_4^+ in the N application, exchanging with H^+ in the mor-layer, resulting in a leaching of H^+ (associated with NO_3^-) into the mineral soil. This “salt-effect” is thus alkalinizing the mor-layer and acidifying mineral soils in a similar way as Na^+ and Cl^- do in coastal areas receiving sea salt spray (Löfgren et al., 2015). Thus, we expect soil adsorbed NH_4^+ to have made up a significant fraction of CEC and acting as a non-acidic cation similar to K^+ in the mor-layers of N2 and N3 treatments. Unfortunately, NH_4^+ is not included in the CEC measurements. When extractable NH_4^+ was measured in 1988 (Tamm et al., 1999) it was, indeed, higher in N2 and N3 than in the control. In the mineral soil, plant uptake of NO_3^- would compensate for the leaching of H^+ (by releasing one OH^- upon uptake of NO_3^-), but uptake and leaching of base cations would acidify the soil with a positive correlation between pH and % base saturation as a consequence. After the N2 and N3 treatments were stopped, the pH-value for both N2 and N3 were approaching that of the control, and the correlations between pH and base saturation in the mineral soil were gradually disappearing.

Continuous data on the chemical composition of water leaching from the different treatments in E55 would have allowed mass balance calculations. We have such data only for 1993–1994 (Lundell et al., 2001). Two kinds of soil cores were studied (both extending 20 cm into the mineral soil and with ion exchange resin bags placed under them), one excluding tree roots and the other allowing ingrowth of them. The data from the ingrowth cores are the most relevant, since they mimic the natural condition with active roots. Leaching of N was far greater from the root-free cores; for NO_3^- the difference was 20 as compared to 5 kg N ha^{-1} and $year^{-1}$ (Lundell et al., 2001) in the on-going N2 treatment. In N3, which had been terminated just a few years before, leaching of N was almost down to the levels in N0 and N1. There were no significant differences in leaching of Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+} and Al^{3+} among the treatments (K^+ was a component of the salt used to extract the other ions from the resins).

The acidification effect of NH_4NO_3 application results from increased uptake of base cations by the trees and by losses of base cations accompanying NO_3^- leaching from the ecosystem. We do not have information on the likely contribution of these potential drivers. Prior studies in Sweden with very high rates of N fertilization estimated that N leaching would be too low to account for a large part of the difference in base cation pools in the soil (Binkley and Högberg, 1997). Based on an extensive sampling in 1988 of N in soils to 20 cm depth, living above-ground tree biomass and dead branches on the ground, Tamm et al. (1999) estimated that 70 %, 45 % and 30 % of the N added was retained in the N1, N2 and N3 treatments, respectively. These rough mass balance estimates for the added N pointed to high rates of N loss (perhaps nitrate leaching) in the N2 and N3 treatments, that might account for the change in base cation pools (Tamm et al., 1999). The greater accumulation of cation nutrients in biomass in the N1 treatment might not be large enough to account for lower base cation pools in the soil, though the quantities per hectare are not known with precision (Tamm et al., 1999). We note that in 2000, exchangeable Ca^{2+} and K^+

were lower in N2 and N3 than in N0 and N1 at 45–55 cm depth in the mineral soil (Högberg et al., 2006), suggesting leaching losses in N2 and N3, in which tree growth should have removed less nutrient base cations than in N1. Our five-decade study indicates the direction and magnitude of soil changes, but the direct processes leading to the changes remain unknown.

We initially asked a question (number 6) about the potential of recovery of trees and soils upon termination of the N load. The underlying expectation was that there would be a recovery from negative effects on tree growth of the N load. However, there were no negative effects in plots subject to high N load, and so this expectation was not met. The only recovery observed in trees was the recovery from the B deficiency and associated growth disorders after the additions of B in 1980. Note that this recovery took place during continued N loads and low soil base saturation in N1, N2 and N3 (Figs. 1 and 5).

Lovett and Goodale (2011) discussed why their experiment, like this one, did not follow the tightly linked sequential phases during N saturation posited by Aber et al. (1989, 1989). Lovett and Goodale explained the discrepancy by distinguishing between a kinetic N saturation, when sinks operate as sinks for N but cannot assimilate all N supplied, as opposed to capacity N saturation in which the sinks are zero or negative. The long-term observation of the effects of the N1 load (the most relevant for discussions about N deposition) at Norrliden is of a forest ecosystem capable of maintaining a sink for N and growth above that of the control. This calls for a better understanding of how trees, especially trees in symbiosis with ectomycorrhizal fungi, adjust to a continued high N supply.

The Norrliden plots also led to insights that were not part of the initial thinking behind the experiments. We have shown large declines in the biomass of ectomycorrhizal fungi and their role in N cycling in N-loaded plots (Högberg et al., 2011, 2014; Hasselquist and Högberg, 2014). The $\delta^{15}N$ of the tree foliage (Högberg et al., 2011) changes accordingly because ^{15}N is more strongly retained by the fungi than ^{14}N, which leads to a transport of ^{15}N-depleted N to the foliage (e.g., Hobbie and Högberg, 2012). Hence, the increased $\delta^{15}N$ of the tree foliage. Some years after the termination of the N load in N3, the $\delta^{15}N$ of the tree foliage started to decrease, a change associated with a recovery of ectomycorrhizal fungi as indicated by the PLFA-biomarker 18:2ω6,9 and analysis of fungal DNA (Högberg et al., 2011, 2014). This recovery was also associated with higher retention of labeled N added to the soil, and higher uptake of labeled N by roots (Högberg et al., 2014). Moreover, Hasselquist and Högberg (2014) found that after two decades of recovery in N3, the taxon richness, the number of sporocarps of ectomycorrhizal fungi and their biomass per 100 m^2 were equal to that of the control, indicating a similar tree belowground C allocation. As shown here, a decline in $\delta^{15}N$ of the tree foliage also occurred shortly after termination of the N load in the treatment N2 (Fig. 4d). This return to the distribution of stable N isotopes typical of ectomycorrhizal symbiosis in N-limited forests (Billings and Richter, 2006; Hobbie and Högberg, 2012) is driven by high fungal N retention, which enhances the N-limitation of tree growth (Näsholm et al., 2013).

One may ask if the above-mentioned increases in $\delta^{15}N$ of tree foliage in N-loaded plots followed by decreases after removal of the N-load involves a strong direct influence of the isotopic signal of fertilizer N, which is commonly around 0–1 ‰ (Hübner, 1986; Vitoria et al., 2004). The answer is no. Obviously the large increase in % N of the foliage upon N-loading was not followed by a direct large change in the $\delta^{15}N$ of the tree foliage; in N1 – N3 it decreased around 0.5–1 ‰ or remained constant for some years (Fig. 5). In N1, it took some 15 years before the $\delta^{15}N$ of the tree foliage started to increase. Furthermore, when the N-load was removed in N3 and N2, the decrease in $\delta^{15}N$ of the tree foliage lagged years behind that in % N, which also demonstrates that another agent than the isotopic signature of the N taken up from the soil is the single cause of the change in $\delta^{15}N$ of the tree foliage. Our record of $\delta^{15}N$ of the tree foliage, analyses of PLFA and DNA, and data from sporocarps of ectomycorrhizal fungi confirm the value of $\delta^{15}N$ in assessing the dynamic role of mycorrhizal symbiosis in N-cycling (Högberg et al., 2011; Hasselquist and Högberg, 2014; Högberg et al., 2014) in northerly pine forests.

5. Conclusions and implications for management

The population of inference for our experiment was on the order of a few ha, but we expect the results would apply broadly to the circumboreal forests at large. The growth of most of these is limited by low supplies of N, with high C:N of soils (especially in the humus layer), and moderate increases in N supply from deposition or fertilization would likely increase growth without risking forest health.

We would emphasize three highlights from this long-term experiment for application to management of forest. The first is the most general: this forest was very dynamic over time, responding strongly and non-uniformly to the shifting N biogeochemistry. The growth of a forest may not be tightly predetermined by inherent site characteristics, when these characteristics (such as climate, biogeochemistry, and management inputs) are themselves not constant and subject to change. Broad average trends might be expected, along with substantial variation around such trends. The second highlight is that forest science by definition occurs at the frontier of current knowledge. Questions are developed that have no current answer and knowledge is developed by proposing new ideas and challenging them with evidence. Concerns about the impacts of air pollution and N deposition in the 20th Century led to ideas about how forests might respond to high, chronic additions of N. These ideas were logically possible, but confidence would be limited until the ideas were challenged by evidence from research projects. Nitrogen biogeochemistry is fundamentally important in all forests, with major influences on species composition, growth, and potentially water quality in streams. Our project demonstrated that long-term questions of the impacts of increased N inputs could not be adequately explored with short-term applications of very large amounts of N (see also Leuzinger et al., 2011, Bebber, 2021). This points to the third highlight: long-term experiments are fundamental for understanding how real forests change across decades. We have excellent long-term records of forest growth from extensive, well-designed forests inventories for many parts of the world, but we have far too few long-term experiments that can challenge our ideas that try to explain forest patterns at the temporal and spatial scales that would provide a sound foundation for successful management. Indeed, we should not be complacent that our historically important long-term research will be sustained, as many have become “extinct” over the decades (e.g., Turner, 2023). Our abilities to understand, predict, and adapt to changing forests of the future will be determined in part by both sustained long-term experiments and how the forest science community engages with forest managers (see Binkley et al., 2018).

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data availability

We have shared data as additional files.

Acknowledgments

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Author contributions

PH: planned the work, analyzed data, drafted the first manuscript and wrote the final version. RWL: sampled and assisted the writing of the final manuscript. MNH: analyzed samples and data, and assisted the writing of the draft and the final manuscript. US: analyzed data and assisted the writing of the final manuscript. EG: analyzed data and assisted the writing of the final manuscript. JL: sampled and assisted the writing of the final manuscript. DB: analyzed data and assisted the writing of the draft and the final manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2023.121644.

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