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ORIGINAL PAPER

Long-term response of soil and stem wood properties to repeated nitrogen fertilization in a N-limited Scots pine stand

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Abstract

Nitrogen is the nutrient mainly limiting forest growth on mineral soil sites in the boreal regions. The objective of this study was to find out the response of stem wood N to repeated fertilizations and to find out their long-lasting effects on soil organic matter composition, focusing on C and N cycling processes and concentrations of condensed tannins. The site was located in a relatively unfertile Scots pine (*Pinus sylvestris* L.) stand in eastern Finland. The treatments were three levels of N fertilization (0, 150, 300 kg/ha) applied four times at 5-year intervals with the last addition 29 years ago. The N additions had not changed the pH of the humus layer but resulted in higher availability of N. The C-to-N ratio of organic matter decreased with increasing N addition. The treatment of 300 kg/ha increased the net N mineralization rate and the ratio of net N mineralization/microbial biomass N and decreased the amount of C in the microbial biomass and its C-to-N ratio and the concentration of condensed tannins. Net nitrification and extractable nitrate were negligible in all soils. In soil diffusive fluxes, NH_4-, NO_3- and amino acid-N were all detected by in situ microdialysis sampling; the results showed large variation but supported higher N availability in N fertilized soil. The N fertilization increased tree-ring widths and the effect lasted for about 10 years after the last fertilization event. Nitrogen content and the N isotopic ratio $^{15}N/^{14}N$ ($\delta^{15}N$) in tree-rings increased both after the first N addition in the treatment of 300 kg/ha. In conclusion, soil properties still indicated higher N availability in the N fertilized soil after three decades since the latest fertilization, but the response of tree diameter growth had faded out after a much shorter period.

Keywords Boreal forest · N cycling · N fertilization · Scots pine · Soil

Introduction

Nitrogen (N) is the main factor limiting tree growth in the boreal forests, except in high N deposition areas (Binkley and Högberg 2016; Högberg et al. 2017). This is because the majority of soil N is bound in complex organic structures and only a small proportion of the large soil N storage is in plant-available form, as inorganic N or as low molecular weight organic N in soil solution (Näsholm et al. 2009). Accordingly, increasing N availability by N additions alone or combined with other main nutrients increases tree growth on mineral soil sites (Kukkola and Saramäki 1983; Kukkola and Nöjd 2000; Prietzel et al. 2008) largely by increasing the leaf area produced (Sigurdsson et al. 2002). Thus, interest in fertilizing with N on mineral soils has risen recently since it is a fast means to increase wood production, which makes it the quickest practical tool to affect the carbon cycle and increase C sequestration (Prescott 2010; Hedwall et al. 2014). However, awareness of the possible environmental risks of N fertilization is also growing as it may increase N leaching or emissions of nitrous oxides.

Addition of N to forest soils via deposition or fertilization may change both soil microbial community structure and functions. Fertilization with fast-release N has been shown to increase net N mineralization and to decrease the mineralization of C, the amounts of C and N in the microbial biomass and the fungal/bacteria ratio (Martikainen et al. 1989; Smolander et al. 1994, 1995; Treseder 2004, 2008; Maaroufi et al. 2019). Due to increased ammonium

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concentrations, either originating directly from the fertilizer or from stimulated net N mineralization, N addition may increase nitrification. Higher nitrate concentrations in soil increase the risk for N losses from the ecosystem. In some soils this only happens if pH is increased by, e.g., liming (Martikainen 1985; Priha and Smolander 1995).

Long-term N fertilization in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) stands in Finland increased the amount of carbon in the humus layer and simultaneously decreased its C-to-N ratio (Saarsalmi et al. 2014). This carbon accumulation in the organic layer, commonly observed after N addition to forest soils (Högberg 2007), appears to be partly due to an increase in the amount of litter and partly due to decreased aerobic C mineralization (Smolander et al. 1995; Janssens et al. 2010). Recent studies suggest that N enrichment leads to a selective accumulation of lignin-derived compounds at the expense of carbohydrate-derived compounds (Hasegawa et al. 2021). However, various changes in soil organic matter composition and degradation after N addition are not well understood. After fertilization, the concentrations of those phenolic compounds important in plant defense are reduced in the foliage (Koricheva et al. 1998; Booker and Maier 2001). In conifers an important group is condensed tannins that may also affect soil N cycling by promoting N retention in soil rather than mobilization (Kraus et al. 2003; Smolander et al. 2012). Recent studies also emphasize their role in carbon sequestration (Adamczyk et al. 2019). Soil tannin concentrations do not necessarily reflect what enters the soil via plant litter, since they are determined also by degradation rates. Altogether, we do not fully understand the response of soil organic matter composition to N addition in general, and—to our knowledge—very little is known about how soil tannins respond to long-term N addition.

Variation of isotopic signatures of N (variation of $^{15}N/^{14}N$ ratios relative to atmospheric N_2, reported as $\delta^{15}N$ values) in tree rings might be useful in determining long-term changes in N availability of a site (Hart and Classen 2003). However, interpretation of the $\delta^{15}N$ signals in tree rings is complicated due to the small size of plant-available N pools in soil, relative proportions of different N-forms uptakes, low N content in wood, translocation of mobile N between tree rings, as well as various fractionation events occurring already during the soil N cycle (Högberg et al. 1999; 2014a, b, c; Balster et al. 2009; Savard 2010). Thus, understanding of soil processes may help in interpretation of tree-ring $\delta^{15}N$ series. Nonetheless, nitrogen fertilization has resulted in shifts in tree ring $\delta^{15}N$ values (Elhani et al. 2005; Balster et al. 2009) and the $\delta^{15}N$ in tree rings has been successfully used to study N deposition (Poulsson et al. 1995; Savard 2010; Doucet et al. 2012; Jung et al. 2013). According to ^{15}N-labelling experiments, $\delta^{15}N$ values in tree rings are highest a year or two after the addition (Hart and Classen 2003; Elhani et al. 2005).

The objective of this study was to increase understanding of long-term effects of repeated N additions to forest soil, the last addition having been performed three decades ago. We aimed to find out the response of stem wood N and $\delta^{15}N$ to different amounts of added N and to analyze whether they can be used to indicate the growth enhancing effects of increasing N availability in the past. We also aimed to find out how soil C and N cycling processes and organic matter composition react to the N additions in the long term, with special focus on condensed tannins. The study site was a relatively unfertile Scots pine stand, where the three levels of N fertilization (0, 150, 300 kg/ha) were applied four times at 5-year intervals. We hypothesized that the repeated N additions in the past can be traced as increased N concentration and $\delta^{15}N$ of stem wood and that the effects are still visible as stimulated N cycling activities in soil.

Materials and methods

Study site and treatments

Two experiments were established in 1975 to the same 40-year-old Scots pine stand in Sotkamo, middle-eastern Finland (64°13′N, 28°46′E, 190 m a.s.l) to study the effects of thinning and fertilization on tree growth (Experiments 501 and 556, Hynynen and Saramäki 1995). The site represented a relatively unfertile *Empetrum-Vaccinium* (EVT) site type, applying the Finnish site type classification (Cajander 1949). The soil was podzol and the humus type was mor. The deposition of N in the area is low, below 3 kg/year (Mustajärvi et al. 2008).

For this study, we selected the plots of delayed first thinning. At the time of establishment, the number of stems on the plots varied from 2770 to 3060 (Table 1). The plots were thinned from below in 1990 (15 years after the establishment) and the removal was 60% of the initial number of stems. The plot size was 1000 m^2 with a 5-m wide surrounding buffer zone with the same treatment. Amounts of fertilizer, distributed evenly by hand, were 0, 750 and 1500 kg/ha NPK fertilizer (20% N as ammonium nitrate, 4% P, 8%

Table 1 Numbers of living and dead trees in the two replicate plots of the fertilization experiment. N0, N150 and N300 refer to amounts (kg/ha) of N in the fertilizations in 1975, 1980, 1985 and 1990

Treatment	Number of living trees in 1975	Number of dead trees in 1975–2000	Dead trees %
N0	2890–3060	330–400	11–13
N150	2830–2850	480–540	17–19
N300	2770–2970	980–1090	35–37

K), corresponding to 0, 150 and 300 kg/ha of N, 0, 30 and 60 kg/ha of P, and 0, 60 and 120 kg/ha of K. The fertilization treatments were assigned randomly to the plots and repeated four times (1975, 1980, 1985 and 1990) resulting in N additions totaling to 600 kg/ha and 1200 kg/ha. In the following, the treatments are marked based on the amount of N given each time, i.e., N0, N150 and N300 kg/ha. Both experiments had one plot for each N addition level. In the following we consider them as replicates since the experiments were overlapping in the same stand.

At the time of establishment in 1974, all trees were numbered, and two perpendicular diameters (*dbh*) from each tree were measured and tree status (dead/alive/to be removed in thinning) was determined. The measurement was subsequently repeated at five-year intervals between 1979 – 1999. In the late 1980s, one of the plots of the treatment N300 had suffered damage, which resulted the death of 20% of trees and created a small opening within the plot. The mean *dbh* growth of living dominant trees was estimated for each plot for the five-year periods 1975–1979, 1980–1984, 1985–1989, 1990–1994 and 1995–1999. Thereafter, the five-year mean *dbh* growth differences between the fertilized and unfertilized plots were estimated for the dominant trees and the results were compared to the corresponding growth differences of the trees sampled for this study in 2019.

Soil sampling and analysis

Soil sampling was performed in August 2019, i.e., 29 years after the last fertilization treatment. Twenty soil cores were systematically (grid design) taken from each plot by a soil corer (diameter 58 mm). The thickness of the humus layer (O_{fh}) was measured, and the humus layer samples were combined to one composite sample per plot. The samples were brought to the laboratory and stored at 4 °C before the analysis.

The samples were homogenized by sieving through 4 mm mesh size sieve. Dry mass, organic matter content, soil water holding capacity (WHC), pH and C-to-N ratio were determined using established methods, described in Smolander et al. (2013). Concentration of condensed tannins was determined from dry soil samples using the spectrophotometric acid butanol assay as described in Smolander et al. (2013).

Microbial biomass and activities were measured from the fresh samples as described by Smolander et al. (2013). Briefly, the flushes of C and N from the microbial biomass were measured with the fumigation-extraction method (Vance et al. 1987) and the conversion formulas by Martikainen and Palojärvi (1990) were used to calculate amounts of C and N in the microbial biomass. Rates of net N mineralization and net nitrification were measured in a 6-week incubation experiment at constant moisture (WHC 60%) and temperature (14 °C). Net N mineralization and net nitrification were determined, after KCl extraction, as accumulation of $(NH_4 + NO_3)$-N or NO_3-N, respectively, during the incubation. Aerobic mineralization of C was estimated by measuring CO_2-C production rate three times during the 6-week incubation (at 2, 8 and 18 days after the incubation started); for this purpose the bottles were closed with gas-tight septa 20 h before the gas chromatograph measurement of CO_2 produced. The ratio net N mineralization/microbial biomass N was calculated to describe the release of N *vs.* N immobilized in the microbial biomass in the organic matter decomposition process.

One-way ANOVA was applied to detect significant differences between the different fertilization amounts (N0, N150, N300). $P < 0.1$ was considered as significant due to the long time elapsed from the last fertilization, probably diluting the fertilization effects. LSD test was used for pair-wise post hoc comparisons between different N amounts.

Determination of plant-available N fluxes

Microdialysis, the in situ sampling technique introduced to soil N studies by Inselsbacher et al. (2011), was used to collect diffusive fluxes of plant-available N in the humus layer simultaneously as soil sampling. This method is based on induced diffusion of soil solutes across small semi-permeable membrane and thus, enables sampling with minimal soil disturbance. On each plot, two sampling miniplots (50 cm × 50 cm each) were located in the middle of the plot at about 10 m distance from each other on the diagonal of the plot. On each miniplot, four CMA 20 microdialysis probes with a polyarylethersulphone membrane (molecular weight cut-off 20 kDa, length 30 mm, diameter 0.5 mm, CMA Microdialysis AB) were vertically inserted at 1.5 cm depth into the humus layer (O_{fh}) after removing above-ground vegetation and coarse litter from the sampling spot. Probes were perfused with Milli-Q water at a flow rate of 5 µl/min for 52 min to collect samples (dialysates). Before and after field sampling, the performance of the membranes was checked as recommended by Inselsbacher et al. (2011). Dialysates were stored at -18 °C until the microplate analyses of different N forms: NH_4-N was determined using a modified indophenol method as described by Hood-Nowotny et al. (2010); NO_3-N was determined by vanadium (III) chloride and Griess method (Miranda et al. 2001; Hood-Nowotny et al. 2010) with modifications by Inselsbacher et al. (2011); and total free amino acid -N was determined by fluorometric method (Jones et al. 2002), with the modifications by Darrouzet-Nardi et al. (2013). Diffusive flux (D) of the N compounds across the membrane is given as nmol N/m^2/s, and is calculated as follows:

$D = c \times V/(A \times t)$, where c is the concentration of N compound in the dialysate, V is the dialysate volume, A is the membrane surface area and t is the sampling time. One-way

ANOVA was performed for plot-wise mean values to detect significant differences between fertilization treatments as above.

Increment coring and analysis

In June 2019, three healthy dominant trees were randomly selected from each plot. Four increment cores of 5 mm diameter were taken from each tree at 1.3 m height. The borer was cleaned with acetone between different trees. The radial increments were crossdated visually and the ring-widths of one core per tree were measured to an accuracy of 0.01 mm using the WinDENDRO™ software (Regents Instruments Inc., Quebec, Canada).

The other three cores were used for analyzing N concentration and δ^{15}N. The cores were covered with aluminum foil and kept frozen until they were dried in their opened foils (48 h at 60 °C). The rings formed in years 1970–1994 were cut in five-year segments: five years before the first N fertilization (1970–1974), followed by the five years after each fertilization occasion (1975–1979, 1980–1984, 1985–1989 and 1990–1994). The corresponding five-year segments of the three cores taken from each tree were pooled together and weighed after drying. To produce homogenized wood samples, the samples were finely ground (5 min, Retsch ball mill MM400). All equipment was cleaned with water and acetone and dried between the samples. We did not remove mobile N fractions from the samples since radial mobilization of N seems to occur without significant isotopic discrimination and does not show any heartwood-sapwood influence (Hart and Classen 2003; Doucet et al. 2011).

Total N concentrations and their corresponding δ^{15}N values of the wood samples were analyzed using Elemental Analysis—Isotope Ratio Mass Spectrometry (EA-IRMS) in the Isotope Science laboratory, Geoscience Department, the University of Calgary, Canada.

Results

Response of soil properties to repeated N fertilization

Effects of repeated N fertilization were detected in several properties of the humus layer 29 years after the last fertilization (Fig. 1, Table 2). The humus layer tended to be thicker

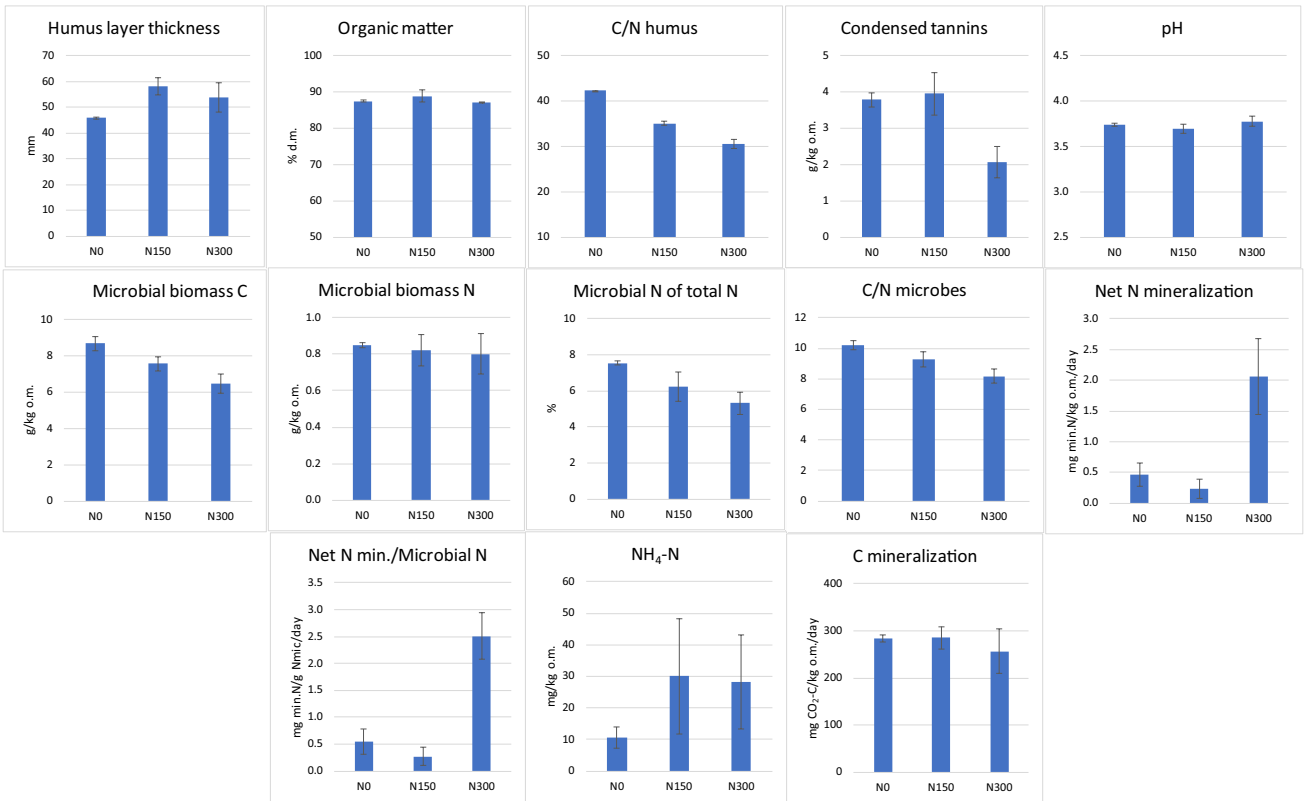

Fig. 1 Thickness of the humus layer and its organic matter concentration and organic matter properties. 0 N, 150 N and 300 N refer to amounts (kg/ha) of N in the fertilizations in 1975, 1980, 1985 and 1990. Mean of the two replicate plots in each fertilization treatment, the error bar shows the values for the two replicate plots. d.m. = dry matter, o.m. = organic matter

Table 2 Probabilities for significant differences ($p < 0.1$) between N0, N150 and N300 from one-way ANOVA; pair-wise differences marked when significant in LSD test

Variable	P	Pair-wise comparison
Humus layer thickness	0.213	
Organic matter %	0.505	
pH	0.518	
Ntot	0.006	N0-N150, N0-N300; N150-N300
C/N humus	0.002	N0-N150, N0-N300; N150-N300
Condensed tannins	0.095	N0-N300
Microbial biomass C	0.087	N0-N300
Microbial biomass N	0.917	
Microbial N % of Total N	0.155	
C/N microbes	0.090	N0-N300
Net N mineralization	0.079	N0-N300; N150-N300
Net N min./microbial N	0.024	N0-N300; N150-N300
NH4-N	0.602	
C mineralization	0.617	
N diffusive flux	0.455	
NH_4-N flux	0.660	
NO_3-N flux	0.873	
Amino acid-N flux	0.489	

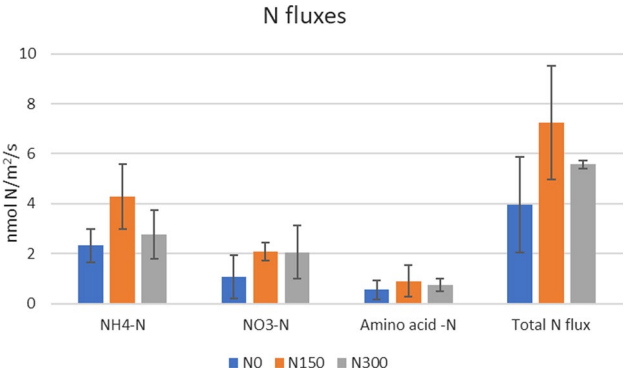

Fig. 2 Diffusive fluxes of NH_4-N, NO_3-N and amino acid -N, total N flux is expressed as the sum of the three N forms. Mean of the two replicate plots in each fertilization treatment, the error bars show the values in the two replicate plots. Treatments explained in Fig. 1

in the fertilization treatments than in the control. The concentration of organic matter in the humus layer was similar irrespective of fertilization treatment. The average amounts of organic matter in the humus layer were 36.2, 50.2 and 52.0 tons/ha for N0, N150 and N300 treatments, respectively. The corresponding values for C were 17.2, 23.4 and 23.8 tons/ha. These values are for sieved humus layer (about 10% loss in sieving) and stoniness is not taken into account.

The C-to-N ratio of organic matter decreased with increasing N addition, by more than 10 units in N300 (Fig. 1, Table 2). The concentration of condensed tannins in the organic matter had halved in N300 treatment. Fertilization did not change humus layer pH.

The heaviest treatment of 300 kg/ha decreased the amount of C in the microbial biomass (Fig. 1, Table 2), as well as its proportion of soil total C which varied from 1.3 to 1.9% (results not shown). Amount of N in the microbial biomass did not change according to the N addition. Its proportion of soil total N, varying from 4.7 to 7.6%, tended to decrease in increasing N addition and so did also the C-to-N ratio of the microbial biomass.

The rate of net N mineralization was fivefold to tenfold higher in N300 as compared to N0 or N150 (Fig. 1, Table 2). This was the case also for the ratio net N mineralization/microbial biomass N. Mean values for NH_4-N concentrations were much higher in both fertilization amounts compared to N0 but there was large variation between the fertilized replicate plots. Both NO_3-N concentrations and the rate of net nitrification were negligible in all soils (results not shown). The fertilization treatments did not affect aerobic C mineralization.

Variation in the diffusive fluxes of plant-available N in the humus layer was large, varying from 0.19 to 30 nmol N/m^2/s in different membrane probes. The fertilization did not significantly affect the fluxes, but the mean values for all N forms were highest on the fertilized plots (Fig. 2, Table 2). Mineral N always dominated the N flux, but the share of NH_4-N and NO_3-N differed. The share of total amino acids in the N flux was 5–17%.

Response of tree rings and tree dbh to repeated N fertilizations

The ring widths of the sample trees on the fertilized plots started to deviate from those on N0 plots soon after the first fertilization in 1975 with both amounts of added N, and radial increment in N150 and N300 remained faster than in N0 after the following fertilization events (Fig. 3). The faster radial increment lasted for 10–15 years after the latest fertilization and thinning in 1990. The response of ring widths to N150 was similar to N300.

The mean differences in five-year *dbh* increment between the fertilized and unfertilized plots are presented in Fig. 4. The differences were calculated both for the 2019 sample (three trees per plot) and for all dominant trees measured between 1974 and 1999 in the successive measurements of the plots. The mean response of *dbh* increment to N300 was higher than to N150 after the first fertilization in 1975. After the third and fourth fertilizations in 1985 and 1990, N300 did not increase *dbh* increment more than N150. After the last treatment in 1990, the effects of fertilization (N150 or N300) combined with thinning were significant for 5–9 years, even though the unfertilized plots were also thinned in 1990. Tree *dbh* increment on the fertilized plots

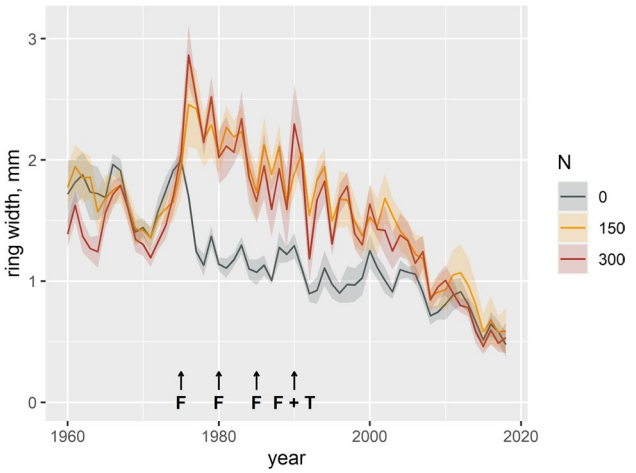

Fig. 3 Mean tree ring widths for the fertilization treatments. Means are calculated over the tree-level observations and the shaded area shows the standard error of the mean. Treatments explained in Fig. 1. F and T show the years of fertilization and thinning, respectively

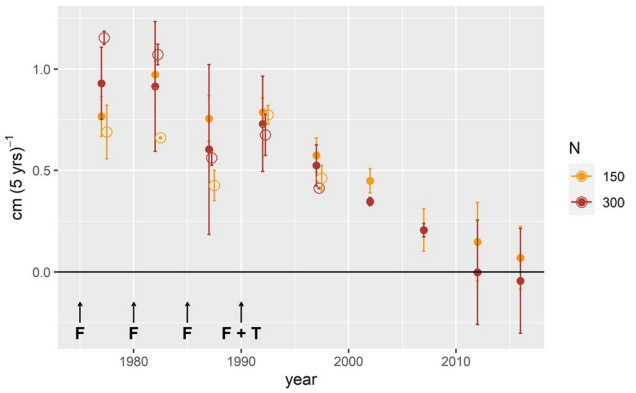

Fig. 4 The mean differences in the five-year *dbh* increments between the fertilized and unfertilized plots. Open circles indicate the dominant trees measured between 1974 and 1999 and solid circles indicate the trees sampled for this study. The error bars show the values in the two replicate plots. Treatments explained in Fig. 1

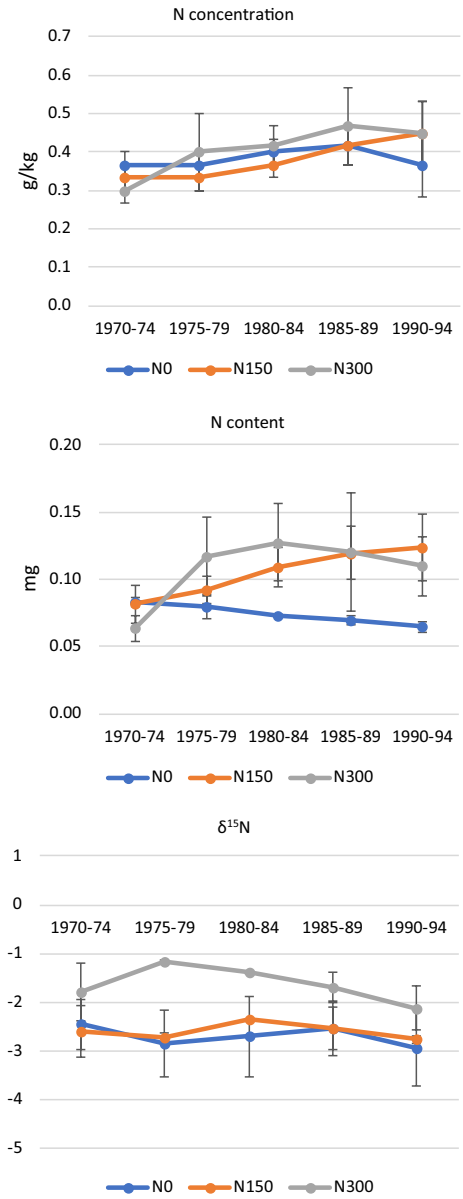

Fig. 5 N concentrations, N contents and $\delta^{15}N$ in the tree rings between 1970–1994 measured in 5-year periods: 1970–1974 before the first fertilization, 1975–1979, 1980–1984, 1985–1989 and 1990–1994 after the four fertilization events. Mean of the two replicate plots in each fertilization treatment, the error bars show the values for the two replicate plots. Treatments explained in Fig. 1

was on average higher than the *dbh* increment on the unfertilized plots for 10–14 years after the last treatment, but the standard errors of the estimates were high, since during this period we only had data from the three trees per plot, sampled in 2019. The differences between the fertilized and unfertilized plots faded out in 15 years after the latest treatment. The dry masses of the tree rings in the increment cores were in accordance with the ring widths during the 5-year periods after the fertilizations; dry mass was higher on the fertilized plots (results not shown).

N content of the tree rings increased due to the fertilization (Fig. 5). The N300 treatment increased the N content sharply during the first 5-year period after the fertilization while the development was slower in the 150 N treatment. The increase in N content was more due to increased wood mass and less due to increased N concentration in the wood.

$\delta^{15}N$ responded to the first N fertilization event in N300 treatment with an increasing isotope ratio, while in N150 the development of $\delta^{15}N$ did not differ much from N0 (Fig. 5). The variation of $\delta^{15}N$ was high between the three sampled trees per plot particularly in N0 treatment (results not shown).

Discussion

In accordance with our hypothesis, several properties of the humus layer of the pine stands still differed due to the repeated N fertilization, although almost three decades had elapsed from the last application. Higher availability of N is indicated by the increased net N mineralization rate, NH_4-N concentration and the decreased C-to-N ratio of both soil organic matter and microbial biomass, as well as the decreased proportion of microbial biomass N from soil total N. Similar changes have been shown also on other Scots pine or Norway spruce stands after a single or repeated N fertilization applied 2–14 years earlier depending on the site (Martikainen et al. 1989; Nohrstedt et al. 1989; Smolander et al. 1994, 1995). The higher N addition (N300) considerably increased the ratio of net N mineralization/microbial biomass N, indicating that the amount of easily-mineralizable N in relation to the amount of N that is immobilized into the microbial biomass is still increased. The increase in humus layer thickness and its organic matter content is also in accordance to the shorter-term studies mentioned above. The soil results were presented by concentration to describe how the N fertilization had affected the properties of organic matter. If we present the results on areal basis (as kg/ha), the differences between the fertilized and unfertilized plots become even more clear because of the thicker humus layer on the fertilized plots.

Soil diffusive fluxes of different plant-available N forms showed large variation, but also these results suggest higher N availability on the fertilized plots. Microdialysis detected NO_3-N in soil solutions although the concentrations of extractable NO_3-N in the soil samples were negligible. Microdialysis measures soil N fluxes with high spatial and temporal resolution, reflecting N dynamics in soil microsites (Inselsbacher et al. 2011; 2014). Thus, one explanation for the highly variable NO_3-N fluxes could be that the in situ microdialysis may detect nitrification microsites that are disturbed by soil sampling, homogenization and extraction procedures (Buckley et al. 2017). The low share of amino acid -N of the total N flux in our study sites contrasts the previous, shorter-term studies, where amino acids dominated the N flux in both unfertilized and fertilized soils, but may also result from the different analysis methods (Inselsbacher and Näsholm 2012; Inselsbacher et al. 2014; Oyewole et al. 2016). Due to large spatial variation, two sampling miniplots on each plot, each having 4 probes, appeared to be inadequate, but the results nevertheless indicated higher N availability due to N fertilization.

The N fertilization had not changed the pH of the humus layer, a result similar to findings from a large number of Scots pine and Norway spruce stands in Finland (Saarsalmi et al. 2014), but contrasting the increased acidity connected to N accumulation in Central-European soils receiving high N load (Prietzel et al. 2006). Despite the exceptionally heavy N fertilization—with N doses of 150 or 300 kg/ha every five years, we detected hardly any net nitrification even with the highest NH_4-N concentrations. In similar soils, N fertilization may either initiate net nitrification, or the initiation requires an increase of pH (Martikainen 1985; Priha and Smolander 1995). We do not know whether intensive net nitrification existed earlier, soon after the treatment. Possibly nitrification had been limited by a very low initial pH. Accordingly, no consistent effects on soil–water chemistry were observed in a Scots pine stand in Sweden 4–8 years after repeated additions of N, totalling to 600 kg N ha^{-1} (Nohrstedt 1998). In practical forestry, the fact that boreal forests are strongly N limited and possess a high N retention capacity means that fertilization of 150 kg/ha N applied once, or twice every 10th year recommended for forestry practice, causes only a temporary low peak in nitrate concentration in soil solution of mature forests (Hedwall et al. 2014).

Evidence from different types of ecosystems suggest that vegetation species composition, below-ground communities and soil processes may be slow to recover whereas some soil variables, such as nitrate and ammonium concentrations, can respond relatively rapidly to reductions in N inputs (Stevens 2016). In boreal N-limited forests, the recovery from N addition is suggested to be driven by increased tree below-ground allocation to ectomycorrhizal roots and fungi (Högberg et al. 2014b) and the recovery time depends on the soil properties in question. A recovery of ectomycorrhizal fungi was indicated in Scots pine and Norway spruce stands about 15 years after termination of repeated N fertilization (Högberg et al. 2011; Blasco et al. 2013). Fungal community structure and N retention capacity of the soils no longer deviated from unfertilized soil in Scots pine stands 14–20 years after the termination of heavy N additions over two decades, but there were still some signs of higher N availability and changes in bacterial community (Högberg et al. 2014a, b).

Lower concentrations of condensed tannins in needles after N fertilization (Booker and Mayer 2001) as well as changes in degradation may explain the decreased concentration of condensed tannins in the soil of N300 treatment. Recently, the role of condensed tannins in increasing C sequestration into soil has been brought up, a possible reason being their ability to form stable complexes with fungal chitin (Adamczyk et al. 2019). In addition to chitin, condensed tannins also form complexes with other N-containing compounds, affect enzyme activities and decrease N mineralization and nitrification (Smolander et al. 2012). The strong accumulation of organic matter in the humus layer after N fertilization, a phenomenon reported for many Scots pine

and Norway spruce stands (Saarsalmi et al. 2014), points to an imbalance between the rates at which litter is produced and decomposed. Thus, N fertilization is considered an efficient tool to increase soil C sequestration (Högberg 2007; Prescott 2010). Our tannin results did not suggest that these compounds would be especially vital in N-addition-induced C sequestration. Lowered rate of aerobic mineralization of C has been reported after N fertilization on several forest soils (Martikainen et al. 1989; Nohrstedt et al. 1989; Smolander et al. 1995; Maaroufi et al. 2015), and recently the accumulation of lignin-derived compounds was shown (Hasegava et al. 2021).

N fertilization increased the width of annual rings already after a year from fertilization. Increased volume growth of Scots pine stands after fast-release N fertilization generally lasts for 7–10 years without any significant residual effects (Laakkonen et al. 1983; Saarsalmi and Mälkönen 2001; Pettersson and Högbom 2004). Valinger et al. (2000) showed that the response of radial growth to N fertilization faded out in eight years in northern Sweden, while the positive effect of thinning could still be seen after twelve years. In our experiment, a weak *dbh* growth enhancing response to the treatments was still visible 10–14 years after the last treatment (N fertilization combined with thinning compared to mere thinning). The thinning treatments were delayed, i.e., done about 15 years later than recommended for forestry practice, which may cause the results to differ slightly from earlier studies. The small difference of *dbh* growth between the different N doses (150 and 300 kg/ha) indicates that there was a change from initial N limitation to a limitation of other growth factors, such as water or nutrients other than N. The fertilizer contained P and K but the contribution of these nutrients to *dbh* growth is unclear. Prietzel et al. (2008) analyzed 40-year effects of different soil amelioration methods in two pine stands with recent high N deposition, and emphasized the important role of other nutrients to compensate N-induced base cation losses caused by leaching and the increased nutrient uptake by faster growing trees. Increased susceptibility to drought with increased N availability has also been suggested (Betson et al. 2007).

Our aim was also to analyze whether tree ring N, and in particular the isotope ratio $\delta^{15}N$, can be used to indicate the growth enhancing effects of increasing N availability in the past. As expected, increase in N content in tree rings due to N addition was more due to increased radial increment than changes in the N concentrations of wood produced. It has also been stated that N concentration in tree rings should not be used as an environmental indicator, because the N stored in tree rings can be translocated to the active cambium even though $\delta^{15}N$ in the wood already formed remains unaffected (e.g., Doucet et al. 2012). Accordingly, N concentration in stem wood did not react as much to fertilization as the $\delta^{15}N$ (cf., Balster et al. 2009). Assuming that N used for the formation of a tree ring is assimilated from the soil during the growing season (Doucet et al. 2012), the tree-ring series may record changes in N availability in soil, despite possible isotopic discrimination in N uptake due the ectomycorrhizal fungi (Högberg et al. 2014a, b, c). This was the case in the heaviest N300 treatment where $\delta^{15}N$ increased due to fertilization, as expected based on previous results (Elhani et al. 2005; Balster et al. 2009). However, the increase was observed only after the first N addition and after that, $\delta^{15}N$ remained at a similar level or even decreased despite of the new N additions. Moreover, we did not observe any clear response of $\delta^{15}N$ to the lower amount of N, i.e., N150 treatment.

The number of replicate plots and the number of sample trees per plot may have been too low to detect differences in $\delta^{15}N$. There were large absolute differences in $\delta^{15}N$ between the three sample trees on each plot, as also found in other studies (Poulson et al. 1995; Hart and Classen 2003). The variation in $\delta^{15}N$ between the sample trees was highest on the unfertilized control plots and the N additions seemed to decrease the between-tree differences. According to Larry et al. (2010), the relationship between $\delta^{15}N$ and N concentration in wood is site specific and becomes stronger with higher soil N input.

Despite the fact that we studied the effects of N addition in an even-aged, mature and pure pine stand, interpretation of $\delta^{15}N$ in tree rings is difficult. The heavier N isotope in the fertilizer may have increased the ^{15}N pool in the wood directly via N uptake of trees, but indirect effects via microbial processes may also have occurred. We cannot exclude a loss of ^{15}N depleted compounds in denitrification or nitrate leaching (Högberg et al. 2014a, b, c) although these processes are generally not very intensive even after high N loads in the N-limited acid soils of the boreal zone (Smolander et al. 2000; Högberg et al. 2017). N deposition and soil acidification have also been reported to affect the ratio (Savard 2010). However, the deposition in the region is very low (N below 3 kg/ha/year, Mustajärvi et al. 2008). Soil acidification is probably not an artefact either, based on the current similar pH values of the unfertilized and fertilized soils, and the fact that N fertilization does not generally acidify similar N limited forest soils (Saarsalmi and Mälkönen 2001; Saarsalmi et al. 2014). In any case, the potential of using the N isotope ratio for retrospective tracing of the N input into forest soil remained unclear in the present study.

In conclusion, the response to repeated N fertilization was still visible after 30 years as changed soil organic matter composition and increased availability of N. The total amounts of N given in 15 years were several times higher than those used in forestry practice. Högberg et al. (2017) concluded in their review that N-poor Fennoscandian forests are rather resilient to these modest N additions. Tree diameter growth had responded only for 10–15 years after

the last N addition and the response to fertilizer dose (N150 *vs* N300) was not large. This points to other growth-limiting factors becoming more important than N. The significance of using the N isotopic ratio for retrospective tracing of the N input into forest soil remained unclear in this study.

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