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*Article*

# Residual Long-Term Effects of Forest Fertilization on Tree Growth and Nitrogen Turnover in Boreal Forest

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**Abstract:** The growth enhancing effects of forest fertilizer is considered to level off within 10 years of the application, and be restricted to one forest stand rotation. However, fertilizer induced changes in plant community composition has been shown to occur in the following stand rotation. To clarify whether effects of forest fertilization have residual long-term effects, extending into the next rotation, we compared tree growth, needle N concentrations and the availability of mobile soil N in young (10 years) *Pinus sylvestris* L. and *Picea abies* (L.) H. Karst. stands. The sites were fertilized with 150 kg·N·ha$^{-1}$ once or twice during the previous stand rotation, or unfertilized. Two fertilization events increased tree height by 24% compared to the controls. Needle N concentrations of the trees on previously fertilized sites were 15% higher than those of the controls. Soil N mineralization rates and the amounts of mobile soil $NH_4$-N and $NO_3$-N were higher on sites that were fertilized twice than on control sites. Our study demonstrates that operational forest fertilization can cause residual long-term effects on stand N dynamics, with subsequent effects on tree growth that may be more long-lasting than previously believed, *i.e.*, extending beyond one stand rotation.

**Keywords:** carry-over fertilization effects; forest growth; Picea abies; Pinus sylvestris; N mineralization; *in situ* incubation; ion-exchange resin (IER)

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## 1. Introduction

Nitrogen (N) availability limits forest growth in most boreal and temperate forests [1,2], and fertilization with N is one of the most cost-effective silvicultural methods to increase boreal forest yield [3]. In fact, timber harvest profitability can increase by nearly 15% if forest stands are fertilized about 10 years before final felling [4]. Fertilization has been practiced commercially since the middle of the previous century in many parts of the boreal and temperate forest regions, for example, in Sweden [5,6], USA [7], Canada [8] and Finland [9]. During the 20th century approximately 10% of the productive forest land in Sweden, *i.e.*, circa (ca) 2 million hectares (ha), was fertilized at least once [3,6,10].

According to previous studies, forest fertilization with N additions corresponding to 150 $kg \cdot N \cdot ha^{-1}$ $application^{-1}$, for up to three times during a forest rotation period [11], will only affect the subjected forest stand for up to 10 years after the last fertilizer application [3,9,12–16]. This conclusion is mainly based on studies of growth effects within the tree rotation period fertilized. Also a recent study, particularly targeting carry-over effects of fertilization between forest stand rotations, showed that growth of five year old pine seedlings was not affected by fertilization of the site three to nine years before harvest of the previous stand rotation [17], *i.e.*, supporting the view that there are no, or only very restricted long-term effects of fertilization. In contrast, a study from North America showed that the height growth of young Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) trees were greater on plots that had been fertilized in the previous stand rotation period than on plots never fertilized [18].

Fertilization effects on species composition of the forest floor vegetation within one forest rotation have been extensively documented, e.g., summarized in Nohrstedt [3] and Saarsalmi and Malkonen [9], but only a few studies have addressed carry-over effects between forest stand rotations. In a study summarizing carry-over fertilization effects on forest floor vegetation, Hedwall, *et al.* [19] reported a higher abundance of graminoids and a lower abundance of dwarf-shrubs on experimental plots fertilized in the previous stand rotation period than on control plots never fertilized. Strengbom and Nordin [20] found carry-over effects between stand rotations on the composition and abundance of forest floor vegetation of operational forest fertilization, *i.e.*, with 150 $kg \cdot N \cdot ha^{-1}$ at one or two occasions during the previous stand rotation.

When fertilizing forests, a large part of the N added is retained in the soil layer [21,22]. Strengbom and Nordin [23] suggested that the disturbance from harvest and site scarification leads to increased mineralization rates of immobilized soil N, explaining why effects of previous (more than 25 years ago) N fertilization was more evident in the early phase of the subsequent stand rotation than within the same stand rotation that were fertilized.

In this study, we revisited young forest stands with documented effects on species composition of the forest floor vegetation from fertilization of the previous stand rotation. We hypothesized that fertilization of the previous forest stand would cause (1) increased tree growth in the following rotation period; and (2) increased soil N mineralization rates, both in comparison to control stands that never had been fertilized. Thus, the aim of the study was to determine fertilization carry-over effects between tree rotations on tree height and diameter growth and on soil N turnover, *i.e.*, mineralization rates and amounts of mobile soil N.

## 2. Materials and Methods

### *2.1. Study Area*

We studied forest sites each sized between 4.7 and 22.4 ha situated within an 8500 ha forest land area owned by the company Sveaskog. The forest land area is in the middle boreal zone [24] in central Sweden (63°00′ N, 16°40′ E). The studied forest sites, either had, or had not, been subjected to N fertilization before harvest and regeneration (Table 1). Control (C) sites ($n$ = 7) were never fertilized; N1 sites ($n$ = 7) were fertilized with 150 kg·N·ha$^{-1}$ once (in 1985), while N2 sites ($n$ = 7) were repeatedly fertilized with 150 kg·N·ha$^{-1}$ (in 1977 and 1985), *i.e.*, our study included seven stands of each treatment. Nitrogen was added as granules of ammonium nitrate ($NH_4NO_3$) spread by tractor or aircraft.

**Table 1.** Number of sites per treatment, elevation (above sea level, a.s.l.), temperature sum (GDD), forest floor slope, the site index (SI), the average numbers of trees ha$^{-1}$ and the average sample tree ages (2010). The sites were fertilized once (N1) with 150 kg·N·ha$^{-1}$ 25 years before the present study, twice (N2) 33 and 25 years before the present study or never fertilized (C).

| Sites (n) | Control | N1 | N2 | *F*-value | *p*-value |
|---|---|---|---|---|---|
| | 7 | 7 | 7 | | |
| Elevation a.s.l. (m) | 334 ± 21 | 358 ± 20 | 334 ± 17 | 0.54 | 0.59 |
| Temperature sum (GDD) | 937 ± 6 | 900 ± 22 | 923 ± 11 | 1.59 | 0.23 |
| Slope (1 to 5) | 2.00 ± 0.22 | 1.86 ± 0.26 | 1.72 ± 0.18 | 0.41 | 0.67 |
| Site index ($H_{100}$, m) | 20.6 ± 0.2 | 19.7 ± 0.3 | 20.4 ± 0.3 | 3.00 | 0.08 |
| Trees (ha$^{-1}$) | 2721 ± 378 | 2982 ± 416 | 2532 ± 206 | 0.43 | 0.65 |
| Tree age (years) | 10.4 ± 0.2 | 10.4 ± 0.2 | 10.3 ± 0.2 | 0.80 | 0.47 |

Forest inventory records describing the forest sites before clear-cut kept by the land owner were used when selecting the sites. In particular care was taken to select sites with similar productivity (site) indices (Table 1). The other criteria for including sites in our study were temperature sum (growing degree days, GDD), *i.e.*, the annual temperature sum of all daily mean temperatures above 5 °C [25]; slope, *i.e.*, the forest floor incline, on a scale from 1 (<10% inclination) to 5 (>50%); current stand age; tree species; and soil conditions. The sites were spread out in the 8500 ha forest land area and spatially separated from each other (>1 km). The annual precipitation in the area is between 600 to 700 mm per year and the atmospheric background N deposition ranges from 2.5 to 3.5 kg ha$^{-1}$ year$^{-1}$ [26].

All stands were on mesic sites with moraine soil, geotechnical nomenclature coarse-grained till [27], with Udic moisture regime [28]. Before clear-felling all sites had a mixed tree species composition with *P. sylvestris* and *P. abies* as dominant trees and *Betula* spp. as subordinate trees. The relative abundance of the two dominant species prior to clear-felling ranged from 25% to 75% with no difference between fertilized and unfertilized stands (Mann–Whitney U-test: U = 83.5, $p$ = 0.348) [20]. The forest field layer was dominated by dwarf shrubs such as *Vaccinium myrtillus* L. and *V. vitis-idaea* L., *i.e.*, the stands were classified as spruce forest of bilberry type [29]. All sites were harvested by clear-felling between 1997 and 2000 and were thereafter treated with soil scarification and regenerated by planting *P. sylvestris* and *P. abies* seedlings at a density of 2200 to 2300 stems ha$^{-1}$. Due natural regeneration, all forest stands at the time of our study had a mix of both coniferous species and a sub population of *Betula pendula* Roth, *Betula pubescence* Ehrh and *Populus tremula* L.

*2.2. Data Collection*

Data on tree growth and needle N concentration were collected in August 2010 from four 100 $m^2$ (r = 5.64 m) circular plots per forest site. The plots were distributed along transects that were approximately stand-centered, described in Strengbom and Nordin [20], to minimize the influence of surrounding stands. Tree height (cm), diameter (mm) at breast height (DBH, at 1.3 m above ground) and species were noted for all trees taller than 1.3 m using cross-callipering and a five meter long, telescopic measuring stick (Teleskopmeter, Skogma, Hammerdal, Sweden). Across all plots, 38% of the trees were *P. abies*, 29% were *P. sylvestris* and 33% *Betula* spp. Less than 1% were *P. tremula.* From each site, 16 undamaged sample-trees (the four tallest from each circular plot) were selected. *P. abies* (56%) and *P. sylvestris* (44%) were exclusively used as sample-trees. Sites were classified as dominated by *P. abies* or *P. sylvestris* (*P. abies* = four C, three N1 and four N2; *P. sylvestris* = three C, four N1 and three N2). There was no statistically significant difference in site species classification between treatments (Kruskal-Wallis one-way analysis of variance test = 0.364, $p = 0.834$). Sample-tree annual shoot growth was measured from first visible node and upwards toward full tree height. Tree age was determined from the number of nodes plus one per sample tree.

Current year needles were collected for C and N analyses in early august 2010. Needles were cut from the sample tree's top branches facing the centre of the circle. All needle samples were dried in 70 °C for 24 h and grinded to a fine powder using a bead mill (five minutes per sample), the powder was stored in clear glass vials. In late September, small amounts (ca 3 mg per sample tree) of the ground needle samples were sealed in small tin foil cups, one per sample tree, and sent to a lab (Umeå Plant Science Centre, UPSC) for C and N content analyses using an AutoAnalyzer 3 (SEAL Analytical, OmniProcess AB, Solna, Sweden). Before statistical analyses replicate samples from the same site were pooled.

The buried bag technique was used to investigate humus N mineralization. At each site seven samples from the organic mor-layer were collected between 1st and 3rd June in 2009 with a cylindrical (diameter 10 cm) soil corer. After gently removing large roots and living green material, half of the sample from each mor-layer core was placed in plastic bags in a cooler and transported to the lab for analyses. The second half was placed in plastic bags and buried in the mor-layer *in situ*, and were retrieved between the 28th and 30th September the same year, stored in a cooler and transported to the lab for analyses. The mor-layer samples were extracted in 1 m KCL and analyzed for $NH_4$ and $NO_3$ using a FIAstar 5012 Analyzer (Tecator, Höganäs, Sweden). Mineralization was calculated through differences in $NH_4$-N and $NO_3$-N content ($mg \cdot g^{-1}$ dry weight [DW] soil) between sample times, *i.e.*, early June and late September. All contaminated samples (*i.e.*, damaged bags) were excluded before estimating mineralization. In total 17 samples were excluded from analyses (2 from C sites, 9 from N1 sites and 6 from N2 sites). Before statistical analyses replicate samples of $NH_4$-N and $NO_3$-N from the same site were pooled to obtain one value of mineralization per site.

Resin ion-exchange capsules (PST-1, Universal Bioavailability Environment/Soil Test, MT, USA) were used to estimate the amount of soil mobile $NH_4$-N and $NO_3$-N (mg per capsule). Six capsules were buried just beneath the mor-layer at each site in early June in 2009 and retrieved in late September in the same year. Following retrieval the capsules were placed in plastic bags in a cooler and transported to the lab for analyses. The ion-exchange resins were brushed off to get them as clean as possible before analyses. The capsules were placed in 50 mL Falcon tubes and 7 mL of 1 m KCL was pipetted into the tubes that thereafter were oscillated for 30 min in room temperature, thereafter the 7 mL was poured into a second collecting falcon tube. This process was repeated three times (3 × 7 = 21 mL), and the tubes were stored in a cooler between oscillations. Soil mobile $NH_4$-N and $NO_3$-N were analyzed using a FIAstar 5012 Analyzer (Tecator, Höganäs, Sweden). Before statistical analyses replicate samples of $NH_4$-N and $NO_3$-N from the same site were pooled to obtain one value of soil mobile N per site.

### *2.3. Experimental Design and Statistical Analyses*

For all statistical analyses the experiment is regarded as a completely randomized design. To test for differences in annual shoot growth among treatments (C, N1 and N2) we used repeated measures ANOVA, with annual growth (cumulative height growth during 10 years) as response and treatment and time as dependent variables. The degrees of freedom were corrected with the Greenhouse-Geisser elipson as the assumption of sphericity was not met, tested by the Mauchly's test of sphericity [30]. Sphericity is not met when the variances of the differences between all combinations of associated groups are not equal; correcting the degrees of freedom with the Greenhouse-Geisser epsilon produces a more accurate, upward adjusted *p*-value in the subsequent analysis. After adjusting the degrees of freedom, Tukey's post hoc HSD method was applied to detect significant ($\alpha = 0.05$) differences between treatments when the analysis of variance showed significant main N treatment effects. For the repeated measures ANOVA we used the software package IBM SPSS (v. 20).

Differences in tree height (in 2010), trees per ha, sample tree age, needle N concentration (%-DW), sample tree DBH, total soil mobile N ($NH_4$-N + $NO_3$-N, mg per ion capsule) and total mineralization rates (ammonification plus nitrification) between the treatments were analyzed with one-way ANOVAs followed by Tukey's post hoc test for pairwise comparison when the analysis of variance showed significant ($\alpha = 0.05$) main N treatment effects. Response variables were checked for normal distribution and homoscedasticity, and when these requirements were not met (for mineralization rate, amount of mobile soil N and needle N), data was transformed with the natural logarithm (LN). For the one-way ANOVAs we used the software package Minitab (v. 16). All data showed in figures and tables are original, untransformed mean values ± 1 standard error (SE).

## 3. Results

On the sites that were fertilized twice (N2) during the preceding stand rotation (fertilizer applied 25 and 33 years before our study), the annual shoot height growth over time was greater ($F_{2.857,25.715} = 5.09$, $p = 0.018$; Table 2) than those of trees on N1 sites and on control sites never fertilized (Figure 1). Annual shoot height growth over time on N1 plots was, however, not significantly greater than that on control plots ($F_{2.857,25.715} = 5.09$, $p = 0.255$). In 2010 the 10 year old trees on N2 sites were on average 24% higher than trees on control sites ($F_{2,18} = 4.50$, $p = 0.026$, Table 3). Average tree height on N1 sites was not different from that on control sites ($F_{2,18} = 4.50$, $p = 0.214$). Tree diameter (DBH) was not affected by the previous fertilization treatments ($F_{2,18} = 0.75$, $p = 0.486$, Table 3).

**Table 2.** Results from repeated measures ANOVA testing the effects of fertilization of the previous stand rotation on annual tree shoot height growth of the current stand rotation.

| Within Subjects | Degrees of Freedom | *F*-value | *p*-value |
|---|---|---|---|
| Year | 1.43 | 948.88 | <0.001 |
| Year · Treatment | 2.86 | 3.33 | 0.037 |
| Error (year) | 25.72 | 1217.1 | |
| Between subjects | | | |
| Treatment | 2 | 5.09 | 0.018 |
| Error | 18 | | |

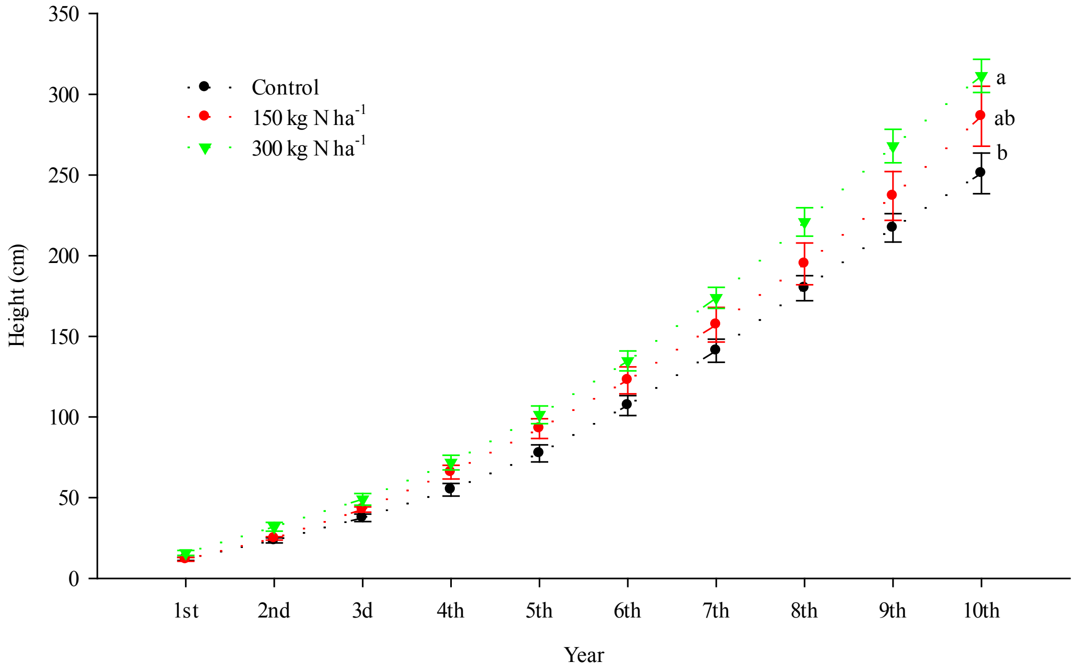


**Figure 1.** The mean (± 1 SE) annual tree height on sites with no fertilization (C), on sites fertilized once with 150 kg·N·ha$^{-1}$ during the previous stand rotation (N1) and on sites fertilized twice with 150 kg·N·ha$^{-1}$ during the previous stand rotation (N2). Different lower case letters (a or b) indicate a significant difference ($p < 0.05$) between sites analyzed with a repeated measures ANOVA followed by a Tukey's post hoc HSD test.

Trees on control sites had lower needle N concentration than trees on both N1 and N2 sites ($F_{2,17} = 5.04$, $p = 0.018$; Table 3). On N1 and N2 sites the needle N concentration was approximately the same, about 16% and 14% higher on N2 and N1 sites respectively compared to the control. There were no differences in needle carbon concentration (data not shown) between sites with the different N treatments.

Although the variation among sites with the same treatment was large, soil N mineralization rates and soil mobile N differed between the treatments (Table 3). Soil N mineralization rates were nearly four times higher on N2 sites than on control sites ($F_{2,18} = 5.22$, $p = 0.016$), whereas there were no differences in N mineralization rates between the control and the N1 sites. The total amount of soil mobile N that were captured on ion exchange resins placed in the soil was 74% higher on N2 sites than on control sites ($F_{2,18} = 3.71$, $p = 0.045$; Table 3).

**Table 3.** The mean (± 1 SE) tree needle N concentration (%-DW), diameter at breast height (DBH, mm), tree height in 2010, the total mineralization rates ($NH_4$-N and $NO_3$-N mg $g^{-1}$ DW soil) and the total soil mobile N ($NH_4$-N and $NO_3$-N) concentration. The 10 year old trees grew on sites never fertilized (C), fertilized once with 150 kg·N·$ha^{-1}$ during the previous stand rotation (N1) and fertilized twice with 150 kg·N·$ha^{-1}$ during the previous stand rotation (N2). Different lower case letters (a or b) besides means in each column indicate significant post hoc differences between treatments (Tukey's HSD test).

| Treatment | Needle N Concentration (%-DW) | DBH (mm, 2010) | Tree Height (cm, 2010) | Total Mineralization (N mg $g^{-1}$ DW soil) | Total Soil Mobile N (mg $capsule^{-1}$) |
|---|---|---|---|---|---|
| C | 1.19 ± 0.05 a | 31 ± 5 a | 251 ± 12 a | 0.083 ± 0.012 a | 0.057 ± 0.023 a |
| N1 | 1.38 ± 0.05 b | 34 ± 5 a | 286 ± 13 ab | 0.090 ± 0.021 a | 0.072 ± 0.022 ab |
| N2 | 1.35 ± 0.04 b | 39 ± 4 a | 311 ± 17 b | 0.293 ± 0.109 b | 0.099 ± 0.020 b |
| *F*-value | 5.04 | 0.75 | 4.50 | 5.22 | 3.71 |
| *P*-value | 0.018 | 0.486 | 0.026 | 0.016 | 0.045 |

## 4. Discussion

According to common forestry practice the growth enhancing effect of N fertilization is expected to diminish within 10 years following treatment [3,9,31]. However, 25 years following the latest fertilization event and 10 years after harvest, scarification and tree planting, we detected positive effects of the previous N fertilization on tree growth in the subsequent young forest stands (Figure 1, Table 2). Our study revealed a fertilizer induced increment in tree growth and needle N concentration. A positive relationship between tree growth and needle N concentration is reported in previous studies, e.g., Bauer, *et al.* [32] and Iivonen, *et al.* [33], and needle N concentration is in turn indicative of the plant available N pool [34]. The higher needle N concentration, in combination with the higher soil N mineralization rates and higher amount of soil mobile N on previously fertilized sites, indicate that N dynamics in the previously fertilized sites remained altered despite that more than 25 years had passed since the last fertilization event.

When fertilizing forests, a large proportion of the N added is normally retained in the soil layer [21,22,35], and disturbances such as clear-felling and soil scarification have been shown to increase soil N mineralization rates and the amount of available soil nutrients [36–38], generally due to increased soil microbial activity. Previously added N retained in the soil layer may thus be accessible to above ground vegetation as N mineralization rates increases after clear-felling and soil scarification. Previous studies of the same sites as used in the present study revealed fertilizer induced shifts in species composition of the forest floor vegetation [20,23]. In combination with the results from the present study this clearly demonstrates that carry-over effects from previous fertilization events influence several components of the ecosystem, resulting in a long-lasting effect on site productivity.

Our results are in contrast to several studies of long-term effects of forest fertilization. For example, Pettersson and Högbom [39] studied old fertilization experiments in boreal forests where 100 to 240 kg of N had been applied one to four times, 14 to 28 years before their study. They found that the previous N addition did not increase *P. abies* or *P. sylvestris* growth in the long term. The majority of the previous studies have, however, addressed the fertilizer effects on site productivity in the forest stand subjected to fertilization, *i.e.*, within the same stand rotation. There are only a few studies targeting carry-over effects of N fertilization across stand rotation periods. In accordance with our results Högbom *et al.* [40,41] found that sites fertilized during the preceding stand rotation had an increased amount of inorganic soil N as well as increased soil N mineralization rates in comparison to unfertilized sites. Worth noting is that this study used N doses largely exceeding the doses used in our study (up to 1800 $kg \cdot N \cdot ha^{-1}$). Generally much higher amounts of N fertilizer than used in operational forestry practice, appears to be a common feature of most previous studies that have addressed carry-over effects between stand rotations, e.g., Smolander, Priha, Paavolainen, Steer and Malkonen [14], Pettersson and Högbom [39] and Olsson and Kellner [42]. Hence, an important contribution from our study is that N doses used in operational forestry practice can also result in a significant positive carry-over effect on tree growth. Moreover, the only other study we are aware of that targets carry-over effects across stand rotation periods from forest fertilization conducted according to the standard practice, report results that contrast ours, *i.e.*, no positive effect on *P. sylvestris* seedlings growth during the initial five year period following plantation [17]. Although there might be several potential explanations to the contrasting results, our study indicates that it may take more than five years to distinguish a consistent effect of previous fertilization on tree height growth. In accordance with our results, a North American study showed that *Pseudotsuga menziesii* height growth in the first couple of years after seedling establishment was unaffected by fertilization of the preceding forest stand, but that the positive response from past fertilization gradually increased over the years [18]. In their study, a significant difference with approximately 15% greater mean height in fertilized stands than in unfertilized stands became evident when the trees were seven to nine years old. In light of current and previous studies it could be argued that growth enhancement is cumulative, *i.e.*, the growth that the fertilization treatments trigger is built up gradually over time, to eventually entail significant differences in tree height between treatments.

Our study system is situated in a forest land area managed according to common operational practices. Since we lack information on initial site productivity conditions and it is not possible to find detailed information on the criteria used to select sites to be fertilized, we cannot exclude the possibility that initial differences may have influenced our final results, *i.e.*, that N2 sites were more fertile than C sites also before fertilization. However, all available information suggests that the sites selected for fertilization were not biased towards more productive sites. For example, the similar site productivity indices, expressed as estimated average tree height at tree age 100 years (20.6 on C sites and 20.4 on N2 sites) [43,44], and the overall similarity in site characteristics between the sites (see Table 1) supports the information provided by the forest companies employees that the sites selected for fertilization in 1977 and 1985 were, from a productivity point of view, randomly distributed over the forest land area. In addition, if results as those obtained in our study, with N1 sites consistently showing intermediate responses compared to the unfertilized controls and N2 sites (Table 3), were to be generated by pre-fertilization differences in productivity, sites selected for being fertilized would always have had to been more productive than unfertilized sites, and N2 sites would always have had to been more productive than N1 sites. Besides that it appears unlikely that sites by coincidence ended up being selected in such a way, we cannot see any logic reasons to why sites intentionally should be selected in such a biased way. Thus, we are confident that our results indicating residual effects of past fertilization are valid and not generated by initial differences in site productivity.

## 5. Conclusions

Our study highlighted that forest fertilization according to the standard practice may cause residual long-term effects on tree growth, needle N concentration, soil mineralization rates and amounts of mobile soil N in the stand rotation following the one fertilized. The findings presented herein emphasize that the effects from operational N fertilization on tree growth and biogeochemical ecosystem features can be of a more long-lasting character than previously thought. Whether this increase in site productivity will persist or in time level off is unclear, and our findings advocate that long-term effects from forest fertilization need to be studied further. Further research is also needed to elucidate the mechanisms on how fertilizer N is immobilized following fertilization, and how it is subsequently released following the disturbance caused by tree harvest and soil scarification to thereafter enhance growth of the young forest stand.

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## Author Contributions

Annika Nordin and Joachim Strengbom conceived and designed the experiment. Fredrik From carried out the field work, data analysis and prepared the manuscript. Annika Nordin and Joachim Strengbom contributed to the data analysis and prepared the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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