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RESEARCH ARTICLE

Boron fertilization in a boreal Norway spruce forest: long-lasting effects on growth and nutrition

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Abstract
Background and aims Boron (B) deficiency is widespread in boreal forests, but it can be prevented by fertilization. As B deficiency reduces root growth, it may affect the uptake of other nutrients. We assessed the persistence of the effects of a one-time B application on growth and nutrition of Norway spruce (*Picea abies*).

Methods A single-tree B-fertilization experiment was established in a highly productive stand in eastern Finland in 2000 and followed until 2018. The applied B dose was 2 kg ha^{-1}.

Results After 19 growing seasons, height growth was higher in B-fertilized trees and the effect was not waning. Diameter growth was not affected. The mean needle-B concentration without B fertilizer was 1.7 mg kg^{-1} and with B-fertilizer, 4.8 mg kg^{-1}. Boron-fertilized trees had higher foliar aluminium (Al), carbon (C), copper (Cu) and sulfur (S) concentrations, and lower soluble silicon (Si).

Conclusions The positive B effect on growth persisted after 19 years. Boron concentrations remained higher in fertilized trees, although lower than at the

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early stages of the experiment. Boron fertilization maintained Cu and S levels above deficiency limits. The small but consistent change in C indicates a change in needle compounds. Increased Al may result from reduced Si accumulation, as Al co-deposition with Si alleviates Al toxicity. Further studies are required to optimize B-fertilization practices and to elucidate the mechanisms behind the effects on height growth and the levels of other elements. A combination of soluble and sparingly soluble fertilizers could further increase the duration of the effect.

Keywords Boron · Copper · Mineral nutrient · Picea · Silicon · Sulphur

Introduction

Boron (B) deficiency is the most common micronutrient disorder in forests worldwide (Stone 1990). Globally, forests tend to grow on relatively acid, coarse-textured soils with downward water movement. These factors result in leaching of B from the rooting zone, as plant-available B is very soluble. In boreal soils, accumulation of organic matter with high B retention capacity reduces leaching on one hand but it reduces B availability to plants on the other hand (Lehto 1995). Drought during the growing season can result in temporary B deficiency even in humid boreal conditions (White and Krause 2001; Sutinen et al. 2006). This explains at least partly the earlier observations that B-deficiency symptoms are found in some years, and in other years the trees may recover (Saarsalmi and Tamminen 2005).

Boron deficiency occurs typically in fertile sites, characterized by optimal or near-optimal nitrogen (N) availability and thereby vigorous growth (Lehto et al. 2010a, b). Norway spruce (*Picea abies* (L.) Karst.) is often the dominating tree species in these sites. In less fertile boreal mineral-soil forest sites, N availability is the most common growth-limiting factor (Saarsalmi and Mälkönen 2001), and therefore B deficiency may remain latent. Nitrogen fertilization or liming can trigger B deficiency (Aronsson 1983; Mälkönen 1990; Lehto and Mälkönen 1994), but deficiency can occur on otherwise fertile sites also without management-related reasons (Sutinen et al. 2006; Tamminen et al. 2012). The mechanism in the case of N fertilization is probably a dilution effect induced by the increased growth. Trees with higher N supply retain more needle year classes, and B is sequestered in needles and increasing trunk growth (Tamminen et al. 2012). Moreover, anthropogenic N deposition diminishes the N limitation in forests, resulting in other nutrient shortages (Jonard et al. 2015). Liming reduces B availability because B is adsorbed on soil particles in poorly available forms at higher pH (Lehto 1995).

The function of B in plants is in the formation and stability of the primary cell wall, and therefore B is essential for the structural development of plant tissues (O´Neill et al. 2004). There is increasing evidence also of other primary functions related to the membrane structure and function or the interplay between membranes, cytoskeleton and cell walls (Wimmer et al. 2020). Many secondary effects of B deficiency follow from the inadequate development of cell walls. In severe cases, apical dominance is disrupted, and side branches compete with each other. In extreme cases, tree height growth can be completely arrested (Lehto et al. 2010a, b).

Boron deficiency symptoms occur first in the meristematic tissues of roots (Dell and Huang 1997). Sufficient B supply is known to enhance root growth and mycorrhiza formation in many species, including Norway spruce seedlings (Möttönen et al. 2001; Räisänen et al. 2007) and mature trees (Lehto 1994; Räisänen 2009). While N or phosphorus (P) fertilization often reduces root growth, B fertilization of deficient plants leads to increased root extension, root tip formation and biomass growth. As roots are affected before aboveground symptoms are visible, this may explain why vigorously growing, healthy-looking trees can suddenly suffer from shoot dieback. The uptake of other nutrients can be expected to increase by B fertilization through the increased root and mycorrhiza exploration of the soil or improved tissue structures. On the other hand, increased growth resulting from fertilization could lead to a dilution effect of other nutrients with low availability.

There are few previous studies reporting nutrient analyses after B fertilization. Previously, B fertilization has increased spruce needle magnesium (Mg) concentrations both in seedlings in controlled environment (Möttönen et al. 2005) and in larger trees in managed forests (Braekke 1983; Lehto and Mälkönen 1994). In the Hammaslahti experiment of the current study, needle copper (Cu) concentrations were very low in the non-fertilized controls, 2.1 mg kg^{-1} in 2012 (13 growing seasons after fertilization), and increased slightly by B fertilization (Kilpeläinen et al. 2013). Both Mg and Cu deficiencies have been suggested in Finland, and their improved supply would be an additional benefit from B fertilization.

Boron deficiency has been shown to reduce tree height growth while diameter growth is not affected (Saarsalmi and Tamminen 2005). The effect on height growth is partly due to the loss of apical dominance in severe B deficiency, but it is not the only reason (Kilpeläinen et al. 2013). Increased height growth, rather than diameter or aboveground dry mass, has been shown also in B-fertilized young Norway spruce seedlings that did not have growth disorders (Möttönen et al. 2001; Räisänen et al. 2007). A single B fertilization affected needle B concentrations and height growth of Norway spruce still 13 years after application (Kilpeläinen et al. 2013). Elevated needle B concentrations were found 20 years after B fertilization of Scots pine in a drained peatland experiment (Rummukainen et al. 2013), and therefore also long-lasting growth effects can be expected. Growth measurements in this time frame are still lacking in Scots pine, Norway spruce as well as other tree species.

We hypothesized that the effect of a single B application in a B-deficient Norway spruce forest leads to an increased height growth rate still 19 years after fertilization; and that other nutrient concentrations remain higher in B-fertilized trees. Additionally, we expected that N fertilization alone and in combination with B fertilization would further exacerbate the B deficiency symptoms. We report the growth results of a single B fertilization and the effects of B on other elements in a high-yielding boreal Norway spruce stand. The experiment was factorial, with B and N fertilization in all combinations in a single-tree setup. Earlier results of the experiment have been published by Räisänen et al. (2006a, b; 2009) and Kilpeläinen et al. (2013).

Materials and methods

The study area and treatments

The experimental site was located in Hammaslahti, Joensuu, Finland (62° 24.9' N, 29° 54.9' E), with an area of about 1.5 ha. The topography was undulating, causing variability between microsites. The site type was herb-rich forest (Cajander 1949), which is the most productive site type in the region. The soil was a cambic podzol (FAO Soils Portal n.d.) developed on glacial till. The Norway spruce (*Picea abies*) stand was planted in 1979 with two-year-old seedlings, and thus the trees in the upper canopy layers were 41 years old after the growing season 2018. However, the seedling survival was poor because of competition of surface vegetation, and complementary seedlings were planted two times afterwards. Therefore, the stand was uneven-aged. The overstorey shelter trees and part of the understorey trees were felled in the first thinning in 1991. At the beginning of the study in 2000 there were smaller broadleaved trees in the stand, mostly silver birch (*Betula pendula*), grey alder (*Alnus incana*) and rowan (*Sorbus aucuparia*). In the second thinning in early 2012 the alders, rowans and a part of the birches and spruces were felled. Needle analyses from five spruce trees showed B concentrations of 2–6 mg kg^{-1} before application of B and N, and leader dieback was observed in the stand.

For the fertilization experiment, 148 trees of 3–6 m height were selected in 2000. The minimum distance between two experimental trees was 8 m, or 7 m if there was another tree in between. The selected trees were grouped into 18 blocks of eight trees and a block with four trees (Räisänen et al. 2006b; 2009). The treatments were factorial combinations of B and N, and the treatment combinations were 0 (control), B, N and N+B, denoted NB. The fertilizers were applied manually to circular plots of a 2.5 m radius around individual Norway spruce trees. Nitrogen was applied as urea at a rate of 180 kg ha^{-1}, and B was applied as borax at a rate of 2 kg ha^{-1}. Fertilization took place 6th–7th June 2000.

After the thinning in early 2012, there were 81 experimental trees: 19, 21, 17 and 24 trees in control (0), B, N and BN treatments, respectively.

Needle sampling and element analysis

Shoots of each tree were collected from the upper third of the live canopy, from the southern side of the sample trees using garden scissors with a telescopic handle in early April 2019, when there was still snow on the ground. Current-year needles (denoted italic *C* to distinguish from C for carbon) were collected from all trees, and additionally, two or three older needle year classes (denoted $C+1$ etc.) from 52 trees. Needles were dried to constant mass at 40 °C and ground to powder with a ball mill (Planetary Monomill "Pulverisette 6", Fritsch GmbH, Idar-Oberstein, Germany) separately for each tree and needle age class.

Nitrogen and carbon in the needle samples were analyzed with an element analyzer (Varian VarioMAX Cube). Other elements were analyzed by ICP-OES (iCAP7200, Thermo Elemental, Franklin, MA, USA) after MARS5 microwave wet digestion in nitric acid and H_2O_2 in Teflon containers (method based on Epa 3051).

Control samples prepared from standard needle samples were run along the sample series in the element analyses (the laboratory's own spruce needle standard, verified internally against the Peach leaves standard of the NIST, USA). All concentrations are expressed per needle dry matter.

Tree measurements

Trees were measured after the growing seasons of the years 1999, 2002–2003, 2005–2007, 2012 (Kilpeläinen et al. 2013) and 2018. Tree heights and the heights of two previous years were measured with a digital Haglöf Vertex III hypsometer (Haglöf, Långsele, Sweden) and diameters at 1.3 m height (dbh) with mechanical Haglöf callipers. Trunk volume was calculated using the diameter- and height-based equation of Laasasenaho (1982).

The condition of the treetops was assessed visually simultaneously with tree height measurements. Trees were allocated into three growth disorder classes: 1) healthy top with a single leader; 2) moderate growth disturbance with loss of apical dominance and several competing leaders in the current year's growth; 3) severe, repeated dieback and obvious wilting of the leader (Saarsalmi and Tamminen 2005). The growth disorder index was calculated as the cumulative count of growth disorders for a certain tree in a certain year (Kilpeläinen et al. 2013).

Statistical analyses

Analysis of variance (ANOVA) using the factorial design B×N on the initial tree dimensions (height, diameter, and volume) showed no difference between the treatments in 1999, before the fertilization for all experimental trees ($P \geq 0.291$ for treatments and their interaction), and for those trees that would remain after thinning in 2012 ($P \geq 0.499$). The silvicultural thinning was carried out independently of the experimental design. The 19 blocks of the original design were not used in the current statistical analysis because after the thinning not all blocks contained trees of all treatments. The large number of blocks and the small number of trees within each block together with a design that accounts for the repeated measurements in each tree can be expected to mitigate bias on the between-trees variance estimates.

Only data for trees alive in 2012 were used for assessing differences in the growth rate in height, diameter, and volume. The same mixed effects model was fitted separately to each of the three response variables, with the volume transformed into the square root.

The linear mixed effects model fitted to the growth data was:

$$\begin{aligned} y_{ijkl} = {} & \beta_0 + \beta_1 D0_k + \beta_2 CGD_{kj} + \beta_3 t_{jk} + \beta_4 t_k^2 + \beta_5 N_i \\ & + \beta_6 \times B_j + \beta_7 N_i \times B_j + \beta_8 N_i \times t_k + \beta_9 B_j \times t_k \\ & + \beta_{10} N_i \times B_j \times t_k^2 + v_{l,0} + v_{l,1} t_k + e_{ijkl} \end{aligned}$$

where y_{ijkl} is the value of the growth response variable for tree l at time k (the number of years since fertilization), with i and j giving the N and B factor levels assigned to it (N0 or N1 and B0 or B1); β are coefficients for fixed effects, v for the random effects and e_{ijkl} are the residuals. β_0 is the intercept, β_1 is the coefficient for the covariate $D0_k$ the stem diameter at $t_k = 0$, β_2 is for covariate CGD_{kj} the cumulated growth disorder index, β_3 and β_4 are the coefficients describing the growth in time as a second-degree polynomial, β_5, β_6 and β_7 are the coefficients describing the effects of the N and B fertilization and their interaction, and β_8 β_9 and β_{10} are the coefficient describing the interaction between time and fertilization (the difference in growth rate with fertilization); $v_{l,0}$, $v_{l,1}$ are tree-level, zero-mean normal random effect for the intercept and linear slope. Nitrogen fertilization and its interactions with B and time were included in the fitted models, but afterwards the levels of N were pooled for plotting, as the interaction N×time was not significant.

The nutrient data of current-year (C) needles were subjected to ANOVA according to the factorial design of the experiment.

$$y_{ijk} = \beta_0 + \beta_1 B_i + \beta_2 N_j + \beta_3 B_i \times N_j + e_{ijk}$$

where y_{ijk} is the nutrient concentration in tree k, B fertilization level i and N fertilization level j. β_i are the model coefficients, and e_{ijk} are the residuals. In the case of some nutrients, weights from a power of variance function of the fitted values were used to describe variance heterogeneity.

When analyzing the different age classes, the linear mixed effects model fitted to the nutrient concentration data was:

$$y_{ijk} = \beta_0 + \beta_1 B_i + \beta_2 a_j + \beta_3 B_i \times a_j + v_k + e_{ijk}$$

where y_{ijk} is the nutrient concentration in tree k and needle age class j, β_i are the model coefficients, B_i are the levels of the B fertilization treatment, a_j are the needle age classes, v_k are tree-level, zero-mean normal random effect for the intercept, e_{ijk} are the residuals.

The data analyses and plotting were done in R 4.3.2 (R Core Team 2023). Data were read with package readxl (Wickham and Bryan 2023) and organized and summarized with package dplyr (Wickham et al. 2023). Mixed models were fitted with packages nlme (Pinheiro and Bates 2000), lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017); plotting was done with packaged ggplot2 (Wickham 2016), ggpmisc (Aphalo 2023) and patchwork (Pedersen 2024).

Results

Tree growth, growth disorders and branches

After 19 years, a single B fertilization still increased the height growth rate (assessed as treatment × time interaction) of the trees clearly and significantly ($P_{B\times T}<0.001$), and there were no signs of waning of the effect (Fig. 1, Table 1). By contrast, diameter and diameter growth rate were not affected. Volume growth rate was slightly larger in B-fertilized trees but not significantly ($P_{B\times T}=0.19$). The statistical model gave nearly the same result, whether the growth disorder index was included or not. Nitrogen fertilization did not affect the growth rates during the study period, and the interactions with N were not significant (Table 1). Therefore, N results are not shown separately.

At the final measurements after the growing season 2018, the proportion of trees with visible apical disorder (class 2) was 27.0% and severe disorder percentage (class 3) was 5.4% in the non-B fertilized trees (treatments 0 and N). In the B-fertilized trees (B and BN) the visible disorder percentage was 18.6%, and there were no severely damaged trees.

Boron and other elements in current-year needles

The effect of N fertilization on nutrient concentrations in current-year (C) needles was significant only for potassium (K), and the B × N interaction was not significant for any element (Table 2). The K concentration was higher in N-fertilized trees, being 4.5 g kg^{-1} and near 4 g kg^{-1} in non-N fertilized ($P=0.010$).

The B effect was significant for Al, B, C, Cu, S and Si in the current needles. Boron fertilization increased Al concentration ($P=0.020$). The B treatment effect on B concentrations was highly significant ($P<0.001$). The C concentration was 2% higher in B-fertilized trees ($P<0.001$). Copper concentrations were 1.6 in non-B fertilized, and over

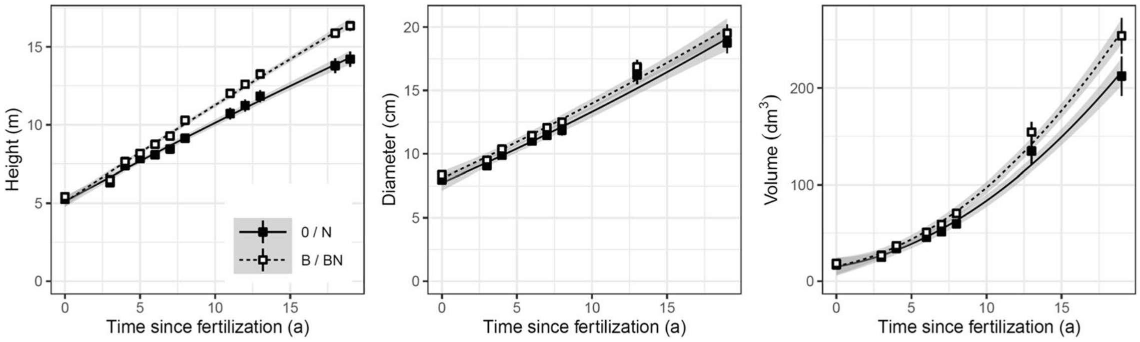

Fig. 1 Height, diameter, and volume growth of experimental trees. Nitrogen-fertilized and non-N fertilized combined, as the N effect was not significant. Means ± standard errors, 2nd degree polynomial fit showing 95% confidence bands. Until 10 a, $n=72$ (0 and N combined, B and BN combined), after 10 a, $n=36$ not B fertilized, 44 B fertilized

Table 1 Statistical significance test results from linear mixed effects (LME) model fit to tree height, diameter at 1.3 m (DBH) and volume (Fig. 1)

Source of variation	DenDF, height	*P*, height	DenDF, DBH	*P*, DBH	DenDF, volume	*P*, volume
DBH 2000	76	**$<2.2\ 10^{-16}$**	76	**$<2.2\ 10^{-16}$**	77	**$<2.2\ 10^{-16}$**
Growth disorder index	911	**$8.7\ 10^{-15}$**	526	0.894	229	**$2.7\ 10^{-4}$**
Time	225	**$<2.2\ 10^{-16}$**	178	**$<2.2\ 10^{-16}$**	117	**$5.1\ 10^{-12}$**
Time^2	830	**$<2.2\ 10^{-16}$**	524	**$1.28\ 10^{-9}$**	528	**$<2.2\ 10^{-16}$**
N	75	**0.04**	75	0.673	65	0.551
B	75	0.365	76	0.708	65	**0.119**
B × N	75	0.436	75	0.996	65	0.479
N × Time	77	0.809	77	0.671	76	0.892
B × Time	77	**$2.3\ 10^{-5}$**	77	0.433	76	0.195
B × N × Time	77	0.634	77	0.278	76	0.590

Factors: DBH 2000 is tree diameter before fertilization (spring 2000) as a covariate, Time is years since fertilization, Time^2 is the square of Time as used in the model, N, B are the fertilization treatments and B × N their interaction, growth disorder index, interactions of the fertilization treatments with Time. Significant *P* values in bold, between 0.05 and 0.10 in italics

Table 2 Element concentrations (means ± standard errors) in Norway spruce needles per unit dry mass after the growing season 2018

Element	0 ($n=19$)	B ($n=20$)	N ($n=17$)	BN ($n=23$)
Al (mg kg^{-1})	**63.3 ± 5.46b**	**72.2 ± 6.31a**	**65.0 ± 5.7b**	**83.7 ± 5.92a**
B (mg kg^{-1})	**1.73 ± 0.142b**	**4.93 ± 0.510a**	**1.73 ± 0.156b**	**4.73 ± 0.453a**
C (g kg^{-1})	**491 ± 1.1b**	**500 ± 11a**	**492 ± 1.0b**	**500 ± 0.6a**
Ca (g kg^{-1})	3.91 ± 0.252	4.15 ± 0.220	4.30 ± 0.258	4.07 ± 0.266
Cu (mg kg^{-1})	**1.61 ± 0.061b**	**2.49 ± 0.384a**	**1.58 ± 0.048b**	**2.09 ± 0.199a**
Fe (mg kg^{-1})	29.6 ± 1.32	29.5 ± 1.16	27.0 ± 1.05	29.9 ± 1.01
K (g kg^{-1})	**4.33 ± 0.131a**	**4.73 ± 0.296a**	**3.91 ± 0.156b**	**4.17 ± 0.133b**
Mg (g kg^{-1})	1.19 ± 0.075	1.19 ± 0.043	1.17 ± 0.064	1.20 ± 0.050
Mo (µg kg^{-1})	15.3 ± 1.49	19.3 ± 1.76	21.4 ± 3.11	21.5 ± 3.84
Mn (g kg^{-1})	0.617 ± 0.047	0.545 ± 0.042	0.678 ± 0.058	0.613 ± 0.045
N (g kg^{-1})	11.4 ± 0.233	11.9 ± 0.232	11.9 ± 0.250	11.7 ± 0.211
Ni (mg kg^{-1})	4.02 ± 0.352	4.52 ± 0.427	4.25 ± 0.461	5.69 ± 1.927
P (g kg^{-1})	1.37 ± 0.066	1.33 ± 0.037	1.25 ± 0.064	1.25 ± 0.048
S (g kg^{-1})	**0.729 ± 0.020b**	**0.874 ± 0.014a**	**0.737 ± 0.018b**	**0.871 ± 0.014a**
Si (mg kg^{-1})	**309 ± 26.9a**	**206 ± 24.5b**	**303 ± 33.9a**	**205 ± 21.7b**
Zn (mg kg^{-1})	27.7 ± 2.13	28.0 ± 3.39	30.7 ± 2.23	27.5 ± 1.76

Treatments: B, boron fertilization, N, nitrogen fertilization in 2000. $P<0.001$ for the main effect of B in B, C, S and Si; $P=0.020$ for Al; $P=0.002$ for Cu. $P=0.010$ for the main effect of N in K. Interaction not significant for any element ($P<0.05$). Means followed by the same letter or without a letter are not significantly different from each other ($P<0.05$)

2 mg kg^{-1} in B fertilized trees ($P=0.002$). Sulfur concentrations were 0.73 g kg^{-1} in non-B fertilized and 0.87 g kg^{-1} in the B-fertilized trees ($P<0.001$). Silicon concentrations were clearly lower in B- fertilized trees ($P=0.001$).

Other nutrients, Ca, Fe, Mg, Mn, Mo, Ni, P and Zn, were not affected by the fertilization treatments in the *C* needles (Table 2).

Variation among needle year classes

When the four sampled needle year classes were taken together, the year class factor was significant and there were higher concentrations of C, N, Mg, P, Zn and K in the current needles and higher concentrations of B, Ca, Mn, and Si in older needles than *C* (Fig. 2a, b).

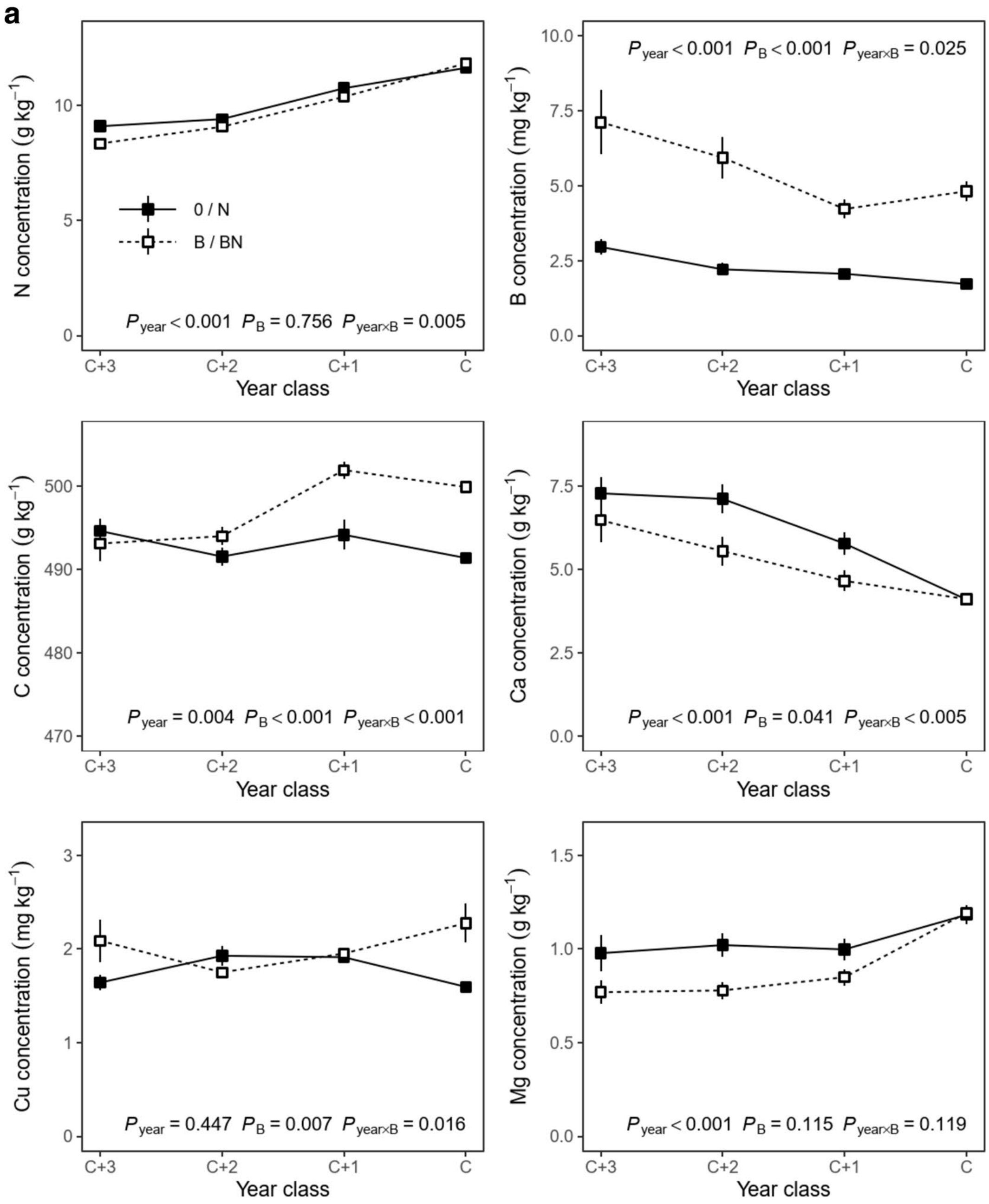

Fig. 2 **a** Nitrogen, B, C, Ca, Cu and Mg concentrations in four needle age classes, *C* represents growing season 2018. Nitrogen- fertilized and non-N fertilized combined, when N not significant, and B-fertilized and non-fertilized combined when B not significant. For *C* needles n = 19 trees in control, 20 in B-fertilized, 17 in N-fertilized, and 23 in N- and B-fertilized trees, in *C* + 1 and *C* + 2 respectively 15,12, 11 and 14, and in *C* + 3, 10, 9, 5 and 5. *P*-values from linear mixed effect model fit shown for main effects of year class (year), B fertilization (B) and their interaction (year × B). **b** Manganese, P, S, Si, Zn and K concentrations in four needle age classes, *C* represents growing season 2018. For details, see Fig. 2a

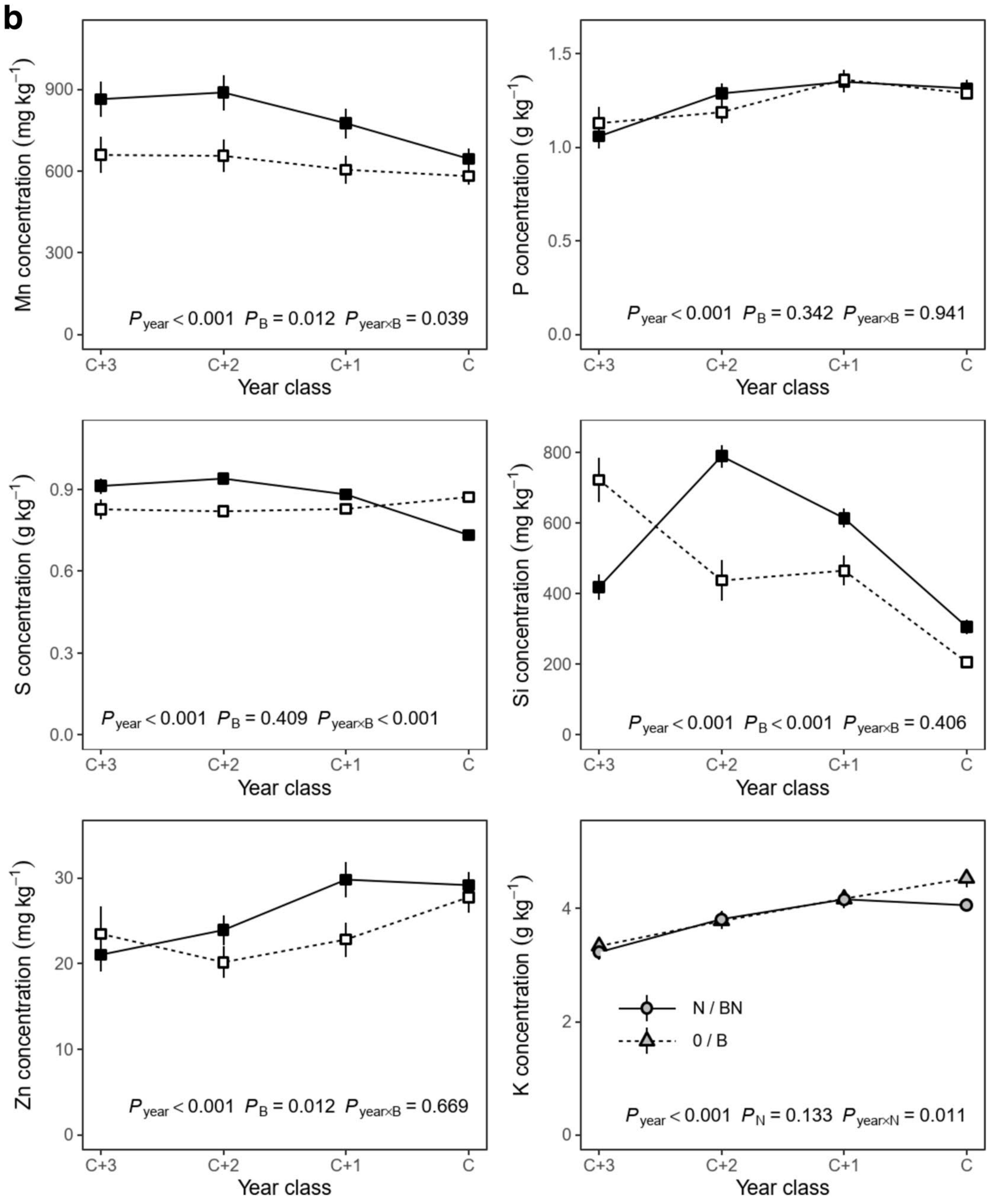

Fig. 2 (continued)

The Si and Zn concentrations were lower in B-fertilized than non-fertilized trees. The main effect of B was significant, but the interaction with time was not, although Zn concentrations were lower only in older needles than *C*.

The interaction between B fertilization and age (needle year class) was significant in the cases of C, N, B, Ca, Cu, Mn, S and Si. The B concentration was elevated the most in the old needles of the B treatment. The C concentration was higher in B treatment in the *C* needles but not the old ones, and the Cu concentrations showed a similar pattern. Additionally, the S concentrations were lower in the older needles in the B-fertilized trees, but higher in the current ones. The Ca, Mg and Mn concentrations were lower in the older needles in the treatments with B (not significant for Mg), but in the current needles the concentrations were similar. In the case of N, the difference in the old needles was marginal. Of the measured elements, only K and P did not respond to B fertilization (Fig. 2b).

Trends in nutrient concentrations during the 19-year experiment

In Fig. 3, the present data on N, B and Cu are combined with earlier data. Earlier results of B, N and Cu after the first growing season, year 2000 are from Räisänen et al. (2006b). Boron and N after 2002 are from Räisänen et al. (2009), and nutrients after growing seasons 2002 and 2012 from Kilpeläinen et al. (2013). Statistical analysis between the different times would not have been reliable, as the sampled trees were different in 2002.

Boron fertilization increased the B concentration in *C* needles from 5 to nearly 60 mg kg^{-1} in the B treatment and 40 mg kg^{-1} in the NB treatment after the first growing season. This was the only time when there was a B×N interaction on B (Fig. 3). The foliar B concentrations decreased rapidly in time but remained higher in the B fertilized treatments until the last sampling. However, the distribution of B concentration overlapped somewhat at the two latest samplings. The range in non-B fertilized was 0.8–3.2 mg kg^{-1}, and in B fertilized 1.4–11.2 mg kg^{-1} in 2018, and in 2012, the ranges were 1.9–6.0 in non-B fertilized and 3.0–16.3 in B fertilized.

After the first growing season from the fertilization, there was an increase in the foliar N from 16.6 to 19.0 g kg^{-1} in response to N fertilization, which did not persist afterwards (Fig. 3). The N concentration decreased steadily with time, to less than 12 g kg^{-1} at the last sampling.

The decrease in Cu concentrations with time was strong. Initially, there was no B effect on Cu, but in 2012, there was a B×N interaction, as the Cu concentration was the same in 0 and BN but higher in B- and N-fertilized treatments (Fig. 3, Kilpeläinen et al. 2013). In 2018, Cu was higher in both B-fertilized treatments than in non-fertilized (Fig. 3).

Discussion

Tree growth and growth disorders in relation to B

The persistence of the effect of a single B application on boreal mineral-soil forests has not been assessed previously. We found a clear and positive effect of B fertilization on Norway spruce height growth still 19 growing seasons after the fertilization. The diameter was not affected, similarly as observed in previous shorter-term experiments (Saarsalmi and Tamminen

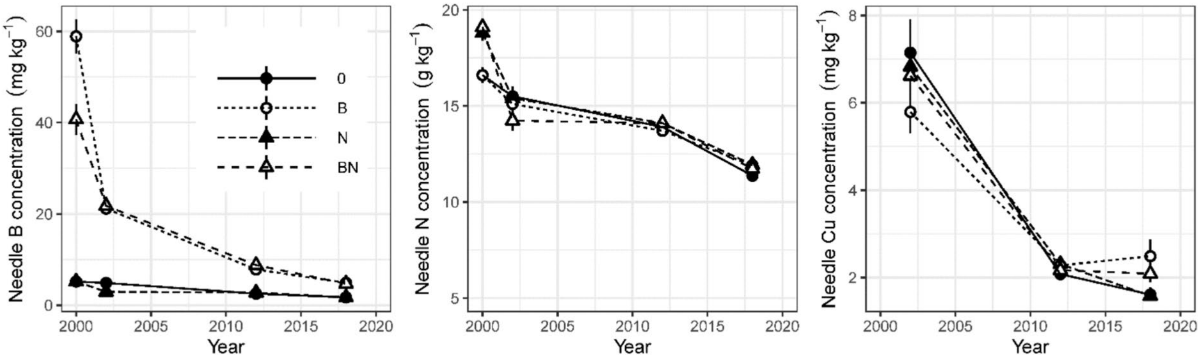

Fig. 3 Boron, N and Cu concentrations in current needles of Norway spruce trees after fertilization in 2000. For B, November 2000, B main effect $P<0.001$, N main effect 0.087, B×N interaction $P=0.003$. For N, November 2000, N main effect was significant ($P<0.001$). For Cu, in 2002, no significant difference. In 2012, the Cu concentration was significantly higher in B and N than in control, and intermediate in NB (B×N interaction $P=0.006$), in 2018 B main effect was significant. Data for 2000 and 2002 are from Räisänen et al. (2006b and 2009), and 2012 from Kilpeläinen et al. (2013)

2005). Therefore, the B effect on volume was not significant, while the B effect on the height growth rate did not show any signs of waning. There are two possible mechanisms for a persistent growth effect: 1) continued improvement in B nutrition and possibly other nutrients; and 2) a carry-over effect of an earlier difference in the size of the trees due to fertilization. In the first case, higher B concentrations and milder disorder symptoms would be expected, while in the second case dilution effects could result in the opposite. As there was still a significant difference in the mean B concentrations of current needles and no severe growth disorders in B-fertilized trees, the difference in growth rate is mainly explained by persisting improved B nutrition.

The increase in height growth is partly caused by avoidance of growth disorders, but not completely. An increase in height growth following B fertilization has been shown also in Norway spruce seedlings before there was any visible apical disorder (Möttönen et al. 2001; Räisänen et al. 2007), as well as in mature trees on a shorter term (Saarsalmi and Tamminen 2005) and in lodgepole pine (*Pinus contorta*; Brockley 2002). Visual damage in Norway spruce is not always shown even at needle B concentrations below 4–5 mg kg^{-1}. Further complicating factors are the variation between individuals (see below), and temporal variability within the same tree, caused by variable water availability (Lehto et al. 2010a, b). Consequently, as a clear-cut criterion for B deficiency is lacking, there is also no general agreement on critical B deficiency limits.

Here, the occurrence of growth disorders was still much lower in B-fertilized trees than non-fertilized, and there were none in the most severe type. However, B-fertilized trees were not completely free of apical disorders, as found before (Saarsalmi and Tamminen 2005; Kilpeläinen et al. 2013). Therefore, the growth disorder alone is not an adequate proof of B deficiency, as insect and other damages in buds may cause loss of apical dominance as well (Carter and Brockley 1990). As the disorder commences by deformation of the apical bud, it takes months before the damage is visible as competing branches, and at that time it is practically impossible to identify if the initial cause was insect or fungus or B deficiency. Nevertheless, very severe, repeated growth disorder may be more likely to be caused by B deficiency than by other causes, as this type did not occur in the B-fertilized trees. Ungulate browsing may cause equally severe branching in seedlings, but Norway spruce is generally not eaten by them. Foliar analyses are recommendable for the detection of B deficiency, particularly at an early stage before significant damage and loss of height growth takes place.

Boron concentrations of current needles were still substantially higher in the B-fertilized trees although they had decreased considerably since the first growing season after fertilization. Now the mean B concentration in the B treatment was 4.9 mg kg^{-1}, which is about the same as the mean B concentration in the unfertilized trees at the beginning of the experiment (Räisänen et al. 2006b). However, in a variable tree population, means are not always good indicators, as the range of values in fertilized trees was 1.4–11.2 mg kg^{-1}, and in the non-B fertilized trees 0.8–3.2 mg kg^{-1}. Therefore, a few of the B-fertilized trees were already in the deficient regime, and this was not a case of a single outlier. Given the large variability, the growth effect in the whole tree population may not be much more long-lasting than the currently measured 19 years after B fertilization, although in many trees it will most likely persist for years.

The variability in B concentrations among trees may have been related to the variable size of the trees; however, the height, diameter, or relative height growth rates between 2012–2018 did not correlate with the current-year B concentrations (data not shown). Large genetic variability in B efficiency has been shown in several species, including conifers (Turner et al. 2021). Boron-efficient genotypes grow and reproduce well on soils where other genotypes experience B deficiency (Rerkasem and Jamjod 1997). The mechanism may be efficient uptake from soil, translocation, internal distribution or efficiency in use. Differences in uptake and translocation would manifest as different B concentrations, and this may be the case in this study. By contrast, physiological differences do not necessarily show in B concentrations. There was a remarkably higher cytokinin and lower abscisic acid level in a B-efficient *Brassica napus* cultivar compared to a non-efficient one, even though B concentrations were similar, as well as the amounts of B sequestered in the cell wall structure (Pommerrenig et al. 2022). Cytokinin is associated with active function and meristem growth, and abscisic acid is a stress hormone. Boron deficiency is well known to affect especially plant reproduction

(Rerkasem and Jamjod 1997). This is likely to affect spruce as well, although this is not in the scope of the present study. It would be worth identifying by modern genetics tools, if there are within-species genetic differences in B efficiency, so they could be taken into account in tree breeding.

The B fertilizer was borax, which is very soluble and thereby has a fast effect. This is necessary, because the growth effects tend to lag behind the B uptake, even for several years (Fig. 1; Saarsalmi and Tamminen 2005). The development of the following-year buds commences already at budburst, and sufficient B is needed during the whole period until shoot growth is complete (Sutinen et al. 2007). Slow-release B fertilizers are so far not in common use in Finland, but a combination of borax and a slow-release fertilizer such as colemanite could extend the fertilization effect without compromising the early response after application.

Most previous studies have lasted a shorter term, and B has often been applied together with other nutrients. Turner et al. (2021) referred to unpublished results showing an increase by 20 mg kg^{-1} above levels of untreated trees over eight years after slow-release ulexite fertilization in Australia, and in British Columbia, lodgepole pine trees maintained elevated B levels and growth nine years after fertilization (Brockley 2002).

In Scots pine in a drained peatland experiment, the needle B concentrations were still 12 mg kg^{-1} as opposed to 5.7 mg kg^{-1} in non-fertilized trees 20 years after fertilization (Rummukainen et al. 2013). The larger effect in B concentrations compared to the present results may be caused by different soil conditions or the high growth rate of Norway spruce in the favourable site and thereby higher B demand in the present study. Although both species have been shown to retranslocate B (Lehto et al. 2004), the extent of retranslocation appears to be greater in Scots pine than in Norway spruce (Helmisaari 1992; Möttönen et al. 2003; present study Fig. 2a). Spruce trees retain more needle year classes than pine trees, which as such can lead to more immobilization. It has been assumed that the part of B that is bound in the cell wall structure cannot be mobilized, and recently Begum et al. (2023) showed it experimentally. When plant B concentrations are extremely low, practically all B is in cell walls. If the plant is still able to grow, the concentrations remain about the same over time, as here in the non-B treatment: growth was taking place, even though with deformations, at concentrations 2–3 mg kg^{-1}.

The experiment comprised an N fertilization treatment in factorial combinations with B to clarify if N would exacerbate the B deficiency in the naturally N-rich site. The B concentrations were lower in the N-treated trees after the first growing season from the fertilization, but afterwards this effect was not observed. Needle N concentrations were also elevated by the N fertilization in the first year after the treatment, but this did not have a detectable effect on growth, as the N levels were as high as 16.6 g kg^{-1} also in controls in the beginning. Yet, N fertilization may have increased the biomass growth sufficiently to cause a dilution effect of B at the early stage. In other experiments with a growth response to N, the N fertilization has decreased B concentrations (Aronsson 1983; Mälkönen 1990). In an experiment with three levels of N fertilizer in Scots pine on sandy soil, the B concentrations declined along with increasing N dose (Högberg et al. 2006). This was the clearest effect of the N supply on any nutrient other than N, and the decline in B with increasing N application persisted 20 years after B fertilization. However, all the plots were fertilized with B, so there were no controls for the B treatment. As a result, an assessment of the effect of the N supply on its own was lacking.

Boron effects on other elements

In addition to higher B concentrations, the B-fertilized trees had higher current-year foliar concentrations of Al, C, Cu and S, and lower concentrations of Si. The carbon concentrations of C and $C+1$ needles were about 10 g kg^{-1} higher in B-fertilized trees. This may be caused by defects in the development of cell wall structures in the B-deficient trees or some other difference in the chemical composition of needles. If there is a similar effect of B on the composition of wood material, it may affect wood quality and should therefore be studied. Boron deficiency has been associated with abundant resin flow in Scots pine and radiata pine (Raitio 1983; Turner 2021), increase of tannins in vacuoles in Norway spruce (Sutinen et al. 2006) and other changes in secondary compounds (Wimmer et al. 2020) which may be the reason for the altered C concentration.

Aluminium concentrations in *C* needles were higher in B-fertilized trees, although the highest concentration, 83 mg kg^{-1} was not higher than previously reported in Norway spruce (Majdi and Rosengren-Brinck 1994; Palviainen et al. 2004). The availability of Al is high in acid forest soils and forest plants need to exclude Al from cells. Co-deposition of Al with Si in roots is a known mechanism (Hodson and Evans 2020), and here, the reduced Si in the B treatments may be related to the increased Al levels. The lower soluble-Si concentrations were shown also in an earlier sampling after the growing season of 2012 (Kilpeläinen et al. 2013). We analyzed only the H_3NO_3-soluble Si concentrations, which were 0.3 g kg^{-1} in control *C*-needles and 0.2 g kg^{-1} in B-fertilized trees, and 0.8 and 0.4 g kg^{-1} in $C+2$ needles. However, this Si fraction is only part of the total, and there was probably much more Si deposited in forms that do not dissolve in HNO_3. In other studies, the $C+2$ Norway spruce needles contained 5 g kg^{-1} total-Si (Lisztes-Szabó et al. 2020). Further studies should consider the insoluble Si proportion, and examine the interactions among B, Al, and Si in detail. In more sensitive plant species, the increase in Al may be critical. Moreover, Si has also other beneficial effects in plants in addition to metal immobilization (Hodson and Evans 2020).

Sulfur concentrations were significantly higher in the B-fertilized trees, 0.87 g kg^{-1}, than in non-B fertilized, 0.73 g kg^{-1}. The S deficiency limits are not well known in most forest trees, but levels below 1.1 g kg^{-1} have been considered deficient in conifers (Coleman et al. 2014), and 0.8 g kg^{-1} is given as a deficiency limit in Norway spruce (Zethof et al. 2024). Low B concentrations together with low S concentrations occur in Douglas fir (*Pseudotsuga menziesii*) and lodgepole pine in British Columbia (Carter and Brockley 1990) and in radiata pine (*Pinus radiata*) in New Zealand (Lambert and Turner 1977). Sulfur uptake in *Brassica napus* seedlings was shown to depend on B supply, but the mechanism remained unknown (Pommerrening et al. 2019). In the 1970s to early 1990s, high levels of anthropogenic, acidic S deposition were a concern in industrialized regions. From then, the deposition has decreased, and S deficiencies are now found in forest trees in Europe (Jonard et al. 2015; Zethof et al. 2024).

Foliar Cu was also higher in the B-fertilized trees at the last samplings when the Cu level had decreased from earlier adequate levels to deficient or near-deficient levels. The critical deficiency concentration for Cu in conifers is considered to be about 2 mg kg^{-1} (Turvey and Grant 1990) but this has not been verified for Norway spruce (Reinikainen et al. 1998). Low Cu levels have not been reported in boreal mineral-soil forests, and both S and Cu nutrition of forest trees require further attention in the region. By contrast, foliar Mg was not affected by the treatments after 19 years, although three years after treatment it was increased by B fertilization (Kilpeläinen et al. 2013). The previous effects agree with the higher Mg concentrations observed in other B-fertilized spruce stands (Lehto and Mälkönen 1994), in needle litter (Lehto et al. 2010a, b) and in seedlings (Möttönen et al. 2005). Moreover, Braekke (1983) found that Mg concentrations in conifer needles were low in same peatland sites as low B and increased after B fertilization.

One possible explanation for the nutrient effects is that B, Cu, Mg and S deficiencies tend to occur in similar soil and climatic conditions, and the B fertilization effect on other nutrients is through larger and better-developed root systems with more mycorrhizas (Möttönen et al. 2001). Other possible general explanations are that B-deficient plants have defects in the structure of the vascular system and cellular functions. These mechanisms would potentially improve the uptake of all nutrients, and the effect could be clear for those nutrients that are at or near deficiency levels. However, specific relationships between B and other nutrients cannot be excluded, as discussed for S above.

Phosphorus and K were not affected by B, but Ca, Mg, Mn, N, S and Zn concentrations of older needle classes were higher in the non-B fertilized treatment than B fertilized, although the current-year levels were similar or lower. This may be due to reduced retranslocation in B-deficient trees, possibly due to malformed vascular systems and membranes. However, this did not lead to obvious differences in the other nutrients in the *C* needles, as only S concentration was highest in B-fertilized *C* needles.

Trends in nutrient concentrations during the 19-year experiment

The initial increase in N was over and the concentrations were similar to controls four growing seasons after N fertilization. However, N levels decreased with the growth of the trees, and at the last sampling, the concentrations were below 12 g kg^{-1}; therefore, N fertilization at this stage could have a positive effect on growth applied together with P (Saarsalmi and Mälkönen 2001) and B.

The concentrations of several other nutrients decreased with time despite the thinning in early 2012, which would have diminished the competition for nutrients. The mean P concentrations were about 2.0 g kg^{-1} in 2002, about 1.5 g kg^{-1} in 2012 and now 1.3 g kg^{-1} (previous results from Kilpeläinen et al. 2013). This decrease suggests that P limitation is possible in this site, as shown previously for fertile upland spruce stands in Finland (Mälkönen 1990; Saarsalmi and Mälkönen 2001). Potassium concentrations decreased as well, from 7–8 to 5–6 to about 4 g kg^{-1}. It has been assumed that K deficiencies do not occur in boreal mineral-soil forests (in contrast to peatlands), yet the most recent value is low. Calcium concentrations also decreased slightly from about 5 g kg^{-1} in 2002 to about 4 g kg^{-1}. The heavy-metal nutrients Cu, Fe, Mn and Zn also showed a larger drop in concentration between 2002 and 2012 than between 2012 and 2018. The results suggest that the nutrient issues on fertile upland soils may be more complex than has been considered, As the growing seasons are becoming longer and warmer in the boreal zone, the N limitation is expected to become less acute. Our results suggest that several nutrients may become scarce with the growth of trees, and their availability and physiological interactions need to be studied in different tree species and soil conditions.

Conclusions

The effect of a single B fertilization persisted for 19 years, but the variability in B concentration between individual trees was large. Applying a mix of a slowly dissolving fertilizer such as colemanite and fast-release borax could both induce a rapid initial response and extend the duration of a single-fertilization effect.

Both S and Cu were at or below their deficiency limits and increased by B fertilization, as far as these limits are known for Norway spruce. Boron fertilization may make a difference between deficiency and sufficiency of these nutrients. Therefore, the effects of B on other nutrients require further attention, as well as the interactions between B, Si and Al.

While global warming and continuing N deposition and fertilization increase N availability and tree growth in boreal forests, the frequency of both B and other nutrient deficiencies are increasing (Kauppi et al. 2014; Jonard et al. 2015). Here, the trends between years suggest that multiple nutrients can become scarce in the most productive boreal forest sites simply as a function of time and increasing biomass.

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Data availability The data will be made available to scientists at a reasonable request.

Declarations

Conflict of interest The authors do not have conflict of interest.

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