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Increased tree growth following long-term optimised fertiliser application indirectly alters soil properties in a boreal forest

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Abstract

It is well established that nutrient addition influences ecosystem features such as productivity, carbon storage, soil acidification and biodiversity. Less studied are long-term effects of sustained fertiliser application on forest soil characteristics and nutrient supplies, and especially direct and indirect mechanisms underlying changes. We investigated effects of three decades vs. one decade of optimised fertiliser application on soil properties and nutrient supplies in a 30-year-old nutrient optimisation experiment in a Norway spruce plantation in northern Sweden. We tested for direct and indirect effects of fertiliser use through structural equation models and correlations among tree and soil variables. Results showed that soil characteristics, especially organic carbon and nutrient concentrations, were significantly affected by 10- and 30-year fertiliser application. Soil C:N was similar for the short-term vs controls, but decreased for the long-term vs short-term treatment. Although not explicitly measured, it was clear from our analyses and earlier studies at the site that litter accumulation played a key role in explaining these changes in soil properties, while foliar stoichiometry data suggest long-term effects of litter quality. Nutrient supply rates increased more after 30 than 10 years of fertiliser application. Summarized, we showed that the interplay of direct and indirect effects can yield nonlinear patterns over time, as exemplified by soil C:N. Furthermore, we conclude that lagged, indirect effects of fertilisation through altered litter quantity and quality dominate changes in soil characteristics in this forest. These soil characteristics have further relevance to nutrient availability, suggesting nutrient optimisation can influence soil fertility also indirectly.

Keywords: fertiliser, Flakaliden, forest soil, ion exchange resin membrane, Norway spruce, plant-soil feedback

Introduction

In many terrestrial ecosystems, increased nutrient inputs occur, either because of deliberate fertiliser use, or as side effects of human activities. The most common form of unintended nutrient enrichment is atmospheric nitrogen (N) deposition, which is primarily related to loss of N from agriculture and fossil fuel combustion (Paulot et al. 2013). Intended nutrient additions are most common in agriculture *sensu stricto*, but also in forestry, fertilisers have been applied to optimise yields (e.g. Tamm et al. 1999; Nohrstedt 2001; Hedwall and Bergh 2013). Irrespective of the purpose or type of nutrient additions, modified nutrient inputs initiate a cascade of changes in biogeochemical cycles, and therefore in general ecosystem structure and functioning (Tamm et al. 1999; Gao et al. 2015; Binkley and Högberg 2016; Homeier et al. 2017).

Nutrient additions typically have strong effects on various ecosystem characteristics and processes. Common responses are, for example, reduced soil respiration (Olsson et al. 2005; Janssens et al. 2010), along with an increase in tree, soil and total carbon storage (Nohrstedt 2001; Magnani et al. 2007; Reay et al. 2008; Fernández-Martínez et al. 2014). Influences on biodiversity include shifts in species composition (Nohrstedt 2001; Novotný et al. 2016), and species richness usually declines due to competitive exclusion by species adapted to nutrient rich environments (Mulder et al. 2013). Moreover, in the case of N deposition, there is the acid deposition of N oxides (NO_x) (Keene et al. 1983; van Breemen et al. 1984; Lamarque et al. 2013) and the potential oxidation of deposited ammonium (NH_y) (Nohrstedt 2001), which both drive acidification of soils with related problems such as base cation leaching (Zeng et al. 2017), Aluminum toxicity (van Breemen et al. 1984) and disturbed microbial community structure and functioning (Chen et al. 2015).

Besides its effects on elemental cycling and the environment, fertiliser addition also influences soil characteristics. When nutrients are applied, total soil concentrations (Schlesinger 2009; Mulder et al. 2013; Sardans et al. 2016) of the elements in the fertiliser mix respond directly and positively. From a soil perspective, increased (inorganic) nutrient availability can be detected as greater “bio-available” concentrations of elements such as N, phosphorus (P) and exchangeable base cations, as derived from soil extractions. Also nutrient supply rates to plant roots increase in response to fertiliser application (Qian and Schoenau 2002; Andersen et al. 2014). The direct influence of fertiliser use on soil characteristics and tree nutrition is thus rather well understood, although the important role of micronutrients and organic molecules in tree nutrition remains an active field of research (e.g. Näsholm et al. 1998; Hedwall et al. 2018). Short-term fertiliser effects on soils have been extensively studied, and also several results from multi-year experiments in forests have been reported (e.g. Tamm et al. 1999; Goodale and Aber 2001;

Högberg et al. 2006; Smaill et al. 2008; Libiete et al. 2016; Addison et al. 2019). However, in particular our understanding of long-term changes in soil characteristics and the mechanisms underlying such changes is still limited.

Beyond the direct influence of fertiliser application on soil characteristics, indirect feedback effects involving plant related processes also occur. For instance, increased productivity may promote litter production, eventually resulting in elevated soil organic matter (SOM) and carbon concentrations (SOC) (Smaill et al. 2008). Furthermore, not only the quantity, but also quality of litter may change with fertiliser application (Berg and Matzner 1997). Plant tissues exhibit stoichiometric flexibility, i.e. elemental concentrations and ratios vary within a given genotype, depending on environmental conditions, including nutrient availability (Ingestad 1987; Magill et al. 2004; Sardans et al. 2016; 2017). Fertiliser-induced shifts in tissue stoichiometry may translate into changes in litter stoichiometry (Ukonmaanaho et al. 2008), and eventually alter the elemental composition of fresh SOM. Hence, fertiliser application can not only alter soil characteristics directly, but also indirectly through stimulation of tree productivity and shifts in tissue stoichiometry.

Direct effects of nutrient addition on key soil properties such as soil C:N ratio (Mulder et al. 2013; de Vries et al. 2014) and pH (van Breemen et al. 1984; Chen et al. 2015) are relatively well understood, but indirect effects much less so, despite their potential relevance. Greater SOM following increased litter production, for instance, can in turn cause a shift in soil pH, and also increase the cation exchange capacity (CEC - e.g. Tamm et al. 1999), because the organic matter colloids serve as cation (and anion) exchange sites (IIASA and FAO 2012), with relevance to soil nutrient retention. As an example regarding altered litter quality, N addition can reduce the litter C:N ratio, which may, together with the added inorganic N, result in a drop in soil C:N ratio. Relevance of properties such as soil C:N ratio and pH lies in that they not only respond to shifts in nutrient availability, but also further govern the soil nutrient status itself (Van Sundert et al. 2018; 2019; Vicca et al. 2018). The soil C:N ratio for example modifies the decomposition rate (Wilkinson et al. 1999; Roy et al. 2006), while soil pH governs chemical (de)sorption of P (Chapin et al. 2002; Bol et al. 2016) and loss of base cations through leaching (Högberg et al. 2006). Indirect mechanisms through which fertiliser application influences soil characteristics can thus further influence nutrient availability by initiating a cascade of shifts in key soil properties.

While direct fertiliser effects on soil characteristics and nutrient availability are widely recognised, tree-mediated indirect influence on key soil characteristics governing nutrient availability is often ignored. In the current study, we therefore investigate whether direct or indirect pathways dominate short- and long-term changes in soil properties and nutrients, and nutrient supplies in a long-term nutrient optimisation experiment in a Norway spruce (*Picea abies* (L.) H. Karst.) plantation in Flakaliden, northern Sweden. We hypothesise that with sustained addition of mineral elements such as N, P, K, Ca and Mg, soil characteristics and nutrient availability are not only affected directly, but also indirectly through mechanisms altering soil properties such as altered productivity and needle stoichiometry, eventually modifying the quantity and quality of litter input.

Materials and methods

Study site and experimental design

In summer 2016 we collected soil samples at the Flakaliden nutrient optimisation experiment in northern Sweden (64°07'N, 19°27'E, mean annual temperature = 2.5 °C and mean annual precipitation ~ 600 mm for the period 1990-2009, background N deposition = 3 kg ha^{-1} yr^{-1}, Sigurdsson et al. 2013). The experiment was initiated in a 28-year old Norway spruce (*Picea abies* (L.) H. Karst.) plantation in 1986 (Bergh et al. 1999). The forest grows on a mineral silty-sandy till soil, classified as haplic podzol sensu FAO (Olsson et al. 2005), with an organic litter-fermenting-humified (LFH) layer of 2-6 cm on top (Lim et al. 2019). The understorey and forest floor vegetation mainly consists of *Vaccinium myrtillus* L., *Deschampsia flexuosa* (L.) Trin., *Dryopteris carthusiana* (Vill.) H.P.Fuchs and mosses, with strongly reduced understorey cover (and thus more bare soil) in nutrient treated plots (Hedwall et al. 2013).

The Flakaliden experiment was designed to investigate (i) potential productivity under optimal nutrition (Linder 1995), and later also (ii) forest responses to global change, and the role of nutrients therein (e.g. Ryan 2013). For the current study, we considered the plots that received "optimal nutrition" during the period 1987-2016 (treatment "30y-IL", *n* = 4), 2007-2016 (treatment "10y-IL", *n* = 4), and untreated controls (treatment "Control", *n* = 4 - Bergh et al. 1999). Prior to 2007, 10y-IL plots were irrigated (former treatment "I"). Since no significant influence of irrigation was observed on leaching or forest growth (Bergh et al. 1999; 2005; Jarvis and Linder 2007), the 10y-IL plots can be considered as former controls. Optimal nutrition consisted of a nutrient mix (with N in ammonium nitrate (NH_4NO_3), plus phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), manganese (Mn), iron (Fe), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo) – Table S1) that was supplied with irrigation water directly on the ground every two days in every growing season (including the year of sampling), except in 2002 and 2003 (for 30y-IL). The amount of each element supplied was re-evaluated annually, based on convergence of needle nutrient concentrations and stoichiometry to set target values, avoidance of leaching (thus maximising nutrient use efficiency) and expected productivity responses (Bergh et al. 1999). More details about the experiment are provided in Linder (1995).

Field sampling

Since litterfall was not monitored at the site during the period considered in this study, and no accurate model for estimating litterfall at treatment 10y-IL exists (since litterfall has never been measured for this treatment), proxies for aboveground biomass and productivity were compared among the nutrient addition treatments as an acceptable alternative (e.g. Berg and Meentemeyer 2001; Starr et al. 2005). To this end, we used data on tree height, basal area and volume from the start of the experiment in 1986 (Bergh et al. 1999), the year before the 10y-IL treatment was initiated (i.e. 2006) and the sampling year 2016. We also searched in literature for earlier studies reporting on litterfall at the site, to reinforce our use of proxies.

Needles were collected in October 2016 (i.e. the time of year when starch levels are low, reducing its potential influence on needle stoichiometry - Linder 1995) for foliar nutrient analyses. In each plot, one branch from each of five trees was sampled in the upper third of the crown. From each branch, three second order, one-year-old shoots were sampled and pooled. The shoots were then immersed in liquid nitrogen and stored at -20 °C until further analyses (Linder 1995).

In each of the plots, we collected two soil samples at 0–10 cm and 10–20 cm depth from the top of the LFH layer for analyses of key soil characteristics and nutrients. While one sample was used to determine soil bulk density based on soil mass and dimensions of the excavation (Blake and Hartge 1986 - because of gravel, using a standard corer was not feasible at the site), the other was sieved (mesh size = 2 mm) to exclude mosses, roots and rocks, and eventually air-dried at 30 °C. Even though separately sampling organic and mineral soil layers is a more common practice, we opted for this sampling protocol because fixed depth intervals give a better picture of the soil from a nutrient availability perspective (e.g. a thicker organic layer can be observed from results as greater CEC). Moreover, this way of sampling allows direct comparisons with ion exchange membrane supply rates.

Supply rates of inorganic ions were assessed with four plant root simulator (PRS®) probe pairs (cathode + anode - Western Ag Innovations, Saskatoon, Canada) per plot, installed for exactly seven days (26-07 until 02-08-2016). The probes collected NH_4^+, NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Mg^{2+} at a soil depth of roughly 3-9 cm. After retrieving the probes from the field and cleaning with distilled water, they were returned to the manufacturer for colorimetric analyses on the eluent using automated flow injection analysis (NH_4^+, NO_3^-) and inductively-coupled plasma spectrometry (PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}). Details on ion exchange membranes can be found in Qian and Schoenau (2002). In brief, the method consists of installing charged resin membranes in the soil for a fixed period of time, during which ions of opposite charge adsorb to the membranes. Ion exchange membranes have been shown to successfully capture treatment effects on nutrient supply rates under field conditions (e.g. Van Sundert et al. 2020).

Laboratory analyses

After drying for 48 hours at 85 °C, the needle samples were ground in a ball mill (MM200, Retsch GmbH, Haan, Germany) and analysed for C and N using an elemental analyser (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany). Other chemical elements were analysed with an inductively coupled plasma-optical emission spectrometry (ICP-OES) analyser (Spectro Ciros Vision, SPECTRO analytical Instruments GmbH, Germany).

We measured pH in water (pH_w) and KCl solution (pH_{KCl}) on the fresh soil samples before sieving. To this end, 10 ± 0.5 g of fresh soil was weighed into a plastic tube and subsequently, 25 ml H_2O or 1 M KCl was added. Then, the solutions were shaken and rested for an hour before measuring the pH with a direct soil pH meter (no. 99121 Hanna Instruments, Temse, Belgium).

After sieving and air-drying, the soil samples were analysed for various soil properties and nutrients. Firstly, total C and N concentrations were determined on ground samples, using an Elemental Analyser (Flash 2000 CN Soil Analyser, Interscience, Louvain-la-Neuve, Belgium). Brown's procedures (1943) were used for CEC and total exchangeable bases (TEB), for which 1 M NH_4Ac at pH = 7 served as the extractant. Extractable phosphorus was measured following the method of Bray (Dickman and Bray 1940; Bray and Kurtz 1945), which requires a 0.03 M NH_4F + 0.025 M HCl solution. P_{Bray} generally correlates well with other common indices of bio-available P, and is particularly recommended for acidic soil types (Renesson et al. 2016). Extracts were evaluated with an iCAP6300 Duo ICP-OES (for CEC and TEB - Thermo Fisher Scientific, Waltham, USA) or a San++ Automated Wet Chemistry Analyser (for available P - Skalar Analytical, Breda, Netherlands). Lastly, we determined the soil texture (percentages of sand, silt and clay) with the hydrometer method (Gee and Bauder 1986) after removing organic matter by regularly adding dilute H_2O_2 until the chemical reactions stopped.

Statistical analysis

We first explored the correlation structure of the data through principal component analyses and correlation matrices (based on Pearson's *r*). This allowed us to visualise the influence of treatments on needle stoichiometry, soil characteristics (of the upper 10 cm, where most change occurred) and nutrient supplies (using the ggfortify R package - Horikoshi and Tang 2016; Tang et al. 2016). Treatment effects on forest stand characteristics (biomass and productivity proxies: tree height, basal area and volume (increment)), needle chemistry, soil characteristics and nutrient supply rates (pooled per plot) were then analysed using one-way ANOVAs, or non-parametric Kruskal-Wallis tests in cases where assumptions of normality or homoscedasticity were not met. If main effects of nutrient addition were significant, Tukey's parametric post hoc test or the non-parametric Bonferroni corrected pairwise Wilcoxon rank-sum test was employed to assess two-by-two differences.

To test whether soil characteristics (C:N ratio, total N, available P, CEC, TEB, bulk density) were predominantly altered through direct or through indirect pathways (through changes in productivity and/or needle stoichiometry), we applied structural equation modeling (SEM) and correlations on path diagrams. Version 0.5-23.1097 of the lavaan package in R (Rosseel 2012) was used to assess SEM model performance and estimate standardised SEM parameters.

Data were log-transformed if distributions were right-skewed. For the ANOVA analyses, we checked linear model assumptions (linearity, residuals normality, absence of influential outliers and homoscedasticity) with standard functions of R. Whenever confidence intervals are given, they represent standard errors of the mean. For all analyses, $\alpha = 0.05$ was taken as significance level, whereas P-values between 0.05 and 0.10 were considered as marginally significant. All statistical analyses were performed in R version 3.4.2 (R Core Team 2017).

Results

Changes in forest stand characteristics and needle chemistry

When the Flakaliden experiment was established in 1986, tree height, basal area and volume did not differ among the treatments (Table 1). The first two decades of fertiliser application increased productivity at 30y-IL compared to Control and I (later 10y-IL). During the last decade, when also 10y-IL received optimised nutrient application, productivity at both 10y-IL and 30y-IL was greater than at Control. While in the last decade height growth and basal area increment did not significantly differ between 10y-IL and 30y-IL, tree volume increment was still significantly greater at 30y-IL. As a consequence, in 2016, tree height, basal area and volume still differed between the 10y-IL and 30y-IL treatment. Altogether, nutrient additions clearly stimulated tree growth, and stand characteristics such as tree height, basal area and volume differed strongly also between the treatment plots for which fertiliser application was initiated three decades vs one decade ago ($P < 0.05$).

A principal component analysis (Fig. 1) and correlation matrix (Table S2) on the needle chemistry data from 2016 suggested a positive association between duration of fertiliser use and foliar N concentrations, and a negative influence on needle carbon to nitrogen (C:N) ratio. ANOVA analyses confirmed that needle N concentrations were significantly greater for 30y-IL and 10y-IL than for Control, and that needle C:N ratio decreased with fertiliser application (Table 2). An overall significant treatment effect was found on needle Ca concentrations, with Control values marginally significantly greater than values for 10y-IL plots (P = 0.09 (*)), and 10y-IL marginally significantly greater than 30y-IL (P = 0.09 (*)). Fertiliser application also exhibited an overall marginally significant influence on needle P concentrations, although no significant group-by-group differences were detected ($P > 0.10$). Concentrations of K and Mg did not significantly differ among treatments. In summary, of the most relevant nutrients, N was the only element for which needle concentrations substantially increased following fertiliser applications (Table 2), but in relation to N all other nutrient elements were within the set target values (cf. Linder 1995; Table 2).

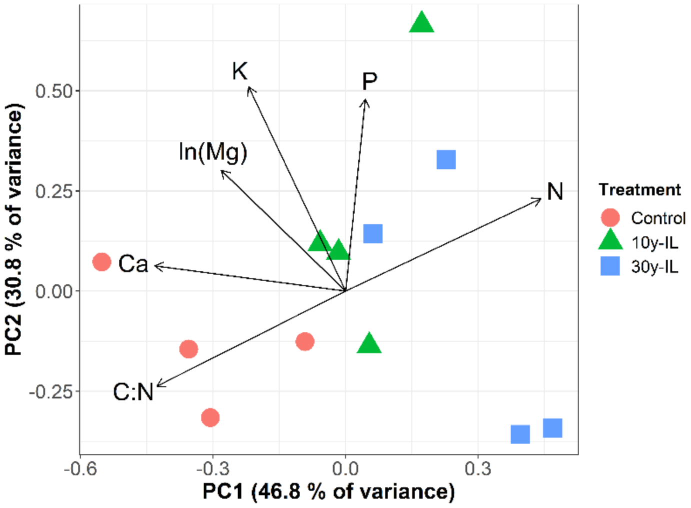

Fig. 1 Principal component analysis on the needle nutrient concentration (%) and stoichiometry data of 2016 at the Flakaliden experiment (sd for PC1 = 1.68, sd for PC2 = 1.36). Treatments were: Control, 10y-IL = "optimal" nutrient mix since 2007, and 30y-IL = "optimal" nutrient mix since 1987. Variables were log-transformed in case of positive skewness. The corresponding correlation matrix is given in Table S2.

Changes in soil characteristics through direct and indirect mechanisms

In the untreated plots, SOC, soil C:N ratio, CEC, TEB and "bio-available" phosphorus (P_{Bray}) all decreased with depth, while soil pH increased ($P < 0.05$, Table 3). Changes in soil characteristics with fertiliser application were mainly observed for the 0-10 cm layer: total nitrogen (TN), CEC, TEB and bio-available P (tended to) increase following nutrient addition (Fig. 2, Table 3). During sampling, we observed markedly thicker organic layers at the nutrient treated plots in comparison to the Control plots, which was reflected in a near-significant increasing trend for SOC with nutrient addition. The response of soil C:N ratio to fertiliser application depended on the time since the start of the treatment: for the recently initiated 10y-IL treatment, C:N did not significantly differ from controls, although three out of four 10y-IL plots exhibited greater C:N than any of the control plots. In contrast, C:N values for the 30y-IL treatment were significantly reduced compared to 10y-IL. In general, soil properties in the 10–20 cm layer did not differ between Control, 10y-IL and 30y-IL, except for CEC, which was significantly greater for the plots applied with fertiliser as compared to the controls.

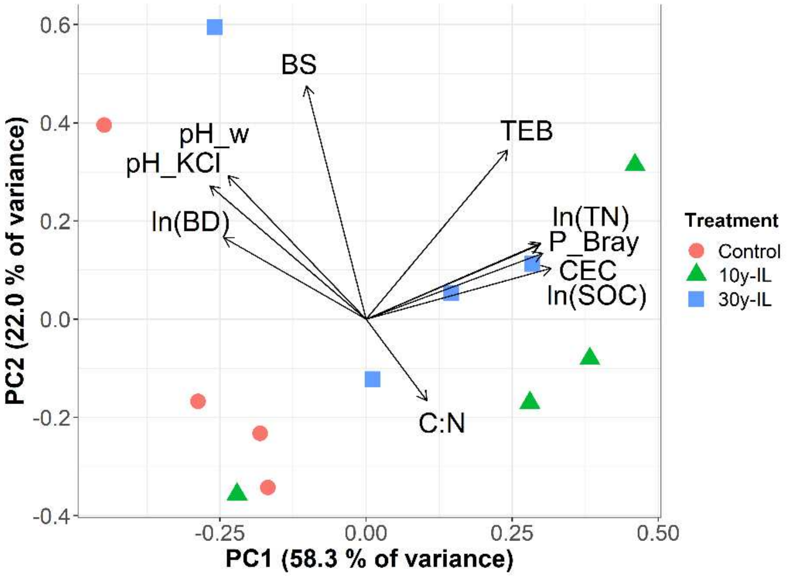

Fig. 2 Principal component analysis on the upper 10 cm soil property and nutrient data of 2016 at the Flakaliden experiment (sd for PC1 = 2.58, sd for PC2 = 1.49). Treatments were: Control, 10y-IL = "optimal" nutrient mix since 2007, and 30y-IL = "optimal" nutrient mix since 1987. Variables were log-transformed in case of positive skewness. Abbreviations: SOC = soil organic carbon concentration (%); TN = total nitrogen concentration (%); C:N = soil carbon to nitrogen ratio; pH_w = soil pH measured in water; pH_KCl = soil pH measured in KCl solution; BD = bulk density (kg m^{-3}); CEC = cation exchange capacity (mmol$_+$ kg^{-1}); TEB = total exchangeable bases (mmol$_+$ kg^{-1}); BS = base saturation (%); P_Bray = "bio-available" phosphorus (mg kg^{-1}) following the extraction method of Bray (Dickman and Bray 1940; Bray and Kurtz 1945). The corresponding correlation matrix is given in Table S3.

We investigated correlations among key variables and constructed structural equation models (SEMs) to elucidate whether nutrient treatments influenced 0-10 cm soil characteristics either directly or indirectly, through altered litterfall (not explicitly measured) and/or needle stoichiometry. Correlations and standardised SEM parameters suggested links between fertiliser application, productivity (+), SOC (+), CEC (+) and bulk density (-) (Fig. S1 and Tables S5,6). Indirect effects through stimulated productivity and SOM/SOC input probably dominated one-decade impacts of fertiliser use on soil C:N ratio (pathway a in Fig. 3, Table S5), whereas under longer-term nutrient addition, also stoichiometry effects and direct reduction of soil C:N ratio through added N would gain importance (pathways b and c in Fig. 3, Table S5). Finally, for TN, bio-available P and TEB, SEMs could not inform on the dominance of either direct or indirect pathways (Table S4, Figs. S2-4), despite rather strong relationships between TN, PBray, TEB, SOC and CEC as indicated by the PCA and correlation analyses ($|$Pearson's r$|$ ≥ 0.8; Fig. 2 and Tables S3 and S6).

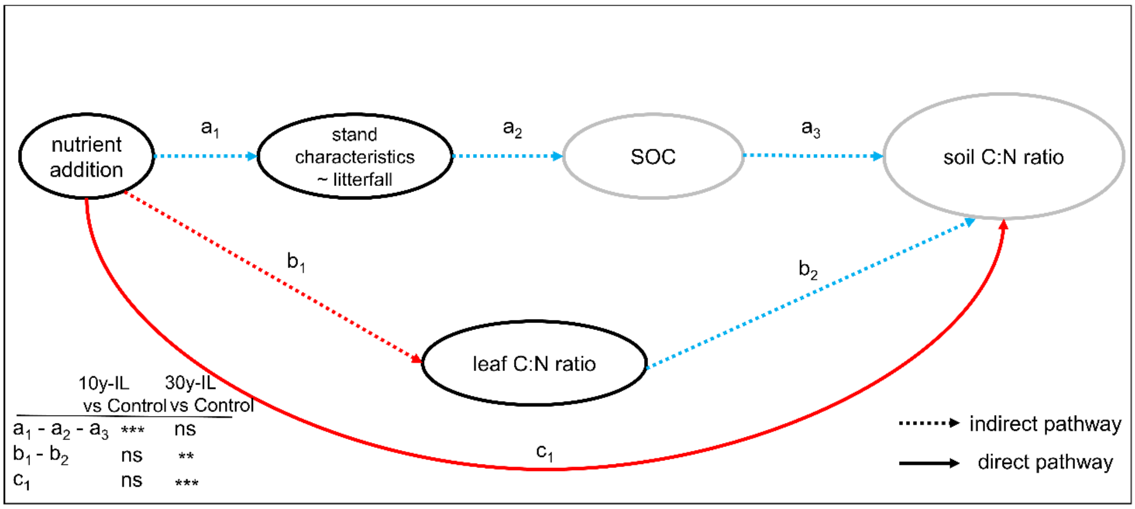

Fig. 3 Path diagram for direct vs indirect nutrient addition effects on soil carbon to nitrogen (C:N) ratio in 2016. Structural equation models (SEMs) were constructed to compare pathways of changes in plots fertilised since one decade (10y-IL, contrasted with Control) with changes in plots fertilised since three decades (30y-IL, contrasted with Control). Statistical evidence for the SEM parameters and correlations (represented by letters) are provided in Tables S4-6. Volume increment for the period 1986-2016 (Table 1) was used as a forest stand characteristic positively correlated with integrated litterfall (e.g. Berg and Meentemeyer 2001; Starr et al. 2005). Abbreviation: SOC = soil organic carbon concentration (%). Red (blue) arrows indicate a negative (positive) effect. Asterisks and ns indicate the level of significance of pathways (ns: $P > 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

Changes in soil nutrient supplies

Ion exchange resin derived supply rates of inorganic N (NH_4^+ and NO_3^-), P and K, and the supply of NO_3^- relative to total inorganic N significantly increased with nutrient addition at the Flakaliden experiment (Fig. 4 and Table 4). Moreover, N and P supply rates also tended to be greater in the 30y-IL than in the 10y-IL treatment despite greater application rates of N and P at 10y-IL since 2007. Although the nutrient mix contained (small) amounts of Ca and Mg (Table S1), only a marginally significant increase in Ca supply occurred between 10y-IL and 30y-IL ($P = 0.08$).

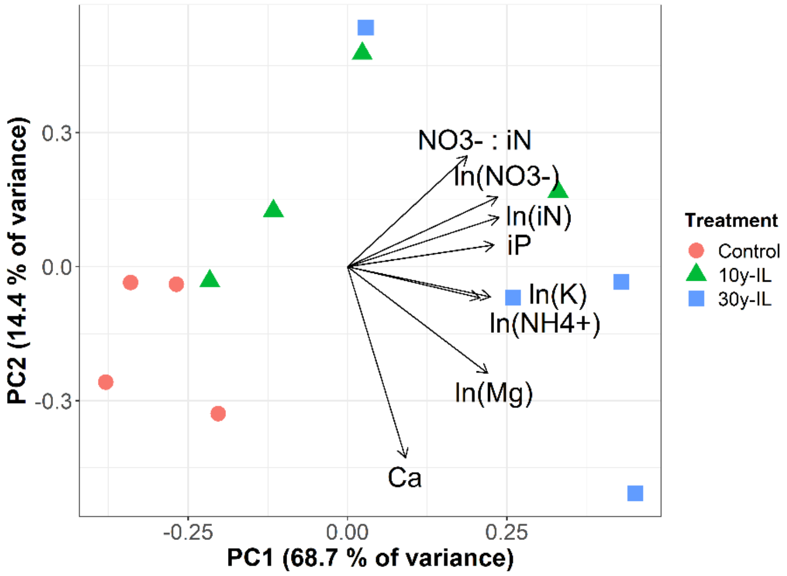

Fig. 4 Principal component analysis on plant root simulator (PRS) probe derived soil mineral nutrient supply rates (µg of element 10 cm^{-2} wk^{-1}; *n* = 4 per plot) in 2016 at the Flakaliden experiment (sd for PC1 = 2.34, sd for PC2 = 1.07). Probes were installed 26-07-2016, and retrieved one week later. Treatments were: Control, 10y-IL = "optimal" nutrient mix since 2007, and 30y-IL = "optimal" nutrient mix since 1987. Variables were log-transformed in case of positive skewness. Abbreviations: iN = inorganic nitrogen (NH_4^+ + NO_3^-); iP = inorganic phosphorus; ":" = ratio. The corresponding correlation matrix is given in Table S7.

Discussion

Litter quantity and quality

Combined evidence from our results and earlier studies at the experimental forest showed differences in the development of biomass related stand characteristics and needle stoichiometry among treatments. Prior to initialisation of fertiliser use at 10y-IL, biomass increased significantly more at 30y-IL than at 10y-IL and Control (Table 1). During the decade following 2006, also 10y-IL was fertilised, leading to similar height and basal area growth at both 10y-IL and 30y-IL by 2016, while volume increment was still greater at 30y-IL. As a result, the investigated biomass proxies (tree height, basal area and volume) were still greater at 30y-IL than at 10y-IL and Control in 2016. In line with our observations, Leppälammi-Kujansuu et al. (2014) showed in an earlier study at the site that the long-term fertiliser application (30y-IL) indeed stimulated both above- and belowground litter production. Long-term productivity and very likely also litterfall (not explicitly measured, but related to productivity – Berg and Meentemeyer 2001; Starr et al. 2005) thus correlated positively with the duration of fertiliser application, with possible consequences for soil properties.

Besides litter quantity, the changes in foliar stoichiometry suggest that also litter stoichiometry and hence quality was very likely affected by long-term optimised fertiliser application. Our analyses performed for the present study confirmed nutrient addition effects on fresh needle N stoichiometry, but concentrations of elements other than N did not significantly differ among the treatments (P, K, Mg) or even decreased over time with fertiliser application (Ca), despite their regular addition (Table 2; in all treatments, needle Ca was above the minimum target value relative to N, such that the Ca concentration was allowed to reduce through a dilution-effect). Somewhat in contrast, Maaroufi et al. (2018) reported increased needle litter concentrations in 2013 not only of N, but also of P in the 30y-IL and 10y-IL plots compared to Control, while no differences between 30y-IL and 10y-IL were observed. This apparent discrepancy may be explained by changes in nutrient resorption under fertiliser application (e.g. Mayor et al. 2014). This implies that indirect effects through stimulated productivity and modified needle stoichiometry need to be taken into account when investigating mechanisms underlying fertiliser-induced shifts in soil characteristics.

Changes in forest soil characteristics through direct and indirect mechanisms

In the experimental boreal forest studied here, (i) the mere occurrence of changes in the upper 10 cm of soil (Table 3), (ii) successful application of SEMs to path diagrams indicating indirect effects of fertiliser application (Figs. 3 and S1), and (iii) strong correlations among variables such as SOC, CEC, bulk density and nutrient concentrations (Fig. 2 and Table S6) all provide evidence for a key role of indirect effects of nutrient addition on soil characteristics through stimulated productivity, and associated with that (but not explicitly measured), litterfall (Berg and Meentemeyer 2001; Starr et al. 2005). Our analyses suggest that both increased litter production (Leppälammi-Kujansuu et al. 2014) and reduced soil respiration (Olsson et al. 2005; Hyvönen et al. 2007; Janssens et al. 2010) following nutrient addition resulted in organic matter accumulation (see also Fröberg et al. 2013). The accumulation of organic matter presumably initiated a further cascade of changes through modification of CEC and bulk density, and by representing a source of fresh material with a stoichiometry different from that of the soil (see also below in the discussion on soil C:N ratio). Besides this indirect pathway and influence through altered needle and litter stoichiometry, we also observed a direct influence of fertiliser application on soil nutrient concentrations of TN, TEB and bio-available P in this study, since N, P and exchangeable bases were all added in the nutrient mix (Fig. 5).

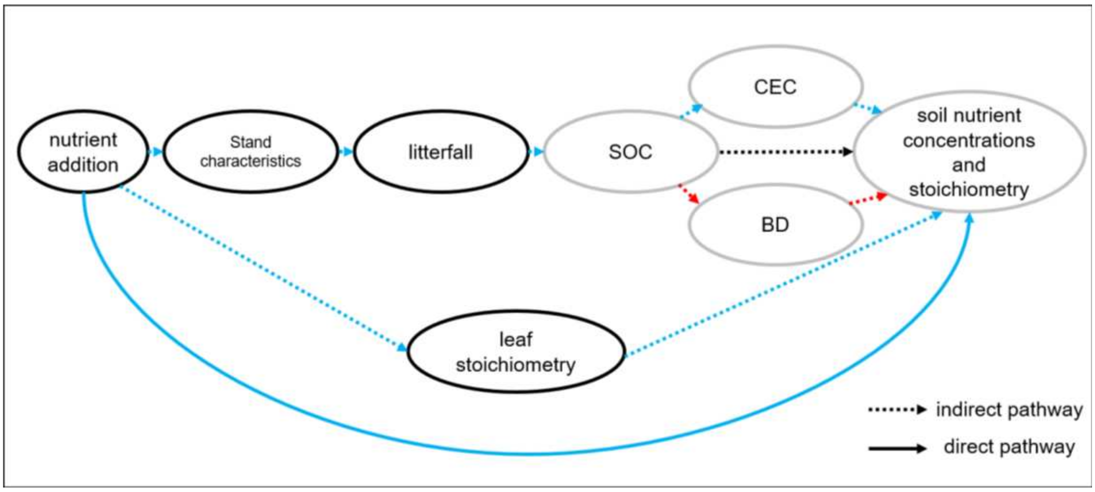

Fig. 5 Generalised path diagram for direct vs indirect nutrient addition effects on soil properties and nutrients. Abbreviations: SOC = soil organic carbon concentration (%); CEC = cation exchange capacity (mmol$_+$ kg^{-1}); BD = bulk density (kg m^{-3}). Red (blue) arrows indicate a negative (positive) effect. The black arrow indicates a direct influence of altered SOC on soil nutrient stoichiometry (e.g. elevated SOC following input of typically N-poor organic matter increases the soil C:N ratio).

An interesting case of time-dependent dominance of different pathways influencing a soil characteristic is shown by the pattern of soil C:N ratio. Soil C:N was lower in 30y-IL compared to 10y-IL, 10y-IL did not significantly differ from the control plots and even tended to be greater (with in three out of four 10y-IL plots having greater C:N than any of the control plots; Table 3). As shown by the structural equation model in Fig. 3, and its parameters in Table S5, elevated soil C:N in 10y-IL was most likely indirectly driven by increased productivity and associated litterfall (Berg and Meentemeyer 2001; Starr et al. 2005), thus increasing the proportion of typically N-poor organic matter (Vesterdal et al. 2008) in the soil samples (cf. the black arrow in Fig. 5). The decrease in 30y-IL as compared to 10y-IL, however, presumably reflects a combination of indirect and direct pathways: while fresh organic matter input through litterfall was most likely still greater, a reduced needle C:N ratio (Table 2) as well as the regular addition of inorganic N gained importance. Both can reduce the C:N ratio of the organic FH layer (e.g. Andersson et al. 2002) and hence of the top 0-10 cm soil. The example of soil C:N ratio thus shows that for a given forest, the dominant driver behind fertiliser-induced soil property changes may depend on the duration of nutrient addition.

Altogether, changes in soil characteristics over one to three decades of nutrient optimisation treatment were mostly limited to variables related to the organic matter content, and to soil C:N ratio, which were to a great extent driven by indirect modifications through stimulated productivity and hence litterfall. Other soil properties exhibited a high stability in response to long-term fertiliser addition, as exemplified by the near-complete absence of changes in the 10-20 cm layer. This result is in line with earlier fertilisation studies in conifer forests where altered litter input was suggested to affect mainly the organic soil layer and upper mineral soil (Huang et al. 2011; Jones et al. 2011). Pronounced modifications of pH and other variables in deeper soil can occur, but are then typically associated with direct effects or acidification (Tamm et al. 1999). We propose that, as long as there is no nutrient oversaturation, soil characteristics in northern coniferous forests exhibit a remarkably high stability under nutrient optimisation programmes and likely also moderate levels of N deposition, and that, if changes occur, effects are mainly indirect and slow through stimulated tree productivity. Finally, we note that fertiliser addition can also modify soil characteristics and nutrient availability by influencing microbial (Demoling et al. 2008; Smaill et al. 2010; Long et al. 2012; Maaroufi et al. 2018) and soil faunal (Lindberg, 2003; Remén et al. 2008; Maaroufi et al. 2018) community structure and function, for example through negative effects on decomposition (Olsson et al. 2005), but soil microbes and fauna were not assessed in our study.

Sustained changes in soil nutrient supplies

In contrast to soil properties, the PRS nutrient supply rates responded strongly and positively to the fertiliser treatments (Table 4 and Fig. 4); supply rates of most added elements increased, and these increases sustained in the long-term as long as the treatment continued (i.e. supply rates at 30y-IL were greater than at 10y-IL). This result implies that it is not just the current load of nutrients that determines their availability to plants; while nutrient applications were similar for 30y-IL and 10y-IL over the last few years, the accumulated load was evidently larger at 30y-IL, where the treatment started two decades earlier (Table S1). In line with what has been reported in other studies on fertiliser application in boreal forests (e.g. Högberg et al. 2006), we therefore note the importance of taking into account both the rate of nutrient application and the accumulated dose when studying effects on ecosystem structure and function. Even better is to estimate the availability of relevant nutrients and compare among treatments (see also Vicca et al. 2018), as performed in this study among others with PRS probes. However, we note that the PRS nutrient supply rates provide only a rough estimate for (changes in) the supply of mineral elements to plant roots, and should therefore be seen as a proxy for nutrient availability only. It is for example well established that boreal forest species take up organic molecules (Näsholm et al. 1998), and trees exhibit nutrient uptake mechanisms other than diffusion, such as mass flow (McMurtrie and Näsholm 2018). Summarized, our observation of sustained and increased supply rates under long-term nutrient optimisation remains valid, but caution is needed when interpreting PRS probe results as actual quantifications of nutrient availability.

Direct vs indirect effects of nutrient addition: implications

In the present study, we identified a plant-soil feedback (e.g. Ehrenfeld et al. 2005) by investigating how long-term fertiliser application indirectly modified soil characteristics by influencing tree function and structure (i.e. productivity and needle stoichiometry, used as proxies for litter production and stoichiometry). Although investigating implications of this feedback was out of the scope of this paper, we suggest a few aspects for which our observations may be relevant. While effects of fertiliser use on (soil) C cycling and storage is a well investigated topic (e.g. Nadelhoffer et al. 1999; Pregitzer et al. 2008; Maaroufi et al. 2016; Bracho et al. 2018), and also effects on nutrient cycling are frequently studied (e.g. Marklein and Houlton 2012), much less is known about the significance of fertiliser-induced thicker organic layers on nutrient retention and general nutrient availability. An emerging question is, for instance, to what extent the increase in SOM can improve nutrient availability, and how much this indirect effect contributes to the overall effect of fertiliser application. Changes in the soil profile may also be relevant for microbial communities and soil fauna with abundances depending on soil layers (Remén et al. 2008). The case of promoted tree growth, and consequent organic matter accumulation thus indicates that fertiliser-induced indirect modifications of soil characteristics may have further consequences for the structure and functioning of the forest ecosystem.

Our observations also hint as to what may happen to nutrient availability and soil conditions if the nutrient treatments would stop, or more generally, if N deposition on boreal forests would decrease (e.g. Lamarque et al. 2013). Considering the direct and sustained positive response of soil nutrient supply rates to fertiliser application, we propose that when nutrient addition ceases, supplies would also decrease within a few years. In the absence of harvests, the nutrient stocks of the ecosystem will remain increased for a long time (given that nutrient leaching was comparable and limited in both control and treated plots; Table S8), but these stocks are not efficiently recycled in these boreal forests, where a large part ultimately ends up in slowly decomposing soil organic matter. The cold climate would again become the dominant control on nutrient (especially N) availability, and we expect that N limitation would quickly return (see also Nohrstedt 2001 and Högberg et al. 2017). We thus suggest a return to N limitation within years after cessation of fertiliser use. However, given the primarily slow changes in soil characteristics, indirectly governed by stimulated growth and litterfall, we suggest that some soil properties (organic layer thickness, SOC, CEC and soil C:N ratio in particular) may be in a new stable state, or it would at least take decades for these to evolve back to the original situation, with potential consequences for soil processes. Earlier studies have indeed shown that even without fertiliser application, soil C stocks in Sweden are generally increasing, given the current disequilibrium between litter inputs and C mineralisation (Ågren et al. 2007), such that it is unlikely that the fertiliser-induced thicker organic layers would decrease within decades.

Conclusions

Long-term nutrient additions in the boreal spruce forest in Flakaliden resulted in direct, sustained changes in soil nutrient supplies, and slow, occasionally non-linear changes in soil characteristics that have further relevance to nutrient availability. Our dataset suggested that these slow changes were dominated by indirect lagged effects through altered productivity and needle stoichiometry. In addition to providing insights into the long-term changes in soil following fertiliser application, our results can also be taken to suggest that long term effects of moderate N deposition on boreal forest soil characteristics (if any) mainly occur indirectly through increased litter production. If this is confirmed by future studies, it would imply that N deposition effects on soil characteristics will persist for years in case N deposition diminishes.

Declarations

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Competing interests

The authors declare that they have no conflict of interest.

Code and data availability

Datasets and an R script with statistical analyses are available at https://github.com/KevinVanSundert/Flakaliden_EUFR_2020_KVS.

Authors' contributions

S.V. and K.V.S. planned the study and performed the field and lab work. K.V.S. analysed the data and wrote the manuscript. All authors contributed to the discussions and the writing of the manuscript.

Electronic supplementary material is available online at doi: XXX.

Table S1 Amount of nutrients supplied to the three-decade (30y-IL) and one-decade (10y-IL) nutrient optimisation treatments in Flakaliden, during the years 1987 - 2006, 2007 - 2015 and 2016, the year of the present study.

Table S2 Correlation matrix of the needle nutrient concentration and stoichiometry data of 2016 at the Flakaliden experiment.

Table S3 Correlation matrix of the upper 10 cm soil property and nutrient data of 2016 at the Flakaliden experiment.

Table S4 Fit measures for structural equation models applied on path diagrams presented in the figures.

Table S5 Significance and estimates of standardised parameters in structural equation models.

Table S6 Spearman correlations (for numerical vs numerical data) and ANOVA-based significance (for numerical vs group data) applied on path diagrams.

Table S7 Correlation matrix of plant root simulator (PRS) probe derived soil mineral nutrient supply rates in 2016 at the Flakaliden experiment.

Fig. S1 Proposed path diagram for indirect nutrient addition effects on cation exchange capacity and bulk density in 2016.

Fig. S2 Proposed path diagram for direct and/or indirect nutrient addition effects on soil total nitrogen in 2016.

Fig. S3 Proposed path diagram for direct and/or indirect nutrient addition effects on soil extractable phosphorus in 2016.

Fig. S4 Proposed path diagram for direct and/or indirect nutrient addition effects on soil total exchangeable bases in 2016.

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Table 1 Development of basic stand characteristics of the Flakaliden experimental spruce forest between the start of the experiment in 1986 and the year of sampling for the current study (2016). Treatments were: Control, 10y-IL = “optimal” nutrient mix since 2007, and 30y-IL = “optimal” nutrient mix since 1987. Change over time in the variables was first calculated per plot and then averaged per treatment. Statistical significance of treatment effects was determined with one-way ANOVAs, or the non-parametric Kruskal-Wallis rank sum test in case assumptions of normality or homoscedasticity were not met. Tukey’s test (parametric) or the Bonferroni corrected pairwise Wilcoxon rank-sum test (non-parametric) was used for post-hoc analysis. Letters in superscript indicate significant differences among the three treatments (overall $P < 0.05$). At the start of the experiment, tree age was 28 years (Bergh et al. 1999). Data for 1986 were taken from Bergh et al. (1999).

Treatment		1986			2006			2016			Growth 1987 - 2006			Growth 2007 - 2016		
		H	BA	V	H	BA	V	H	BA	V	H	BA	V	H	BA	V
		m	$m^2\ ha^{-1}$	$m^3\ ha^{-1}$	m	$m^2\ ha^{-1}$	$m^3\ ha^{-1}$	m	$m^2\ ha^{-1}$	$m^3\ ha^{-1}$	m	$m^2\ ha^{-1}$	$m^3\ ha^{-1}$	m	$m^2\ ha^{-1}$	$m^3\ ha^{-1}$
Control	*Mean*	2.8	2.6	7.3	8.6^{1}	18.3^{1}	84.7^{a}	11.2^{a}	25.2^{a}	150.0^{a}	5.8^{a}	15.7^{a}	77.4^{a}	2.6^{a}	6.9^{a}	62.1^{a}
	SE	0.1	0.3	0.9	0.2	0.6	3.7	0.3	1.0	8.1	0.2	0.8	4.3	0.2	0.4	3.9
Irrigated (I) 1987 - 2006; Irrigated-fertilised from 2007 (10y-IL)	*Mean*	3.0	3.0	8.5	8.4^{1}	17.2^{1}	77.1^{a}	12.8^{b}	31.3^{b}	198.4^{b}	5.4^{a}	14.1^{a}	68.7^{a}	4.4^{b}	14.1^{b}	121.3^{b}
	SE	0.1	0.3	0.9	0.2	0.6	2.4	0.2	0.9	6.8	0.2	0.7	2.2	0.1	0.6	5.5
Irrigated - fertilised (30y-IL)	*Mean*	3.0	2.6	7.4	13.2^{1}	46.1^{1}	305.2^{b}	17.6^{c}	59.3^{c}	503.0^{c}	10.6^{b}	42.2^{b}	275.7^{b}	4.0^{b}	14.5^{b}	185.3^{c}
	SE	0.1	0.1	0.4	0.9	1.5	12.3	0.5	1.2	12.7	0.9	1.5	12.0	0.4	0.5	6.7

[1] Significant overall treatment effect, but no significant differences among treatments detected according to post hoc tests.

Table 2 Needle nutrient concentrations (letters) and stoichiometry (symbol ":") per fertiliser treatment in October 2016 (Control, 10y-IL = "optimal" nutrient mix since 2007, 30y-IL = "optimal" nutrient mix since 1987), nutrient optimisation stoichiometry targets as specified in (Linder 1995), and trends in these characteristics over time with nutrient addition at the Flakaliden experiment ($n = 5$ per plot). For parameter estimates, the mean ± standard error is given, unless statistical tests were performed on log-transformed, positively skewed data. In these cases, intervals represent back-transformed values within the range of one standard error from the mean on a log-scale. Statistical significance of treatment effects was determined with one-way ANOVAs, or the non-parametric Kruskal-Wallis rank sum test in case assumptions of normality or homoscedasticity were not met. Tukey's test (parametric) or the Bonferroni corrected pairwise Wilcoxon rank-sum test (non-parametric) was used for post-hoc analysis. Letters in superscript indicate significant differences between treatments. ↑ and ↓ indicate an overall increasing or decreasing trend of the characteristic. Arrows between brackets illustrate trends that were only marginally significant or ambiguous when comparing values for either 10y-IL or 30y-IL vs Control.

Foliar characteristic	Control mean ± SE or interval	10y-IL mean ± SE or interval	30y-IL mean ± SE or interval	Treatment target	Statistical significance	Trend
N (mg g^{-1})	12.2 ± 0.4^a	14.4 ± 0.7^b	15.6 ± 0.4^b	18	$F_{2,9} = 10.25$; $P = 0.005$ **	↑
P (mg g^{-1})	1.71 ± 0.06	1.98 ± 0.10	1.71 ± 0.08	0.1 x [N]	$F_{2,9} = 3.62$; $P = 0.07$ (*)	
K (mg g^{-1})	5.9 ± 0.2	6.0 ± 0.3	5.6 ± 0.4	0.35 x [N]	$F_{2,9} = 0.63$; $P = 0.55$ ns	
Ca (mg g^{-1})	6.90 ± 0.53^1	5.58 ± 0.29^1	3.78 ± 0.03^1	0.025 x [N]	$\chi^2_2 = 9.85$; $P = 0.007$ **	(↓)
Mg (mg g^{-1})	1.05–1.28	1.06–1.11	1.00–1.14	0.04 x [N]	$F_{2,9} = 0.38$; $P = 0.69$ ns	
C:N	44 ± 2^a	38 ± 2^b	35 ± 1^b	n/a	$F_{2,9} = 9.12$; $P = 0.007$ **	↓

[1] Significant overall treatment effect, but no significant differences among treatments detected according to post hoc tests.

Table 3 Soil properties and extracted nutrients per fertiliser treatment in 2016 (Control, 10y-IL = "optimal" nutrient mix since 2007, 30y-IL = "optimal" nutrient mix since 1987), and trends in these characteristics over time with nutrient addition at the Flakaliden experiment. The mean ± standard error is given, unless statistical tests were performed on log-transformed, positively skewed data. In these cases, intervals represent back-transformed values within the range of one standard error from the mean on a log-scale. Overall statistical significance of treatment effects was determined with one-way ANOVAs, or the non-parametric Kruskal-Wallis rank sum test in case assumptions of normality or homoscedasticity were not met. Tukey's test (parametric) or the Bonferroni corrected pairwise Wilcoxon rank-sum test (non-parametric) was used for post-hoc analysis. Letters in superscript indicate significant differences between treatments. ↑ and ↓ indicate an overall increasing or decreasing trend of the characteristic, ≈ indicates no significant shifts in values, before or after a change up or down. Arrows between brackets illustrate trends that were only marginally significant or ambiguous when comparing values for either 10y-IL or 30y-IL vs Control. Abbreviations: SOC = soil organic carbon concentration; CEC = cation exchange capacity; TEB = total exchangeable bases; BS = 100*TEB/CEC = base saturation; P_{Bray} = "bio-available" phosphorus following the extraction method of Bray (Dickman and Bray 1940; Bray and Kurtz 1945).

Soil characteristic	Control mean ± SE or interval	10y-IL mean ± SE or interval	30y-IL mean ± SE or interval	Statistical significance	Trend
SOC (%)					
0–10cm[1]	2.9–3.5	7–21	7–15	$F_{2,9} = 3.44$; $P = 0.08$ (*)	(↑)
10–20 cm	2.4 ± 0.7	2.8 ± 0.2	2.9 ± 0.2	$\chi^2_2 = 1.85$; $P = 0.40$ ns	
TN (%)					
0–10 cm	0.08–0.11^{a}	0.19–0.53^{ab}	0.29–0.59^{b}	$F_{2,9} = 4.62$; $P = 0.04$ *	↑
10–20 cm	0.07–0.10	0.09–0.20	0.10–0.11	$\chi^2_2 = 2.35$; $P = 0.31$ ns	
C:N					
0–10 cm	33 ± 2^{ab}	39 ± 4^{a}	25 ± 2^{b}	$F_{2,9} = 5.78$; $P = 0.02$ *	≈ → ↓
10–20 cm	27.6 ± 2.2	25.7 ± 6.7	26.7 ± 0.9	$\chi^2_2 = 1.08$; $P = 0.58$ ns	
pH_{water}					
0–10 cm	4.3 ± 0.3	3.9 ± 0.1	4.2 ± 0.3	$\chi^2_2 = 2.19$; $P = 0.33$ ns	
10–20 cm	5.0 ± 0.3	5.1 ± 0.1	4.8 ± 0.2	$F_{2,9} = 0.33$; $P = 0.73$ ns	
pH_{KCl}					
0–10 cm	3.1 ± 0.2	2.5 ± 0.1	2.8 ± 0.3	$\chi^2_2 = 4.79$; $P = 0.09$ (*)	
10–20 cm	3.9 ± 0.3	3.7 ± 0.2	3.4 ± 0.2	$F_{2,9} = 1.20$; $P = 0.35$ ns	
BD (kg m^{-3})					
0–10 cm	770–1410	230–640	450–700	$F_{2,9} = 1.93$; $P = 0.20$ ns	
10–20 cm	1600 ± 300	1000 ± 100	1500 ± 100	$\chi^2_2 = 2.92$; $P = 0.23$ ns	
CEC ($cmol_+$ kg^{-1})					
0–10 cm[1]	50 ± 10	220 ± 60	130 ± 20	$\chi^2_2 = 6.96$; $P = 0.03$ *	(↑) → (↓)
10–20 cm	33 ± 9^{a}	62 ± 9^{b}	61 ± 6^{ab}	$F_{2,9} = 4.50$; $P = 0.04$ *	↑
TEB ($cmol_+$ kg^{-1})					
0–10 cm	13 ± 5	50 ± 20	43 ± 6	$F_{2,9} = 3.23$; $P = 0.09$ (*)	(↑)
10–20 cm	6–11	7–9	10–16	$F_{2,9} = 1.13$; $P = 0.36$ ns	
BS (% of CEC)					
0–10 cm	28 ± 9	20 ± 4	34 ± 6	$F_{2,9} = 1.13$; $P = 0.36$ ns	
10–20 cm	33 ± 10	15 ± 4	23 ± 5	$F_{2,9} = 1.92$; $P = 0.20$ ns	
P_{Bray} (mg kg^{-1})					
0–10 cm	12 ± 5	48 ± 14	37 ± 10	$F_{2,9} = 3.25$; $P = 0.09$ (*)	(↑)
10–20 cm	3 ± 2	11 ± 8	19 ± 7	$F_{2,9} = 1.43$; $P = 0.29$ ns	

[1] Significant overall treatment effect, but no significant differences among treatments detected according to post hoc tests.

Table 4 Plant root simulator (PRS) probe derived soil mineral nutrient supply rates ($n = 4$ per plot) per fertiliser treatment in 2016 (Control, 10y-IL = "optimal" nutrient mix since 2007, 30y-IL = "optimal" nutrient mix since 1987), and trends in these supply rates over time with nutrient addition at the Flakaliden experiment. Probes were installed 26-07-2016, and retrieved one week later. For parameter estimates, the mean ± standard error is given, unless statistical tests were performed on log-transformed, positively skewed data. In these cases, intervals represent back-transformed values within the range of one standard error from the mean on a log-scale. Overall statistical significance of treatment effects was determined with one-way ANOVAs, or the non-parametric Kruskal-Wallis rank sum test in case assumptions of normality or homoscedasticity were not met. Tukey's test (parametric) or the Bonferroni corrected pairwise Wilcoxon rank-sum test (non-parametric) was used for post-hoc analysis. Letters in superscript indicate significant differences between treatments. ↑ and ↓ indicate an overall increasing or decreasing trend of the characteristic. Arrows between brackets illustrate trends that were only marginally significant or ambiguous when comparing values for either 10y-IL or IL vs Control. Abbreviations: iN = inorganic nitrogen ($NH_4^+ + NO_3^-$); iP = inorganic phosphorus; ":" = ratio.

PRS supply rate	Control mean ± SE or interval	10y-IL mean ± SE or interval	30y-IL mean ± SE or interval	Statistical significance	Trend
iN (µg N 10 cm^{-2} wk^{-1})	8–12^{a}	23–54^{b}	86–139^{b}	$F_{2,9} = 15.77$; $P = 0.001$ **	↑
NH_4^+ (µg N 10 cm^{-2} wk^{-1})	6–10	13–23	17–46	$F_{2,9} = 3.50$; $P = 0.07$ (*)	(↑)
NO_3^- (µg N 10 cm^{-2} wk^{-1})	2.1–2.5^{a}	5.7–28.1^{ab}	64.4–91.8^{b}	$\chi^2_2 = 7.04$; $P = 0.03$ *	↑
NO_3^- : iN	0.24 ± 0.04^{a}	0.45 ± 0.15^{ab}	0.71 ± 0.07^{b}	$F_{2,9} = 6.07$; $P = 0.02$ *	↑
iP (µg P 10 cm^{-2} wk^{-1})	2.1 ± 0.7^{a}	8.8 ± 2.8^{ab}	11.3 ± 2.5^{b}	$F_{2,9} = 4.65$; $P = 0.04$ *	↑
K (µg K 10 cm^{-2} wk^{-1})	84–126^{a}	211–278^{ab}	211–419^{b}	$F_{2,9} = 5.40$; $P = 0.03$ *	↑
Ca (µg Ca 10 cm^{-2} wk^{-1})	69 ± 7	50 ± 4	78 ± 11	$F_{2,9} = 3.31$; $P = 0.08$ (*)	
Mg (µg Mg 10 cm^{-2} wk^{-1})	14–17	15–20	21–35	$F_{2,9} = 3.00$; $P = 0.10$ ns	