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Effects of long-term nitrogen addition on phosphorus cycling in organic soil horizons of temperate forests

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Abstract High atmospheric nitrogen (N) deposition is expected to impair phosphorus (P) nutrition of temperate forest ecosystems. We examined N and P cycling in organic soil horizons of temperate forests exposed to long-term N addition in the northeastern USA and Scandinavia. We determined N and P concentrations, enzyme activities and net N and P mineralization rates in organic soil horizons of two deciduous (Harvard Forest, Bear Brook) and two coniferous (Klosterhede, Gårdsjön) forests which had received experimental inorganic N addition between 25 and 150 kg N ha^{-1} $year^{-1}$ for more than 25 years. Long-term N addition increased the activity of phosphatase (+ 180%) and the activity of carbon (C)- and N-acquiring enzymes (cellobiohydrolase: + 70%, chitinase: + 25%). Soil N enrichment increased the N:P ratio of organic soil horizons by up to 150%. In coniferous organic soil horizons, net N and P mineralization were small and unaffected by N addition. In deciduous organic soil horizons, net N and P mineralization rates were significantly higher than at the coniferous sites, and N addition increased net N mineralization by up to 290%. High phosphatase activities concomitant with a 40% decline in P stocks of deciduous organic soil horizons indicate increased plant P demand. In summary, projected future global

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increases in atmospheric N deposition may induce P limitation in deciduous forests, impairing temperate forest growth.

Keywords Chronic nitrogen deposition · Exoenzymes · Long-term experiments · Nutrient mineralization · Phosphatase · Soil stoichiometry

Introduction

Future increases in atmospheric nitrogen (N) deposition are expected to alter not only N, but also phosphorus (P) cycling in temperate forest ecosystems. This trend is already apparent in decreased foliar P concentrations observed in many temperate forests over the last several decades (Houdijk and Roelofs 1993; Flückiger and Braun 1998; Duquesnay et al. 2000; Ilg et al. 2009; Jonard et al. 2015; Talkner et al. 2015). Although the implementation of air pollution control measures has reduced N emissions in Europe and the USA (Xing et al. 2013; Vet et al. 2014; Li et al. 2016b), the amount of N deposition is still well above pre-industrial levels in these regions (Galloway et al. 2004; Simpson et al. 2014) and global rates of deposition are projected to double by 2050 (Galloway et al. 2004; 2008). Temperate and boreal forests, which are often N-limited, are particularly sensitive to high N inputs (Aber et al. 1989; Vitousek and Howarth 1991). High N inputs might cause P limitation in temperate forests (Mohren et al. 1986; Tessier and Raynal 2003; Gress et al. 2007; Talkner et al. 2015) because N-induced forest growth can increase plant P demand (Gradowski and Thomas 2006, 2008; Li et al. 2016a). For example, P limitation of trees has recently been reported in hardwood forests of the northeastern USA (Goswami et al. 2018).

Net mineralization of N and P, defined as the difference between gross mineralization and net immobilization, makes these nutrients available for plant uptake (Schimel and Bennett 2004; Bünemann et al. 2007). The mineralization of organic P is catalyzed by phosphatases, which are exoenzymes released by plants and soil microorganisms. Nitrogen addition has been shown to elevate soil phosphatase activity, likely as a result of increased P demand by plants and microorganisms (Treseder and Vitousek 2001; Wang et al. 2007; Marklein and Houlton 2012; Deng et al. 2017). The production of the N-rich phosphatases might be facilitated by high N availability (Treseder and Vitousek 2001; Wang et al. 2007; Marklein and Houlton 2012; Deng et al. 2017).

The activity of other enzymes besides phosphatases may also change in response to N availability. For example, the activities of the cellulose and the chitin degrading enzymes cellobiohydrolase and chitinase (β-1,4-*N*-acetylglucosaminidase) have been reported to increase (Weand et al. 2010a), whereas lignin degrading phenol oxidases are often inhibited by high N concentrations (Frey et al. 2004; Gallo et al. 2004; Waldrop and Zak 2006; Jian et al. 2016). Comparison of the activities of C-, N- and P-acquiring enzymes and their ratios can be used to identify the nutrient cycling processes in which organisms preferentially invest energy (Sinsabaugh et al. 2008, 2009; Sinsabaugh and Follstad Shah 2012; Herold et al. 2014). Such ratios might indicate, for example, whether microbial investment into P acquisition is increased compared to investment into N or C acquisition in response to long-term N addition.

Numerous studies have evaluated the effects of simulated N deposition in N addition experiments in forests of the temperate zones in North America and Europe (Dise and Wright 1992; Aber et al. 1993; Norton et al. 1999). Nitrogen addition has been shown to elevate N concentrations in foliage and soil organic horizons (e.g. Aber et al. 1993; Magill et al. 2004; Kjønaas and Stuanes 2008; Elvir et al. 2010), increase plant biomass (e.g. Aber et al. 1993; Magill et al. 1997; Lovett et al. 2013), stimulate nitrate leaching (e.g. Gundersen 1998; Jefts et al. 2004; Moldan et al. 2006; Lovett et al. 2013), and reduce soil cation concentrations (Currie et al. 1999; Moldan and Wright 2011). Further, net N mineralization often increases following N addition (Aber et al. 1993, 1995; Kjønaas et al. 1998; Jefts et al. 2004; Fatemi et al. 2016; Carrara et al. 2018). Despite this long history of research, the influence of chronic N deposition on P cycling is not well studied and the response of N:P ratios of vegetation and soils to long-term N addition is seldom reported (e.g. in Kjønaas et al. 1998; Kjønaas and Stuanes 2008; Weand et al. 2010a; Crowley et al. 2012). This underlines the need for further research of the influence of high N deposition on P cycling in temperate forests. Special consideration should be given to how element cycling processes in the organic horizon change upon increased N inputs because

organic horizons represent an important pool of P in forest soils that is rapidly mineralized by microorganisms and is very important for forest P nutrition (Ponge 2003; Huang and Spohn 2015; Spohn et al. 2018).

Our objective was to assess changes in N and P cycling and microbial nutrient acquisition in response to long-term N addition to temperate forests soils. Our hypotheses were that (i) N addition would increase N:P ratios of organic soil horizons, (ii) phosphatase activity would increase more strongly in comparison to the activities of C- and N-acquiring enzymes due to N addition and (iii) increased phosphatase activity would lead to increased net P mineralization. To test these hypotheses, we analyzed organic soil horizons of two deciduous and two coniferous long-term N addition experiments in the USA and Europe.

Materials and methods

Study sites and sampling

Two North American hardwood forest sites (Harvard Forest, Bear Brook) and two Scandinavian spruce forests (Klosterhede, Gårdsjön) exposed to long-term, experimental N addition were sampled. Harvard Forest (Massachusetts, USA, N42°32′, W72°10′) is located 330 m a.s.l., with mean annual precipitation and temperature being 1240 mm and 8.5 °C, respectively. The vegetation is dominated by black oak (*Quercus velutina*) and red oak (*Q. borealis*) mixed with other hardwood species including black birch (*Betula lenta*), red maple (*Acer rubrum*) and American beech (*Fagus grandifolia*). The soils are mainly Cambisols (Inceptisols; USDA Taxonomy), developed from sandy tills of the Gloucester series. The organic horizon has an average thickness of 5 cm and a bulk density ranging from 0.17 (N150) to 0.24 cm^{-3} (control), depending on N treatment. There are three N addition treatments with application rates (since 1988) of 0 (N0), 50 (N50) and 150 (N150) kg N ha^{-1} $year^{-1}$ in the form of ammonium nitrate (NH_4NO_3). Each treatment plot has a size of 30 × 30 m. Ambient N deposition at the site is currently 8–10 kg N ha^{-1} $year^{-1}$ (Aber et al. 1995; Magill et al. 2004; Schwede and Lear 2014).

Bear Brook is located in Maine, USA (N44°52′, W68°06′) and has a mean annual precipitation of 1400 mm and a mean annual temperature of 4.9 °C. Its lower elevations, which were sampled in this study, are dominated by American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*) and red maple (*Acer rubrum*). The soils are mainly Podzols (Spodosols) developed from quartzite and gneiss covered by an organic horizon with an average thickness of 4 cm (bulk density: 0.09 g cm^{-3}, both control and N addition plots). The Bear Brook experiment consists of two adjacent watersheds. The 11 ha watershed "West Bear" has received 25 kg N ha^{-1} $year^{-1}$ in the form of ammonium sulfate ($(NH_4)_2SO_2$) since 1989 and the 10 ha watershed "East Bear" has served as a control. Ambient deposition is 3 kg N ha^{-1} $year^{-1}$ (Norton et al. 1999; Fernandez et al. 2010; SanClements et al. 2010).

Klosterhede (Denmark, N56°29′, E008°24′) is situated at 27 m a.s.l., with a mean annual precipitation of 860 mm and mean annual temperature of 9 °C. A 108 year-old Norway spruce (*Picea abies*) plantation grows on Podzols (Spodosols) developed from glacial outwash sands. The organic horizon is on average 11 cm thick and has bulk densities of 0.10 (control) and 0.13 g cm^{-3} (N addition). The experiment was part of the "NITREX project" (Wright and van Breemen 1995) and consists of a 500 m^2 N-addition plot surrounded by three control plots. The N-addition plot has received 35 kg N ha^{-1} $year^{-1}$ in the form of NH_4NO_3 in monthly doses since 1992; ambient N deposition is approximately 25 kg N ha^{-1} $year^{-1}$ (Dise and Wright 1992; Gundersen and Rasmussen 1995; Gundersen 1998). Samples were taken from 15 × 15 m subplots.

The Gårdsjön experiment (Sweden, N58°04′, E12°03′), which was also part of the "NITREX project", is situated at 135-145 m a.s.l. with mean annual precipitation of 1100 mm and mean annual temperature of 6.4 °C. The dominant tree species is Norway spruce (*Picea abies*) with Scots pine (*Pinus sylvestris*) growing in drier areas. The main soil types are Orthic Humic Podzols and Gleyed Humoferric Podzols (Spodosols) developed from glacial till covered by an 8 cm thick organic horizon with a mean bulk density of 0.18 g cm^{-3} (both control and N addition). We sampled the catchment G2 (0.5 ha), which has received 40 kg N ha^{-1} $year^{-1}$ in the form of NH_4NO_3 in small doses (128 mg N l^{-1}) along with each rain event since 1991, and the control catchment F1 (3.7 ha). Ambient N deposition at the site is 12 kg N ha^{-1} $year^{-1}$ (Dise and Wright 1992;

Andersson et al. 1998; Moldan et al. 2006; Seftigen et al. 2013).

The organic horizon at Harvard Forest and Bear Brook was sampled in July 2016. Samples (20 × 20 cm) were collected from six replicate plots and divided into leaf litter (Oi horizon) and organic soil (Oe + Oa horizons). Samples were collected from Klosterhede and Gårdsjön in March–April 2017 following the same methods. Samples were stored at 4 °C and shipped immediately on ice to the University of Bayreuth, Germany. All Oe + Oa horizons were sieved to remove roots and stones and stored moist at 4 °C. They were characterized for total C, total and available N and P concentrations as well as microbial C, N and P concentrations, N and P stocks, pH, exoenzyme activity and net N and P mineralization rates. Leaf litters were homogenized by hand, dried at 60 °C, and subsequently analyzed for total C, N and P concentrations.

Soil characteristics

Gravimetric water content and maximum water holding capacity of the soils was determined following Naeth et al. (1991). Plastic tubes closed with a fine cloth at the bottom were filled with 1 cm of field-moist soil for two replicates from each site, weighed, and water saturated for 48 h. Samples were then drained for 48 h on a water saturated sand bath at 5 °C and weighed again. During draining, the tops of the tubes were covered with a wet cloth to prevent water loss via evaporation. Sample dry weights were determined after 48 h drying at 60 °C. The bulk density was calculated for the organic soil horizons of both N addition and control plots in six replicates.

Soil pH was measured with a gel electrode (WTW) in a suspension of moist soil and deionized water at a ratio of 1:5 (w/v). Total C and N concentrations were measured on dried (60 °C) and finely ground subsamples using a CN analyzer (Vario MAX, Elementar). Total soil P concentrations were measured with an inductively coupled plasma-optical emission spectroscopy (ICP-OES, Vista-Pro radial, Varian) after pressure digestion in concentrated nitric acid. Nitrogen and P stocks were calculated from total N concentrations (total P concentrations) and soil bulk density measurements.

Net mineralization

Net N and P mineralization were determined by incubating soil (ca. 50 g), adjusted to 60% water holding capacity, at 15 °C for 11 weeks. Net N mineralization was determined based on the increase in ammonium (NH_4^+) and nitrate (NO_3^-) concentrations extracted with cold water from 2.5 g dry-weight equivalents of moist soil in weekly (1st month) to biweekly intervals with a ratio of 1:20 (w/v). NH_4^+ and NO_3^- concentrations were measured with flow-injection analysis (FIA-Lab, MLE). The NH_4^+–N + NO_3^-–N concentrations determined at the first measurement were defined as the available N concentration. In the same incubation, net P mineralization was determined based on the increase in phosphate (PO_4^-) concentrations in Bray-1 extracts (0.03 M NH_4F + 0.025 M HCl). For this purpose, 5 g dry-weight equivalent of moist soil were regularly extracted in a ratio of 1:10 (w/v). PO_4^- concentrations in the extracts were measured spectrophotometrically using a microplate reader (M200 pro, Tecan) according to the method of Murphy and Riley (1962). To prevent interference with the color formation of the assay, fluoride ions were neutralized with 0.1 M boric acid before addition of the molybdate blue reagent. The PO_4^-–P concentration determined at the first measurement was termed available P. Net N and P mineralization rates were calculated as the increase in NH_4^+–N plus NO_3^-–N and PO_4^-–P concentrations, respectively, over time.

Soil microbial biomass

Soil microbial biomass C, N and P were determined by chloroform-fumigation extraction (Brookes et al. 1984, 1985; Vance et al. 1987) of subsamples (C and N: 5 g, P: 2.5 g) from the incubation experiment described above. Samples were fumigated for 24 h at room temperature. For microbial C and N, controls and fumigated samples were extracted in 0.5 M K_2SO_4 with a ratio (w/v) of 1:5 (Joergensen et al. 1995). The C and N concentrations in the extracts were determined with a CN analyzer (multi N/C 2100, Analytik Jena). For microbial P, controls and fumigated samples were extracted in Bray-1 solution (0.03 M NH_4F + 0.025 M HCl, Bray and Kurtz (1945)) in a ratio of 1:10. PO_4 concentrations in the extracts were measured spectrophotometrically with a

microplate reader (M200 pro, Tecan) using the method of Murphy and Riley (1962) modified as above. The Bray-1 solution has been shown to be a very efficient extractant for microbial P (Khan and Joergensen 2012). Soil microbial biomass C, N and P were calculated as the difference of C, N and P concentrations in fumigated and control samples, and corrected by a factor of 2.22 for C and N and by 2.5 for P, respectively (Jenkinson et al. 2004).

Exoenzyme activity

The activities of cellobiohydrolase, chitinase and phosphatase were determined using the fluorogenic substrates 4-methylumbelliferyl-β-D-cellobioside, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide and 4-methylumbelliferyl-phosphate according to Marx et al. (2001) and German et al. (2011). Briefly, 1 g moist soil was dispersed in 50 ml deionized water with ultrasound and 50 µl of this soil slurry were transferred to black 96-well microplates in four analytical replicates. These samples were diluted with 50 µl sterile deionized water and amended with 100 µl of a 1 mM substrate solution. The plates were incubated at 30 °C for 210 min and fluorescence was measured after 30, 60, 90, 150 and 210 min. Fluorescence values were corrected for quenching of the soil, as well as for the fluorescence of substrate, and soil enzyme activity calculations were based on end-point measurements (German et al. 2011). The ratios of cellobiohydrolase-to-chitinase, cellobiohydrolase-to-phosphatase and chitinase-to-phosphatase were determined. For this calculation, the natural logarithms of the specific enzyme activities in µmol g organic C^{-1} h^{-1} were used (Sinsabaugh et al. 2008; Herold et al. 2014).

Statistical analyses

Data were tested for significant site-specific differences between N addition treatments and controls. The normality of the data was examined visually by QQ-plots and analytically by Shapiro–Wilk tests, and the homogeneity of variances were checked with Levene's tests. In all cases in which normality assumptions were met, analyses of variance (ANOVA) followed by Tukey's multiple comparisons tests were used for Harvard Forest, which had three treatments, and t-tests were used for Bear Brook, Klosterhede and Gårdsjön, which each had two treatments. Where assumptions of normality were violated, Kruskal–Wallis tests followed by multiple comparisons tests according to Dunn (Pohlert 2014) were calculated for Harvard Forest, and Wilcoxon rank sum tests were used for the other sites. The relationships between cellobiohydrolase, chitinase and phosphatase activity as well as net N and P mineralization and different fractions of soil C, N and P were analyzed with Spearman rank correlations. Where linear regressions were used, all residuals were checked for normal distribution and homoscedasticity. If assumptions for least-squares regression were not met, robust linear regression (Yohai 1987; Koller and Stahel 2011) was used with the R package "robustbase" (Rousseeuw et al. 2015). The influence of control and N addition treatments on regression analyses were examined with analyses of covariance (ANCOVA). All statistical analyses were performed in R version 3.2.2 (R Core Team 2015).

Results

N and P of leaf litters and organic horizons

The total N concentration of the leaf litter increased significantly at all sites due to N addition, on average by 17% (Table 1). The total N concentration of the organic soil horizon also increased at two of the four sites, on average by 42% (Table 2). The total N stock of the organic soil horizon increased significantly at Klosterhede (+ 15%), but decreased at Bear Brook (− 27%) in response to N addition (Table 2). The available N concentration in the organic horizon was significantly elevated at all sites upon N addition (Table 2).

The total P concentration of the leaf litter declined significantly at Gårdsjön, but increased at Bear Brook (Table 1). The total P concentration of the organic horizon decreased at Harvard Forest by 29%, but increased at Klosterhede by 20%. Total P concentrations of leaf litter and the organic soil horizon were significantly lower in the coniferous than in the deciduous forests ($P < 0.001$, Tables 1, 2). The total P stocks of the coniferous organic soil horizons were not affected by N addition. In contrast, P stocks were significantly reduced in the deciduous organic soil horizons due to N addition by 55 and 35% at Harvard

Table 1 Total C, N and P concentrations and C:N:P ratios of the leaf litter from control and N-addition plots at Harvard Forest, Bear Brook, Klosterhede and Gårdsjön

Site	Treatment	Total C (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	C:N ratio	C:P ratio
Harvard Forest	Control	475 ± 04	16.7 ± 1.3	1.16 ± 0.04	32.9 ± 2.8	1039 ± 053
	+ 50 kg N	481 ± 03	**18.9 ± 1.3***	1.24 ± 0.04	29.8 ± 2.1	1001 ± 037
	+ 150 kg N	**493 ± 07*****	17.8 ± 1.1	0.97 ± 0.10	32.3 ± 2.1	**1324 ± 139***
Bear Brook	Control	464 ± 02	16.0 ± 1.1	0.84 ± 0.04	33.8 ± 2.2	1422 ± 056
	+ 25 kg N	469 ± 06	**19.5 ± 1.3*****	**0.95 ± 0.04****	**27.2 ± 2.1*****	**1265 ± 034****
Klosterhede	Control	477 ± 16	17.7 ± 0.5	0.82 ± 0.04	31.4 ± 1.2	1501 ± 060
	+ 35 kg N	**496 ± 05***	**20.0 ± 0.3*****	0.76 ± 0.05	**28.9 ± 0.4****	**1642 ± 049****
Gårdsjön	Control	498 ± 03	13.5 ± 1.3	0.78 ± 0.11	43.2 ± 3.8	1665 ± 226
	+ 40 kg N	**510 ± 09****	**17.5 ± 1.6*****	**0.62 ± 0.13***	**34.3 ± 3.8****	2220 ± 590

All ratios were calculated on a molar basis and all values are given as means and standard deviations. Asterisks mark the level of significance for all significant differences between the N addition treatment and the respective control (bold)

Levels of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Forest and Bear Brook, respectively (Table 2). The available P concentrations of the organic soil horizon was elevated at Harvard forest by 67% due to N addition.

N:P ratios were increased in all leaf litters and organic soil horizons due to N addition, and the increases were significant for all sites except for Bear Brook (leaf litter) and Gårdsjön (organic soil horizon, Fig. 1). Overall, the N:P ratios of leaf litter and organic soil horizons were lower in the deciduous forests than in the coniferous forests (leaf litter: $P < 0.05$, organic soil horizons: $P < 0.001$).

Total C, pH and microbial biomass

Total C concentrations of leaf litter and organic soil horizons increased significantly under N addition at Harvard Forest, Klosterhede and Gårdsjön (Tables 1, 2). Nitrogen addition did not affect total C stocks of the organic soil horizons, with the exception of a significant increase at Gårdsjön (by 9%). Organic horizon soil pH was significantly reduced at Harvard Forest and Bear Brook in response to N addition. Microbial biomass C declined in response to N addition at Harvard Forest in the N150 treatment, but was not affected at the other sites (Table 2). Microbial biomass N and P concentrations and microbial biomass C:N, C:P, and N:P ratios were also unaffected by long-term N addition (Table 3).

Exoenzyme activities in organic soil horizons

Phosphatase activity in the organic horizon increased significantly in response to N addition at all sites, except for Klosterhede (Fig. 2c). Cellobiohydrolase activity increased significantly at Bear Brook and Gårdsjön, and chitinase activity increased significantly at Klosterhede and Gårdsjön upon N addition (Fig. 2a, b). On average, phosphatase activity increased more strongly due to N addition (260%) than chitinase (80%) and cellobiohydrolase activity (150%); however, ratios of phosphatase-to-cellobiohydrolase or chitinase activity were only significantly increased in response to N addition at Harvard Forest (Fig. 2).

Phosphatase activity was negatively correlated with total P concentrations ($R^2 = 0.46$, $P < 0.001$) and positively with total N concentrations ($R^2 = 0.26$, $P < 0.001$) and N:P ratios ($R^2 = 0.65$, $P < 0.001$; Fig. 3). An exponential model best described the negative relationship between phosphatase activity and P concentrations in the organic soil horizons. Phosphatase activity was also strongly positively correlated with N:P and C:P ratios and total C and N concentrations (Online Resource 1). Cellobiohydrolase exhibited similar correlations with these variables, while chitinase activity was only weakly positively correlated with them (Online Resource 1).

Table 2 Characteristics of organic soil horizons of control and N-addition plots at Harvard Forest, Bear Brook, Klosterhede and Gårdsjön

Site	Treatment	Total C (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	Total C stock (kg m^{-2})	Total N stock (g m^{-2})	Total P stock (g m^{-2})
Harvard Forest	Control	168 ± 29	08.3 ± 1.1	1.14 ± 0.10	1.63 ± 0.35	076.1 ± 08.3	11.93 ± 1.33
	+ 50 kg N	215 ± 58	11.5 ± 2.7	**0.89 ± 0.04*****	1.79 ± 0.30	096.7 ± 19.7	**07.83 ± 2.38****
	+ 150 kg N	**245 ± 60***	**13.2 ± 3.2***	**0.74 ± 0.08*****	2.11 ± 0.88	111.6 ± 41.1	**05.39 ± 0.88*****
Bear Brook	Control	278 ± 25	14.8 ± 1.5	0.92 ± 0.18	1.10 ± 0.20	058.6 ± 12.1	03.20 ± 0.45
	+ 25 kg N	297 ± 15	16.7 ± 2.8	0.80 ± 0.10	0.83 ± 0.27	**042.7 ± 13.2***	**02.08 ± 0.74***
Klosterhede	Control	377 ± 26	12.1 ± 1.1	0.42 ± 0.05	4.99 ± 0.35	160.7 ± 14.3	05.56 ± 0.65
	+ 35 kg N	**441 ± 24****	**15.2 ± 0.3*****	**0.50 ± 0.02***	5.38 ± 0.30	**185.3 ± 03.2****	06.08 ± 0.23
Gårdsjön	Control	450 ± 24	14.8 ± 2.6	0.58 ± 0.06	6.30 ± 0.34	207.3 ± 36.6	08.20 ± 0.89
	+ 40 kg N	**491 ± 08****	17.4 ± 1.8	0.56 ± 0.04	**6.87 ± 0.11****	243.8 ± 24.7	07.88 ± 0.53

C:N ratio	C:P ratio	Microbial C (mg g^{-1})	Microbial N (mg g^{-1})	Microbial P (mg g^{-1})	Available N (μg g^{-1})	Available P (μg g^{-1})	pH
23.6 ± 2.0	0380 ± 051	1.20 ± 0.11	0.20 ± 0.02	0.18 ± 0.04	066 ± 012	77.8 ± 29.8	4.7
21.7 ± 1.0	**0629 ± 185***	1.18 ± 0.17	0.21 ± 0.05	0.20 ± 0.03	068 ± 012	**37.9 ± 17.4****	4.4
21.8 ± 1.6	**0877 ± 289****	**0.94 ± 0.17***	0.15 ± 0.01	0.13 ± 0.04	**097 ± 167****	**12.8 ± 04.4*****	**3.5*****
22.0 ± 1.8	0807 ± 167	1.63 ± 0.47	0.32 ± 0.10	0.34 ± 0.09	095 ± 021	39.0 ± 42.1	4.9
22.8 ± 2.8	**1042 ± 100****	1.23 ± 0.19	0.23 ± 0.02	**0.18 ± 0.07****	**188 ± 081***	42.4 ± 20.4	**4.0** **
36.3 ± 1.6	2326 ± 141	1.18 ± 0.19	0.18 ± 0.05	0.13 ± 0.02	006 ± 002	04.8 ± 01.0	4.2
35.0 ± 1.5	2369 ± 139	1.23 ± 0.18	0.18 ± 0.04	0.14 ± 0.03	**015 ± 005****	05.5 ± 01.0	4.1
38.3 ± 3.2	2011 ± 327	2.07 ± 0.29	0.41 ± 0.03	0.24 ± 0.06	003 ± 003	05.3 ± 01.7	4.4
32.2 ± 2.5**	2189 ± 139	1.85 ± 0.35	**0.35 ± 0.05***	0.20 ± 0.07	**054 ± 040****	04.1 ± 02.1	4.3

The stoichiometric ratios were calculated on a molar basis and all values are given as means with standard deviations. Asterisks mark the level of significance for all significant differences between the N-addition treatment and the respective control (bold)

Levels of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Net N and P mineralization rates

Net N and P mineralization was substantially higher in deciduous forests than in coniferous forests ($P < 0.001$). Net N mineralization ranged between 0.09 and 0.56 μmol N g^{-1} day^{-1} at the deciduous forest sites (Harvard Forest, Bear Brook) and between 0.02 and 0.05 μmol N g^{-1} day^{-1} at coniferous forests (Klosterhede, Gårdsjön) across all treatments (Fig. 4). Net P mineralization was between 2.6 and 12.7 nmol P g^{-1} day^{-1} in deciduous forests and 0.0 and 0.4 nmol P g^{-1} day^{-1} in coniferous forests. Net N and P mineralization were only affected by N addition treatments in deciduous organic soil horizons. Net N mineralization increased significantly in the Harvard Forest N150 treatment (+ 290%) and at Bear Brook (+ 210%) compared to the control. Net P mineralization increased significantly in response to N addition at Bear Brook (+ 400%), whereas it decreased significantly in the N50 treatment at Harvard Forest (+ 60%).

Net N mineralization was strongly correlated with several fractions of C, N and P and organic soil horizon C:N:P stoichiometry across all sites and treatments, whereas net P mineralization was only related to P fractions (Online Resource 1). Net N mineralization correlated strongly positively with dissolved organic N (r = 0.89), the N:P and C:P ratios (r = 0.77 and 0.78, respectively), and total N concentrations of the organic horizons (r = 0.59). In addition, net N mineralization was strongly negatively correlated with total P concentrations (r = − 0.74, Online Resource 1).

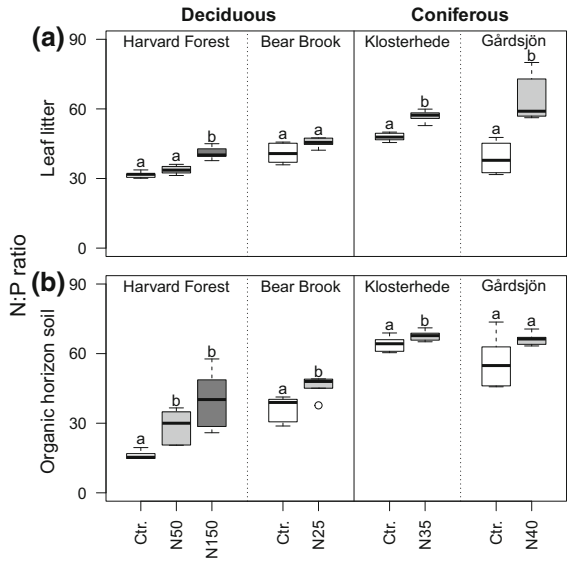

Fig. 1 N:P ratios of **a** leaf litter and **b** organic soil horizons of control and N addition treatments at Harvard Forest, Bear Brook, Klosterhede and Gårdsjön. Different lowercase letters indicate significant site-specific differences ($P < 0.05$)

Discussion

Long-term N addition significantly increased the N:P ratio and phosphatase activity in the organic horizon at three of the four forest sites. This response was particularly obvious in the deciduous forests where the P stock of the organic horizon was also significantly reduced in response to N addition. In the coniferous organic horizons, net N and P mineralization rates were negligible and were not affected by N addition, indicating efficient microbial uptake of these nutrients. In the deciduous organic soil horizons, net N and P mineralization rates were significantly higher than in the coniferous forests and net N mineralization at these sites increased due to high chronic N inputs.

Exoenzyme activity

Phosphatase activity increased strongly in response to long-term N addition (Fig. 2c). This observation, in combination with the positive linear relationship between phosphatase activity and the total N concentrations of the organic soil horizons (Fig. 3), confirms previous findings (Naples and Fisk 2010; Weand et al. 2010b; Marklein and Houlton 2012). The observed relationship between total N concentrations and phosphatase activity may be due to an increased P demand of plants and potentially of microorganisms exposed to long-term N addition (Clarholm 1993; Olander and Vitousek 2000). The high N supply is likely beneficial for the synthesis of N-rich enzymes, including phosphatase (Allison and Vitousek 2005). Phosphatase activity was negatively correlated with total P concentrations and positively correlated with C:P and N:P ratios of the organic soil horizons (Fig. 3, Online Resource 1). This finding is in agreement with previous studies showing that the soil P concentration regulates phosphatase activity (Spiers and McGill 1979; Marklein and Houlton 2012), with declining activity where P is abundant (Juma and Tabatabai

Table 3 Molar microbial biomass C:N, C:P and N:P ratios in the organic soil horizons of long-term N addition experiments at the deciduous forest sites Harvard Forest and Bear Brook and the coniferous forest sites Klosterhede and Gårdsjön

Site	Treatment	C:N ratio	C:P ratio	N:P ratio
Harvard Forest	Control	7.3 ± 1.1	16.8 ± 2.2	2.3 ± 0.3
	N50	6.2 ± 0.4	15.1 ± 1.9	2.3 ± 0.5
	N150	6.6 ± 0.6	19.5 ± 5.9	2.9 ± 0.8
Bear Brook	Control	6.0 ± 0.4	12.6 ± 2.3	2.1 ± 0.5
	N	6.0 ± 0.5	15.7 ± 1.3	2.8 ± 0.4
Klosterhede	Control	8.0 ± 1.7	25.6 ± 2.7	3.3 ± 1.0
	N	7.9 ± 0.8	22.9 ± 3.1	2.9 ± 0.4
Gårdsjön	Control	5.9 ± 0.6	24.4 ± 5.2	4.2 ± 1.2
	N	6.2 ± 0.8	20.9 ± 2.7	3.4 ± 0.5

Values are given as mean with standard deviation (n = 6)

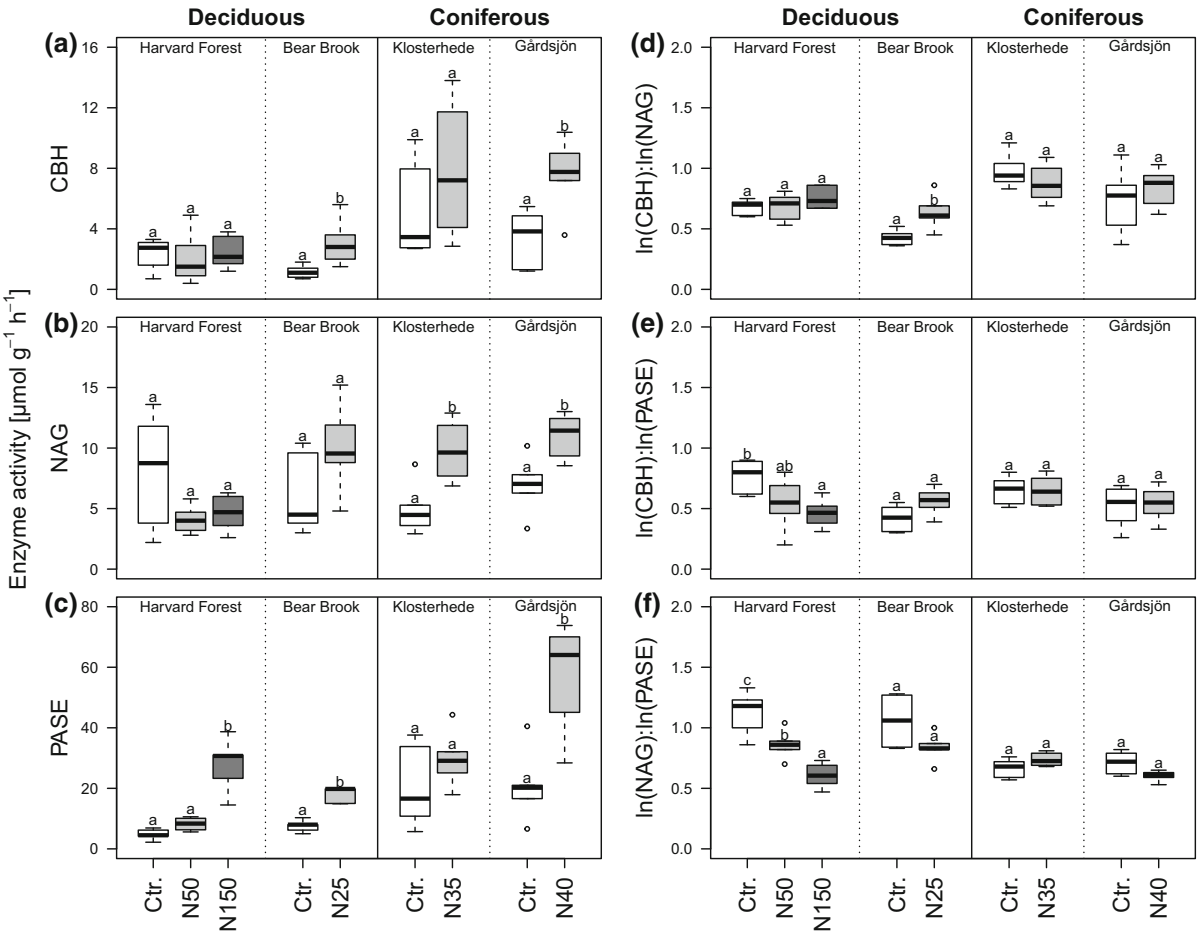

Fig. 2 Activities of the enzymes **a** cellobiohydrolase (CBH), **b** chitinase (NAG), **c** phosphatase (PASE) and ratios of the natural logarithms of specific enzyme activities, **d** CBH:NAG, **e** CBH:PASE, **f** NAG:PASE in the organic soil horizons of control and N addition treatments at Harvard Forest, Bear Brook, Klosterhede and Gårdsjön. Different lowercase letters indicate significant site-specific differences ($P < 0.05$)

1977, 1978; Olander and Vitousek 2000; Moscatelli et al. 2005; Marklein and Houlton 2012).

Cellobiohydrolase and chitinase activity also increased with N addition, mainly in the coniferous forests (Fig. 2a, b), suggesting that N addition at these nutrient poor sites led to increased microbial C demand and facilitated enzyme synthesis of N-rich hydrolytic enzymes. However, the increase in phosphatase activity in response to N addition was much larger than the increase in cellobiohydrolase and chitinase activity, indicating that long-term N addition predominantly increased P demand.

Net N and P mineralization

Net N and P mineralization in the organic horizon were substantially higher in the deciduous forests than in the coniferous forests (Fig. 4). This is most likely because soil microorganisms experienced greater N and P scarcity in the coniferous forest soils compared to the deciduous forest soils, and therefore took up the available forms of the two nutrients almost completely. This is supported by the stoichiometry of the organic soil horizons. The C:N and N:P ratios in the coniferous forests were above the thresholds for net N mineralization in organic soils, which amount to 20–40 for the C:N ratio (Parton et al. 2007; Moore et al. 2011) and 60 for the N:P ratio (Heuck and Spohn

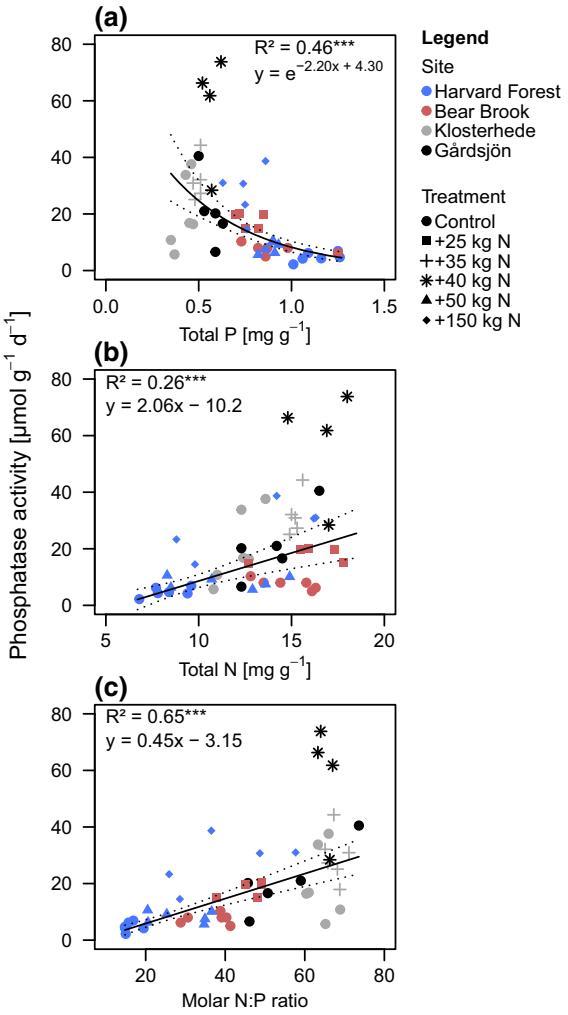

Fig. 3 Relationship between phosphatase activity and **a** total soil P concentration, **b** total soil N concentration and **c** molar N:P ratio of the organic soil horizons. Sites are distinguished by colors (blue: Harvard Forest, red: Bear Brook, grey: Klosterhede, black: Gårdsjön) and treatments are distinguished by symbols (control: circle, + 25 kg N: square, + 35 kg N: plus, + 40 kg N: star, + 50 kg N: triangle, +150 kg N: diamond). An exponential model was fitted for (**a**) and linear models for (**b**) and (**c**). Fitted lines are presented with 95%-confidence intervals (dotted lines), R^2 and the regression equations. Levels of significance are: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

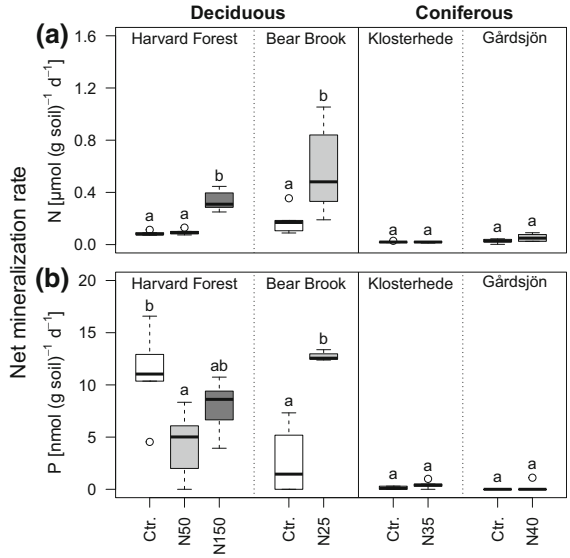

Fig. 4 Net mineralization rates of **a** N and **b** P in organic soil horizons of control and N addition treatments at Harvard Forest, Bear Brook, Klosterhede and Gårdsjön. Different lowercase letters indicate significant site-specific differences ($P < 0.05$)

2016). Above these thresholds, N availability is so low that no net release of inorganic N occurs, and microorganisms immobilize all N when decomposing organic matter (Parton et al. 2007; Moore et al. 2011; Heuck and Spohn 2016; Spohn 2016). Analogously, net P mineralization was not observed in the coniferous forests, presumably because the threshold C:P (300–1700, Blair 1988; Moore et al. 2011) and N:P ratios (40, Heuck and Spohn 2016) for net P mineralization were exceeded. In the deciduous organic soil horizons, N addition increased microbial net N mineralization. This may have occurred because available N concentrations exceeded microbial N demand due to N addition, and thus microbes released surplus N in inorganic forms during organic matter decomposition (Prescott et al. 1992). This likely allowed the microbes to maintain their biomass C:N:P stoichiometry (Table 3) despite changes in organic matter stoichiometry (Table 2), and is in agreement with the theory of the microbial biomass stoichiometry being homeostatic (Cleveland and Liptzin 2007; Xu et al. 2013; Spohn 2016). In addition, variation in the microbial community composition of deciduous and coniferous organic horizons may have contributed to the differences in net N and P mineralization rates because of higher fungal:bacterial ratios in soils of temperate coniferous forests than in deciduous forest soils (Fierer et al. 2009).

Despite a consistent increase in phosphatase activity in response to N addition, we did not find a consistent increase in net P mineralization. In deciduous forests, net P mineralization both increased (Bear Brook) and decreased (Harvard Forest), whereas in the coniferous forests, net P mineralization rates were

very small across all treatments. Elevated phosphatase activity only increases net P mineralization if substrate concentrations are sufficiently high, and the microbial P demand is satisfied. This seemed to be the case at Bear Brook, but not at Harvard Forest. Another possible explanation for these divergent responses of the two decideous sites is that different forms of N were added to soils at the two sites. Ammonium nitrate was added at Harvard Forest, whereas ammonium sulfate was used at Bear Brook. The added sulfate may have contributed to the additional P release, because sulfate might exchange with sorbed phosphate (Geelhoed et al. 1997).

Taken together, in deciduous organic soil horizons high rates of net N and P mineralization (Fig. 4) led to the formation of high concentrations of inorganic N and P (Table 2) that are potentially plant available. In contrast, in the coniferous organic soil horizons, net rates of N and P mineralization, and concentrations of plant available inorganic N and P were much lower (Table 2). Thus, in the deciduous but not in the coniferous forests, increased microbial net N and P mineralization may have facilitated increased plant nutrient uptake. The significant declines in P concentrations and P stocks in the deciduous organic soil horizons in response to long-term N addition (Table 2) most likely resulted from an increased P demand of the plants that led to increased P uptake. The increased P demand was likely caused by the high availability of N. This is supported by increased aboveground biomass and annual net primary productivity of trees in the N addition plots at Harvard Forest (Magill et al. 2004; Savage et al. 2013). Yet, aboveground biomass was unaffected by N addition at Bear Brook (Elvir et al. 2010), which might be due to the addition of sulfate together with N that may have led to desorption of adsorbed phosphate (see above). Substantial net P mineralization in the organic horizons of the deciduous forests likely allowed for transfer of inorganic P from the organic soil horizons into the vegetation by plant uptake or into the mineral soil by leaching. In the long-term, this may force plants to acquire P from the mineral soil, where P strongly adsorbs to minerals and therefore is more difficult to obtain.

Nutrient stocks of the organic horizon

The significant decrease in P stocks of the organic horizon in the two deciduous forests (Table 2) can most likely be attributed to increased plant P uptake caused by the high N availability (see above). The organic horizon is an important pool of P for forest nutrition (Ponge 2003; Huang and Spohn 2015; Spohn et al. 2018), and a significant decline in P stocks of this pool might lead to plant P limitation in the long-term if N inputs remain high. Foliar P concentrations of several deciduous tree species are currently decreasing in Europe (Duquesnay et al. 2000; Ilg et al. 2009; Talkner et al. 2015). The reason for this decrease is not known. However, our study indicates that one possible explanation is atmospheric N deposition.

The N:P ratio of leaf litter and the organic soil horizon increased at most sites due to long-term N addition (Fig. 1), which is in agreement with previous results for foliage and the organic soil horizon of the site Gårdsjön (Kjønaas et al. 1998; Kjønaas and Stuanes 2008). The increase in the N:P ratios resulted from the decreased P stocks as well as from the large N additions that increased organic horizon N stocks either directly due to incorporation of added N in the organic horizon (Magill et al. 1997; Gundersen 1998) or indirectly due to increased foliar N concentrations (Aber et al. 1993; Gundersen 1998; White et al. 1999; Magill et al. 2004; Kjønaas and Stuanes 2008; Fernandez and Norton 2010).

Conclusions

Long-term N addition consistently increased phosphatase activities and N:P ratios of the organic horizons in both coniferous and deciduous temperate forests, indicating increased plant P demand, which confirms our first two hypotheses. In the deciduous forests, net N and P mineralization rates were substantially higher than in the coniferous forests. In contrast to our third hypothesis, high phosphatase activities caused by N addition did not consistently translate into high net P mineralization rates. This can be attributed to the organic horizon stoichiometry that differed among deciduous and coniferous forests, and to a very efficient immobilization of P by the microbial biomass in the coniferous organic horizons. We observed a decline in the P stocks of the organic horizons due to N addition in the deciduous but not in the coniferous forests. The decline in P stocks of the deciduous organic horizons indicates that in the long-term, high N inputs might lead to decreased plant P

uptake and even to plant P limitation in deciduous forests.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. Bioscience 39:378–386. https://doi.org/10.2307/1311067

Aber JD, Magill A, Boone R et al (1993) Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecol Appl 3:156–166

Aber JD, Magill A, Mcnulty SG et al (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. Water Air Soil Pollut 85:1665–1670. https://doi.org/10.1007/BF00477219

Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37:937–944. https://doi.org/10.1016/j.soilbio.2004.09.014

Andersson BI, Bishop KH, Borg GC et al (1998) The covered catchment site: a description of the physiography, climate and vegetation of three small coniferous forest catchments at Gårdsjön, South-west Sweden. In: Hultberg H, Skeffington R (eds) Experimental reversal of acid rain effects—the Gårdsjön roof project. Wiley, Chichester, pp 25–70

Blair JM (1988) Nitrogen, sulfur and phosphorus dynamics in decomposing deciduous leaf litter in the southern Appalachians. Soil Biol Biochem 20:693–701. https://doi.org/10.1017/CBO9781107415324.004

Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59:39–45

Brookes PC, Powlson DS, Jenkinson DS (1984) Phosphorus in the soil microbial biomass. Soil Biol Biochem 16:169–175

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Bünemann EK, Marschner P, McNeill AM, McLaughlin MJ (2007) Measuring rates of gross and net mineralisation of organic phosphorus in soils. Soil Biol Biochem 39:900–913. https://doi.org/10.1016/j.soilbio.2006.10.009

Carrara JE, Walter CA, Hawkins JS et al (2018) Interactions among plants, bacteria, and fungi reduce extracellular enzyme activities under long-term N fertilization. Glob Chang Biol. https://doi.org/10.1111/gcb.14081

Clarholm M (1993) Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. Biol Fertil Soils 16:287–292. https://doi.org/10.1007/BF00369306

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235–252

Crowley KF, McNeil BE, Lovett GM et al (2012) Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? Ecosystems 15:940–957. https://doi.org/10.1007/s10021-012-9550-2

Currie WS, Aber JD, Driscoll CT (1999) Leaching of nutrient cations from the forest floor: effects of nitrogen saturation in two long-term manipulations. Can J For Res 29:609–620. https://doi.org/10.1139/x99-033

Deng Q, Hui D, Dennis S, Reddy KC (2017) Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: a meta-analysis. Glob Ecol Biogeogr 26:713–728. https://doi.org/10.1111/geb.12576

Dise NB, Wright RF (eds) (1992) The NITREX project (Nitrogen saturation experiments). Commission of European Communities, Brussels

Duquesnay A, Dupouey JL, Clement A et al (2000) Spatial and temporal variability of foliar mineral concentration in beech (*Fagus sylvatica*) stands in northeastern France. Tree Physiol 20:13–22

Elvir JA, Wiersma GB, Bethers S, Kenlan P (2010) Effects of chronic ammonium sulfate treatment on the forest at the Bear Brook Watershed in Maine. Environ Monit Assess 171:129–147. https://doi.org/10.1007/s10661-010-1523-3

Fatemi FR, Fernandez IJ, Simon KS, Dail DB (2016) Nitrogen and phosphorus limitations to soil enzyme activities in acid forest soils. Soil Biol Biochem 98:171–179. https://doi.org/10.1016/j.soilbio.2016.02.017

Fernandez IJ, Norton SA (2010) The bear brook watershed in maine—the second decade: preface. Environ Monit Assess 171:1–2. https://doi.org/10.1007/s10661-010-1530-4

Fernandez IJ, Adams MB, SanClements MD, Norton SA (2010) Comparing decadal responses of whole-watershed manipulations at the Bear Brook and Fernow experiments. Environ Monit Assess 171:149–161. https://doi.org/10.1007/s10661-010-1524-2

Fierer N, Strickland MS, Liptzin D et al (2009) Global patterns in belowground communities. Ecol Lett 12:1238–1249. https://doi.org/10.1111/j.1461-0248.2009.01360.x

Flückiger W, Braun S (1998) Nitrogen deposition in Swiss forests and its possible relevance for leaf nutrient status, parasite attacks and soil acidification. Environ Pollut 102:69–76

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. For Ecol Manage 196:159–171. https://doi.org/10.1016/j.foreco.2004.03.018

Gallo M, Amonette R, Lauber C et al (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microb Ecol 48:218–229. https://doi.org/10.1007/s00248-003-9001-x

Galloway AJN, Dentener FJ, Capone DG et al (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Geelhoed JS, van Riemsdijk WH, Findenegg GR (1997) Effects of sulphate and pH on the plant-availability of phosphate adsorbed on goethite. Plant Soil 197:241–249. https://doi.org/10.1023/A:1004228715984

German DP, Weintraub MN, Grandy AS et al (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

Goswami S, Fisk MC, Vadeboncoeur MA et al (2018) Phosphorus limitation of aboveground production in northern hardwood forests. Ecology 99:438–449. https://doi.org/10.1002/ecy.2100

Gradowski T, Thomas SC (2006) Phosphorus limitation of sugar maple growth in central Ontario. For Ecol Manage 226:104–109. https://doi.org/10.1016/j.foreco.2005.12.062

Gradowski T, Thomas SC (2008) Responses of *Acer saccharum* canopy trees and saplings to P, K and lime additions under high N deposition. Tree Physiol 28:173–185. https://doi.org/10.1093/treephys/28.2.173

Gress SE, Nichols TD, Northcraft CC, Peterjohn WT (2007) Nutrient limitation in soils exhibiting differing nitrogen availabilities: what lies beyond nitrogen saturation? Ecology 88:119–130. https://doi.org/10.1890/0012-9658(2007)88%5b119:NLISED%5d2.0.CO;2

Gundersen P (1998) Effects of enhanced nitrogen deposition in a spruce forest at Klosterhede, Denmark examined by moderate NH4NO3 addition. For Ecol Manage 101:251–268

Gundersen P, Rasmussen L (1995) Nitrogen mobility in a nitrogen limited forest at Klosterhede, Denmark, examined by NH4NO3 addition. For Ecol Manage 71:75–88. https://doi.org/10.1016/0378-1127(94)06085-W

Herold N, Schöning I, Berner D et al (2014) Vertical gradients of potential enzyme activities in soil profiles of European beech, Norway spruce and Scots pine dominated forest sites. Pedobiologia (Jena) 57:181–189. https://doi.org/10.1016/j.pedobi.2014.03.003

Heuck C, Spohn M (2016) Carbon, nitrogen and phosphorus net mineralization in organic horizons of temperate forests: stoichiometry and relations to organic matter quality. Biogeochemistry 131:229–242. https://doi.org/10.1007/s10533-016-0276-7

Houdijk ALFM, Roelofs JGM (1993) The effects of atmospheric nitrogen deposition and soil chemistry on the nutritional status of *Pseudotsuga menziesii*, *Pinus nigra* and *Pinus sylvestris*. Environ Pollut 80:79–84

Huang W, Spohn M (2015) Effects of long-term litter manipulation on soil carbon, nitrogen, and phosphorus in a temperate deciduous forest. Soil Biol and Biochem 83:12–18

Ilg K, Wellbrock N, Lux W (2009) Phosphorus supply and cycling at long-term forest monitoring sites in Germany. Eur J For Res 128:483–492. https://doi.org/10.1007/s10342-009-0297-z

Jefts S, Fernandez IJ, Rustad LE, Dail DB (2004) Decadal responses in soil N dynamics at the Bear Brook Watershed in Maine, USA. For Ecol Manage 189:189–205. https://doi.org/10.1016/j.foreco.2003.08.011

Jenkinson DS, Brookes PC, Powlson DS (2004) Measuring soil microbial biomass. Soil Biol Biochem 36:5–7. https://doi.org/10.1016/j.soilbio.2003.10.002

Jian S, Li J, Chen J et al (2016) Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biol Biochem 101:32–43. https://doi.org/10.1016/j.soilbio.2016.07.003

Joergensen RG, Anderson T-H, Wolters V (1995) Carbon and nitrogen relationships in the microbial biomass of soils in beech (*Fagus sylvatica* L.) forests. Biol Fertil Soils 19:141–147. https://doi.org/10.1007/BF00336150

Jonard M, Fürst A, Verstraeten A et al (2015) Tree mineral nutrition is deteriorating in Europe. Glob Chang Biol 21:418–430. https://doi.org/10.1111/gcb.12657

Juma NG, Tabatabai MA (1977) Effects of trace elements on phosphatase activity in soils. Soil Sci Soc Am J 41:343. https://doi.org/10.2136/sssaj1977.03615995004100020034x

Juma NG, Tabatabai MA (1978) Distribution of phosphomonoesterase in soils. Soil Sci 126:101–108

Khan KS, Joergensen RG (2012) Relationships between P fractions and the microbial biomass in soils under different land use management. Geoderma 173–174:274–281. https://doi.org/10.1016/j.geoderma.2011.12.022

Kjønaas OJ, Stuanes AO (2008) Effects of experimentally altered N input on foliage, litter production and increment in a Norway spruce stand, Gårdsjön, Sweden over a 12 year period. Int J Environ Stud 65:431–463

Kjønaas OJ, Stuanes AO, Huse M (1998) Effects of weekly nitrogen additions on N cycling in a coniferous forest catchment, Gårdsjön, Sweden. For Ecol Manage 101:227–249

Koller M, Stahel WA (2011) Sharpening Wald-type inference in robust regression for small samples. Comput Stat Data Anal 55:2504–2515

Li Y, Niu S, Yu G (2016a) Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. Glob Chang Biol 22:934–943. https://doi.org/10.1111/gcb.13125

Li Y, Schichtel BA, Walker JT et al (2016b) Increasing importance of deposition of reduced nitrogen in the United States. PNAS 113:5874–5879. https://doi.org/10.1073/pnas.1525736113

Lovett GM, Arthur MA, Weathers KC et al (2013) Nitrogen addition increases carbon storage in soils, but not in trees, in an eastern U.S. deciduous forest. Ecosystems 16:980–1001. https://doi.org/10.1007/s10021-013-9662-3

Magill AH, Aber JD, Hendricks JJ et al (1997) Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecol Appl 7:402–415

Magill AH, Aber JD, Currie WS et al (2004) Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. For Ecol Manage 196:7–28. https://doi.org/10.1016/j.foreco.2004.03.033

Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol 193:696–704. https://doi.org/10.1111/j.1469-8137.2011.03967.x

Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol

Biochem 33:1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7

Mohren GMJ, Van Den Burg J, Burger FW (1986) Phosphorus deficiency induced by nitrogen input in Douglas fir in the Netherlands. Plant Soil 95:191–200. https://doi.org/10.1007/BF02375071

Moldan F, Wright RF (2011) Nitrogen leaching and acidification during 19 years of NH4NO3 additions to a coniferous-forested catchment at Gårdsjön, Sweden (NITREX). Environ Pollut 159:431–440. https://doi.org/10.1016/j.envpol.2010.10.025

Moldan F, Kjønaas OJ, Stuanes AO, Wright RF (2006) Increased nitrogen in runoff and soil following 13 years of experimentally increased nitrogen deposition to a coniferous-forested catchment at Gårdsjön, Sweden. Environ Pollut 144:610–620. https://doi.org/10.1016/j.envpol.2006.01.041

Moore TR, Trofymow JA, Prescott CE et al (2011) Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. Plant Soil 339:163–175. https://doi.org/10.1007/s11104-010-0563-3

Moscatelli MC, Lagomarsino A, De Angelis P, Grego S (2005) Seasonality of soil biological properties in a poplar plantation growing under elevated atmospheric CO_2. Appl Soil Ecol 30:162–173. https://doi.org/10.1016/j.apsoil.2005.02.008

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Naeth MA, Bailey AW, Chanasyk DS, Pluth DJ (1991) Water holding capacity of litter and soil organic matter in mixed prairie and fescue. J Range Manag 44:13–17. https://doi.org/10.2307/4002629

Naples BK, Fisk MC (2010) Belowground insights into nutrient limitation in northern hardwood forests. Biogeochemistry 97:109–121. https://doi.org/10.1007/s10533-009-9354-4

Norton S, Kahl J, Fernandez I et al (1999) The Bear Brook Watershed, Maine (BBWM), USA. Environ Monit Assess 55:7–51

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49:175–190

Parton W, Silver WL, Burke IC et al (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–365

Pohlert T (2014) The pairwise multiple comparison of mean ranks package (PMCMR). R package

Ponge J-F (2003) Humus forms in terrestrial ecosystems: a framework to biodiversity. Soil Biol Biochem 35:935–945. https://doi.org/10.1016/S0038-0717(03)00149-4

Prescott CE, Corbin JP, Parkinson D (1992) Availability of nitrogen and phosphorus in the forest floors of Rocky Mountain coniferous forests. Plant Soil 143:1–10

R Core Team (2015) R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria

Rousseeuw P, Croux C, Todorov V, et al (2015) Robustbase: Basic robust statistics. R package

SanClements MD, Fernandez IJ, Norton SA (2010) Soil chemical and physical properties at the Bear Brook Watershed in Maine, USA. Environ Monit Assess 171:111–128. https://doi.org/10.1007/s10661-010-1531-3

Savage KE, Parton WJ, Davidson EA et al (2013) Long-term changes in forest carbon under temperature and nitrogen amendments in a temperate northern hardwood forest. Glob Chang Biol 19:2389–2400. https://doi.org/10.1111/gcb.12224

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602. https://doi.org/10.1890/03-8002

Schwede DB, Lear GG (2014) A novel hybrid approach for estimating total deposition in the United States. Atmos Environ 92:207–220

Seftigen K, Moldan F, Linderholm HW (2013) Radial growth of Norway spruce and Scots pine: effects of nitrogen deposition experiments. Eur J For Res 132:83–92. https://doi.org/10.1007/s10342-012-0657-y

Simpson D, Andersson C, Christensen JH et al (2014) Impacts of climate and emission changes on nitrogen deposition in Europe: a multi-model study. Atmos Chem Phys 14:6995–7017. https://doi.org/10.5194/acp-14-6995-2014

Sinsabaugh RL, Follstad Shah JJ (2012) Ecoenzymatic stoichiometry and ecological theory. Annu Rev Ecol Evol Syst 43:313–343. https://doi.org/10.1146/annurev-ecolsys-071112-124414

Sinsabaugh RL, Lauber CL, Weintraub MN et al (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Sinsabaugh RL, Hill BH, Shah JJF (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 468:122. https://doi.org/10.1038/nature09548

Spiers GA, McGill WB (1979) Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. Soil Biol Biochem 11:3–8. https://doi.org/10.1016/0038-0717(79)90110-X

Spohn M (2016) Element cycling as driven by stoichiometric homeostasis of soil microorganisms. Basic Appl Ecol 17:471–478

Spohn M, Zavišić A, Nassal P, Bergkemper F, Schulz S, Marhan S, Polle A (2018) Temporal variations of phosphorus uptake by soil microbial biomass and young beech trees in two forest soils with contrasting phosphorus stocks. Soil Biol Biochem 117:191–202

Talkner U, Meiwes KJ, Potočić N et al (2015) Phosphorus nutrition of beech (*Fagus sylvatica* L.) is decreasing in Europe. Ann For Sci 72:919–928. https://doi.org/10.1007/s13595-015-0459-8

Tessier JT, Raynal DJ (2003) Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J Appl Ecol 40:1–12. https://doi.org/10.1046/j.1365-2664.2003.00820.x

Treseder KK, Vitousek PM (2001) Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology 82:946–954

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Vet R, Artz RS, Carou S et al (2014) A global assessment of precipitation chemistry and deposition of sulfur, nitrogen, sea salt, base cations, organic acids, acidity and pH, and

phosphorus. Atmos Environ 93:3–100. https://doi.org/10.1016/j.atmosenv.2013.10.060

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115. https://doi.org/10.1007/BF00002772

Waldrop MP, Zak DR (2006) Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9:921–933. https://doi.org/10.1007/s10021-004-0149-0

Wang Y-P, Houlton BZ, Field CB (2007) A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production. Global Biogeochem Cycles 21:1–15. https://doi.org/10.1029/2006GB002797

Weand MP, Arthur MA, Lovett GM et al (2010a) Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. Soil Biol Biochem 42:2161–2173. https://doi.org/10.1016/j.soilbio.2010.08.012

Weand MP, Arthur MA, Lovett GM et al (2010b) The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization. Biogeochemistry 97:159–181. https://doi.org/10.1007/s10533-009-9364-2

White G, Fernandez I, Wiersma G (1999) Impacts of ammonium sulfate treatment on the foliar chemistry of forest trees at the Bear Brook Watershed in Maine. Environ Monit Assess 55:235–250

Wright RF, van Breemen N (1995) The NITREX project: an introduction. For Ecol Manage 71:1–5. https://doi.org/10.1016/0378-1127(94)06080-3

Xing J, Pleim J, Mathur R et al (2013) Historical gaseous and primary aerosol emissions in the United States from 1990 to 2010. Atmos Chem Phys 13:7531–7549. https://doi.org/10.5194/acp-13-7531-2013

Xu X, Thornton PE, Post WM (2013) A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Glob Ecol Biogeogr 22:737–749

Yohai VJ (1987) High breakdown-point and high efficiency robust estimates for regression. Ann Stat 15:642–656. https://doi.org/10.1214/aos/1176348654