Biogeochemistry (2012) 109:189–202
DOI 10.1007/s10533-011-9619-6

Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests

Jared L. DeForest · Kurt A. Smemo · David J. Burke · Homer L. Elliott · Jane C. Becker

Received: 22 April 2011 / Accepted: 29 June 2011 / Published online: 16 July 2011

Abstract Although northern temperate forests are generally not considered phosphorus (P) limited, ecosystem P limitation may occur on highly weathered or strongly acidic soils where bioavailable inorganic P is low. In such environments, soil organisms may compensate by increasing the utilization of organic P via the production of extracellular enzymes to prevent limitation. In this study, we experimentally increased available P and/or pH in several acidic eastern deciduous forests underlain by glaciated and unglaciated soils in eastern Ohio, USA. We hypothesized that where inorganic P is low; soil microbes are able to access organic P by increasing production of phosphatase enzymes, thereby overcoming biogeochemical P limitations. We measured surface soil for: available P pools, N mineralization and nitrification rates, total C and N, enzymes responsible for C, N, and P hydrolysis, and microbial community composition (PLFA). Increasing surface soil pH a whole unit had little effect on microbial community composition, but increased N cycling rates in unglaciated soils. Phosphorus additions suppressed phosphatase activities over 60% in the unglaciated soils but were unchanged in the glaciated soils. All treatments had minimal influence on microbial biomass, but available pools of P strongly correlated with microbial composition. Microbes may be dependent on sources of organic P in some forest ecosystems and from a microbial perspective soil pH might be less important overall than P availability. Although our sampling was conducted less than 1 year after treatment initiation, microbial community composition was strongly influenced by available P pools and these effects may be greater than short-term increases in soil pH.

Keywords Enzyme activity · Lime fertilization · Phosphorus fertilization · PLFA · Temperate forests

J. L. DeForest (✉) · H. L. Elliott · J. C. Becker
Department of Environmental and Plant Biology, Ohio University, Porter Hall 315, Athens, OH 45701, USA
e-mail: deforest@ohio.edu

K. A. Smemo · D. J. Burke
The Holden Arboretum, Kirtland, OH 44094, USA

K. A. Smemo
Department of Biological Sciences, Kent State University, Kent, OH 44242, USA

D. J. Burke
Department of Biology, Case Western Reserve University, Cleveland, OH 44106, USA

Introduction

In temperate forest ecosystems, the exact relationship between available soil phosphorus (P) and microbial composition and function is poorly understood, especially when compared to soil nitrogen (N). Most P biogeochemistry research is in agricultural or tropical ecosystems (Tanner 1981; Crews et al.

1995; Townsend et al. 2002). This is largely based on the well-recognized observation that N appears to limit productivity in temperate forests (Vitousek and Howarth 1991; Aber et al. 1993), while P appears to be limiting in agricultural (Mitchell et al. 2008) or tropical systems (Walker and Syers 1976; Vitousek and Sanford 1986; Elser et al. 2007). As such, P fertilization experiments in temperate forests are rare, and observational P cycling studies on natural gradients often have confounding factors that strongly correlate with P availability, like soil age or pH, making it difficult to separate P effects from the influence of other soil properties.

Soil pH, in particular, poses a problem because it can strongly influence the structure and function of microbial communities (Bååth and Anderson 2003; Fierer and Jackson 2006; Rousk et al. 2010). However, in an observational study, DeForest and Scott (2010) provide evidence that the influence of soil pH on microbial community composition may be a consequence of P availability in acidic temperate ecosystems. When specifically studied, insufficient microbial P availability altering function and community appears widespread and has been observed in boreal forests (Lagerström et al. 2009), temperate forests (Gallardo and Schlesinger 1994; Gress et al. 2007; DeForest and Scott 2010), and moist tropical forests (Cleveland et al. 2002; Krashevska et al. 2010), but uncertainty with these relationships is also widespread (Rojo et al. 1990; Cruz et al. 2009; Lagerström et al. 2009; Ehlers et al. 2010). Furthermore, P can also limit microbial growth in temperate (Demetz and Insam 1999) and tropical forests (Duah-Yentumi et al. 1998).

Insufficient P availability in terrestrial ecosystems can be caused by a variety of factors (Vitousek et al. 2010). With time, soils can become depleted in inorganic P through the weathering and subsequent leaching of primary minerals (Walker and Syers 1976; Porder et al. 2007) while other ecosystems can have low P due to P deficient parent material or slow weathering rates (Crews et al. 1995). Some ecosystems may have ample P in parent material, but availability is low because the system is N saturated (Walker and Syers 1976; Phoenix et al. 2004) or inorganic P is geochemically bound to aluminum (Al) and iron (Fe) complexes (Goldberg et al. 1996; Giesler et al. 2002). Because the mobilization of these metals increases with acidity, P availability can decrease with soil pH (Thomas and Hargrove 1984). Regardless of the cause, P availability may have a major influence on soil microbial structure and biogeochemical function when in short supply.

If inorganic P sources are limiting, then P from organic sources becomes the most important P pool for soil microbes (McGill and Cole 1981). While soil microbes can utilize organic P via several mechanisms, the most significant is the production of extracellular phosphatase enzymes (Vance et al. 2003). Phosphatases are hydrolytic enzymes that targets phosphate ester bonds. Phosphatase enzyme activity is suppressed by soluble inorganic P (McGill and Cole 1981; Attiwill and Adams 1993; Olander and Vitousek 2000), which suggests phosphatase enzymes are produced in response to insufficient supply of readily available P. Producing these enzymes is likely a significant microbial resource investment and can be used as a biological indicator of bioavailable P. As such, soil microorganisms have the capacity to ameliorate potential P limitation of primary producers and could explain why widespread P limitation is not observed in some ecosystems; especially where P limitation is expected due to high N availability from anthropogenic sources (Elser et al. 2007; Finzi 2009; Weand et al. 2010). It remains to be determined if mediating P biogeochemical cycling significantly impacts microbial C or N cycling.

In this study, we investigated the effects of soil P availability and acidity on microbial community composition and function, and whether soil microbes may be P limited in temperate hardwood forests. We hypothesized that (1) soil microbes in acidic forests experience low P availability and will respond to short-term increases in P availability; (2) where inorganic P is low, soil microbes are able to access organic P using phosphatase enzymes, overcoming biogeochemical P limitation; and (3) where the availability of inorganic P is low, microbial community composition will be more influenced by soil P than soil acidity. Furthermore, because overcoming microbial P limitation by phosphatase production will exert an energetic cost on soil microbes, we expect alleviating the low P availability will result in increases in soil organic matter decomposition as evident by elevated enzyme activities responsible for C and N hydrolysis. We tested our hypothesis by experimentally manipulating soil pH and readily

available phosphorus in several acidic temperate broadleaf forests on glaciated (younger) and unglaciated (older) soils in eastern Ohio.

Materials and methods

Study sites

The study sites are located in two different physiographic regions in eastern Ohio, USA (Fig. 1). The study was conducted in three sites in each region: the glaciated sites are managed by The Holden Arboretum and Case Western Reserve University and unglaciated sites are managed by the Division of Wildlife and Division of Forestry within the Ohio Department of Natural Resources (Table 1). Within each region, forests were selected based on the natural variation found within eastern deciduous forested ecosystems (Table 1). The primary regional difference is the forests in the northern region are on till plains that were deglaciated ~15,000 years ago, whereas the southern region is on unglaciated Allegheny Plateau. For the glaciated region, annual total precipitation averages 120 cm (average of 287 cm snowfall) with an average temperature of 8.1°C. The annual precipitation for the unglaciated region is 100 cm (average of 59 cm snowfall) with an average temperature of 10.7°C.

Fig. 1 Study areas (*dots*) in temperate deciduous forest in glaciated and unglaciated eastern Ohio, USA

Experimental design

The experiment was a randomized complete-block design with two regions (glaciated vs. unglaciated) and four treatments. The treatments included a control (i.e. ambient soil conditions) elevated pH, elevated P, and elevated pH + P. Each region has three forest stands (i.e., blocks) with three replicate plots (Table 1). In August 2009, 800 m^2 (20 × 40 m) plots were established and arranged over approximately a nine hectare area within a block. Distance between regions was 250 km and distance between blocks within a region was at least 1 km, but no more than 15 km. Overall, each treatment within a region had nine replicate plots with a total of 72 plots for the entire experiment. Treatment selection for each plot was chosen randomly.

Prior to leaf fall in October 2009, Hi-Ca lime (The Andersons, Maumee, OH, USA) was dispensed using a hand operated spreader. Enough lime was added with the intent to increase soil pH to at least a range between 5.8 and 6.2 for surface soil (top 7 cm). This pH range is the lowest pH to immobilize most reactive Al in these soils (DeForest and Scott 2010). Lime requirement was plot specific and determined using a SMP single buffer method (Sims 1996). The glaciated sites received an average of 11.4 Mg ha^{-1} of Hi-Ca lime and the unglaciated site received an average of 7.3 Mg ha^{-1} of Hi-Ca lime. Phosphate was added as triple super phosphate (TSP; The Andersons, Maumee, OH, USA) prior to leaf fall in October, 2009 and June, 2010. Phosphate requirement was consistent for the experiment and was determined by averaging P sorption (Tiessen et al. 1991) for each forest block. A total of 41.8 kg P ha^{-1} of TSP was added to each of the elevated P and elevated pH + P treatments.

In July 2009, nine mineral (A horizon) soil samples were combined per plot from a 2-cm core to a depth of 5 cm. Samples were placed in plastic bags, kept cool, and homogenized by passing through a 2-mm mesh sieve within 18 h of collection. Samples were kept 'field fresh' by storing at 4°C until analysis. A subsample was lyophilized shortly after sieving for PLFA analysis.

Table 1 Summary of site and soil properties for study area in eastern Ohio, USA

Region	Site name	Latitude and longitude	Basal Area (m^2 ha^{-1})	Relative dominance			Sand (%)	Clay (%)	Soil classification
				Acer spp. (%)	*Quercus* spp. (%)	*Fagus* spp. (%)			
Glaciated	Gl	41°36′38″N, 81°18′39″W	36.1	30.1	60.4	0.3	21	27	Aquic Hapludalfs
	G2	41°36′44″N, 81°19′15″W	32.6	68.1	16.1	5.6	18	31	Aeric Endoaqualfs
	G3	41°29′60″N, 81°25′15″W	41.0	30.4	34.3	21.4	24	24	Aeric Endoaqualfs
Unglaciated	U1	39°21′10″N, 82°16′05″W	28.5	10.1	61.1	16.8	39	21	Typic Hapludalfs
	U2	39°20′45″N, 82°15′54″W	33.0	16.3	45.1	8.8	18	24	Typic Dystrudepts
	U3	39°16′34″N, 82°18′59″W	32.4	13.7	74.6	0.2	16	25	Typic Hapludults

Soil chemistry and nutrients

Soil pH was measured with a 1:2 dilution of deionized water. Total acidity and moblized Al ($cmol_c$ kg^{-1}) was determined by the fluoride titration method (Sims 1996) and represents exchangeable acidity or Al. Base cations (Ca, Mg, K, Na) were extracted from a 1:15 gravimetric dilution of soil to 1 M NH_4OAc (pH 7) and analyzed on an ICP-MS (XSeries 2, Thermo Scientific, Waltham, MA). Potential cation exchange capacity (CEC) was determined by the sum of total acidity and base cations, and base saturation determined by dividing the sum of base cations by CEC. Total soil C and N was determined using an ECS 4010 CHNSO elemental analyzer (Costech, Valencia, CA). Concurrently with all analyses, a soil subsample from each plot was dried at 105°C for 48 h to determine gravimetric soil moisture content. These moisture values were used to express values gravimetrically based on dry soil weight (μg g^{-1}).

Inorganic N analysis was performed colorimetrically following methods detailed by DeForest and Scott (2010). Briefly, a salicylate-hypochlorite procedure was used for NH_4^+ (Kempers and Zweers 1986), and a VCl_3/Griess procedure was used for NO_3^- (plus NO_2^-), here reported as NO_3^- (Miranda et al. 2001). A subsample of soil was incubated at 20°C for 14 days near field capacity in the lab then analyzed for NH_4^+ and NO_3^- concentration to determine net mineralization and nitrification rates (Robertson et al. 1999).

Phosphorus was sequentially split into the first three of the Hedley P fractions because these fractions are most likely to influence microbial dynamics within a growing season (Johnson et al. 2003). The most readily available fraction (i.e., PO_4^{3-}) was extracted using anion exchange membranes (AEM; GE Infrastructure: Water & Process Technologies, Watertown, MA, USA). Because this P pool is extremely labile and can be sorbed into other fractions after disturbance (Turner 2005), we immediately extracted these fractions after sieving. Adapted from Lajtha et al. (1999), one 2 × 6 cm AEM strip, charged with $NaHCO_3$, was placed in a high density polyethylene centrifuge tube with approximately a 1:3 dilution of soil and deionized water (DI) and shaken for 4 h. Strips were removed from the soil slurry and rinsed with DI water to remove debris and then shaken in a flask for 18 h with 25 ml of 0.5 M HCl. Resin P fraction was determined using the colorimetric Murphy-Riley molybdate solution where absorbance was measured at 880 nm (Kuo 1996) on a microplate reader (Synergy HT, BioTek, Winoski, VT, USA). The remaining soil slurry from the resin P extraction was centrifuged at 2000 rpm for 10 min and supernatant was discarded. The remaining soil was then sequentially extracted with a 0.5 M $NaHCO_3$ (bicarb P), and then 0.1 M NaOH (hydroxide P). Phosphorus from these fractions was analyzed on an ICP-MS (XSeries 2, Thermo Scientific, Waltham, MA).

Extracellular enzyme activity

Potential extracellular enzyme activity was measured on field fresh soil within 24 h of collection. Sieved soil was stored at 5°C prior to analysis. We measured the activity of two C acquiring enzymes (*β*-1,4-glucosidase and cellobiohydrolase), two N acquiring enzymes (*β*-*N*-acetylglucosaminidase and leucine aminopeptidase), and two P acquiring enzymes (phosphomonoesterase and phosphodiesterase). Soil slurries were prepared by blending approximately 1:100 dilution of soil to 50 mM acetate buffer (pH = 5.0) for 1 min using a Biospec Tissue Tearor.

Hydrolytic enzyme activity was measured in black 96-well plates using fluorgenic methylumbelliferone (MUF)-linked substrates as described in DeForest (2009). Leucine aminopeptidase used L-leucine-7-amino-4-methylcoumarin as the fluorgenic model substrate with 7-amino-4-methylcoumarin (AMC) as the reference (Sigma-Aldrich, St. Louis, MO, USA). The model substrate for phosphodiesterase was bis-(4-MUF)-phosphate (Glycosynth Ltd., Warrington, Cheshire, England). Each plate included blanks (buffer); reference standard (MUF or AMC + buffer); negative controls (substrate + buffer); sample controls (soil slurry + buffer); quench (MUF or AMC + soil slurry); and the assay (soil slurry + substrate). All samples were incubated at 20°C for approximately 2 h based on an enzyme kinetics study for these soils (DeForest, unpublished data). Fluorescence was measured (365-nm excitation and 450-nm emission) using a microplate fluorometer (Synergy HT, BioTek, Winooski, VT, USA). Due to the problems associated with adding NaOH just before measuring fluorescence (DeForest 2009), we elected not to add NaOH. We found that after increasing the sensitivity parameters of the Synergy HT, it was not necessary to add NaOH to get a clear signal with a 2-h incubation time. Calculations are described in DeForest (2009), however, when comparing between regions enzyme activities were expressed per gram of soil and total PLFA biomass.

Phospholipid fatty acid analysis

Soil phospholipid fatty acid composition was analyzed to investigate treatment effects on microbial community composition (individual biomarkers and bacterial: fungal ratios) and total microbial biomass (as indicated by total amount of recognized PLFA biomarkers). Total lipids were extracted using 10 ml of methanol, 5 ml chloroform, and 4 ml phosphate buffer from 5 g of lyophilized soil (White et al. 1979; DeForest et al. 2004). Analytical recovery for the procedure was determined by adding a surrogate phospholipid 19:0 (1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine) standard (Avanti Polar Lipids, Inc., Alabaster, AL, USA). Polar lipids were separated from other lipids using silicic acid solid-phase chromatography columns (500 mg 6 ml^{-1}; Thermo scientific, Waltham, MA, USA). The separated polar lipids were converted to fatty acid methyl esters (FAME) through methanolysis (Guckert et al. 1985). The resulting FAMEs were separated using a HP GC-FID (HP6890 series, Agilent Technologies, Inc. Santa Clara, CA, USA) gas chromatograph. External FAME standards (K101 FAME mix, Grace, Deerfield, IL, USA) were used to determine concentrations.

A total of 73 peaks/biomarkers were identified across all samples using the Sherlock System (v. 6.1, MIDI, Inc., Newark, DE, USA). Standard phospholipid fatty acid nomenclature (e.g., 16:1ω5c) was used where the first number refers to the total number of C atoms; the second number refers to the number of double C bonds; and the third number (after the ω) is the location on the fatty acid chain. Prefix notations '*a*', '*i*', '*cy*', and 'Me' refer to anteiso- and iso-branched fatty acids, methyl and cyclopropane groups, respectively. Suffixes 'c' and 't' refer to cis or trans isomer geometry. We removed very rare PLFA biomarkers if they had low biomass (<1% mol fraction) and occurred in less than 10% of the samples (with apparent random distribution) within a region. In all, 58 biomarkers were used for subsequent data analysis. Because microbial biomass often tracks with soil C, data are expressed as absolute PLFA biomass per gram of soil C. Microbial abundance (% mol fraction) was calculated by dividing individual biomarkers by total PLFA biomass. Fungal bacterial ratios were calculated by dividing the sum of 18:1ω9c and 18:2ω6,9c with the sum of 15:0, a15:0, i15:0, i16:0, a17:0, cy17:0, and i17:0.

Data analysis and statistics

A linear mixed-effect (LME) model was used to determine the effect of the treatments on soil chemistry, extracellular enzyme activity, and microbial biomass and bacterial: fungal ratios. Region and treatments are fixed effects and forest stand (i.e. blocks) is the random effect. This analysis was performed using the 'anova' function downstream from the 'lme' function in the 2.12.0 version of R 'nlme' package (R-Project 2010).

The relationship between soil variables and microbial community composition (i.e., the 58 PLFA biomarkers) was analyzed using nonmetric multidimensional scaling (NMDS). The purpose of this approach is to organize complex relationships between measured soil variables and microbial composition graphically. NMDS analysis was performed using R (R-Project 2010) with the 'metaMDS' function in the

Table 2 Mean values, standard error, and *P* values for control soil properties for glaciated and unglaciated eastern deciduous forests in eastern Ohio, USA ($n = 9$)

Variable	Physiographic region		*P* value
	Glaciated	Unglaciated	
CEC ($cmol_c\ kg^{-1}$)	8.83 ± 1.09	3.27 ± 0.19	0.03
Base saturation (%)	8.5 ± 1.7	23.2 ± 9.0	0.16
NH_4^+ ($mg\ kg^{-1}$)	21.38 ± 0.90	22.56 ± 1.73	0.70
NO_3^+ ($mg\ kg^{-1}$)	20.57 ± 5.11	5.11 ± 0.40	0.01
Net N mineralization ($mg\ N\ kg^{-1}\ day^{-1}$)	4.02 ± 0.98	0.75 ± 0.20	0.08
Nitrification ($mg\ N\ kg^{-1}\ day^{-1}$)	3.13 ± 0.81	0.17 ± 0.14	0.03
Total N ($g\ kg^{-1}$)	4.2 ± 0.4	2.2 ± 0.1	0.01
Total C ($g\ kg^{-1}$)	65.5 ± 6.1	38.6 ± 2.2	0.02
Molar C:N	20.7 ± 0.7	23.9 ± 1.5	0.14
PLFA biomass (nmol g soil C^{-1})	245 ± 23	161 ± 13	0.07
Fungal bacterial ratio	0.12 ± 0.03	0.21 ± 0.02	0.06

'vegan' package. Euclidean dissimilarity index was used for this analysis because it produced the lowest stress compared to other indices (e.g., Gower, Bray, or Kulczynski). Using the 'envfit' function allowed the creation of significant environmental vectors to overlay the ordination. To prevent clutter in the figures, certain soil variables were not included if they were auto-correlated with another variable. For example, we included net N mineralization, but not nitrification ($P < 0.01$; $r = 0.99$). Multiresponse permutation procedure (MRPP) was used to determine if soil chemistry, microbial community function or composition differed between the two regions and between the treatments within a region. This analysis was performed using the 'mrpp' function in the 'vegan' package (R-Project 2010).

The Shapiro–Wilk normality test was used to determine if the data was normally distributed and Bartlett's test was used to determine homoscedasticity within a region and among treatments. If necessary, variables were transformed (e.g. $\log_{10}$) to ensure homogeneity of variance. While pH means are reported, statistical analysis was conducted on H^+. All differences discussed were significant at $P \leq 0.05$ probability level, unless otherwise stated.

Results

Physiographic regions

We confirmed that ambient soil chemical properties were significantly different between the two regions using the multivariate MRPP approach. The delta (δ; weighted mean of within-group distance) between regions was significant ($P < 0.01$), glaciated soils were 76.6 and unglaciated soils were 29.4. The chance corrected within-group agreement (A) was 0.30, which gives a high degree of confidence that these soils are different (McCune and Grace 2002). Overall, the glaciated soils were more fertile with higher total C and N than the unglaciated soils and had greater microbial biomass (Table 2). Microbial biomass (nmol PLFA g^{-1}) without adjusting for soil C was 1521 ± 98 (mean ± SE) in the glaciated soils and 609 ± 39 in the unglaciated soils. Fungal bacteria PLFA ratio was 75% greater in the unglaciated regions when compared to the glaciated region.

Soil properties

Elevated pH treatments significantly ($P < 0.01$) increased soil pH at least a whole unit in both regions (Table 3). We also observed a significantly ($P < 0.01$) greater soil pH in the elevated P treatment in unglaciated soils when compared to the control. However, we do not believe this was caused by the P addition because even though pH between control and P plots were similar pre-treatment ($P = 0.25$), the mean pH of the P treated plots were slightly higher ($\sim 5\%$) than the controls. Regardless, this appears to be a temporary effect because we observed no significant changes in pH from samples collected 2 months after this sampling date (DeForest, unpublished data). Treatments with P addition significantly ($P < 0.01$) increased readily available PO_4^{3-} (i.e., resin P) by

Table 3 Mean values and standard error of surface mineral soil properties in response to experimental treatments for glaciated and unglaciated eastern deciduous forests in eastern Ohio, USA

Region	Treatment	Soil pH	Mobilized Al ($cmol_c\ kg^{-1}$)	Resin P ($mg\ P\ kg^{-1}$)	Bicarb P ($mg\ P\ kg^{-1}$)	Hydroxide P ($mg\ P\ kg^{-1}$)
Glaciated	Control	4.34 ± 0.12	9.20 ± 1.23	0.86 ± 0.11	82.9 ± 6.5	106.3 ± 18.3
	Elevated pH	**5.42 ± 0.21**	**3.44 ± 1.03**	0.58 ± 0.04	**67.9 ± 7.1**	82.9 ± 9.9
	Elevated P	4.43 ± 0.12	8.85 ± 1.14	**8.21 ± 2.08**	74.6 ± 6.1	114.1 ± 22.0
	Elevated pH + P	**5.25 ± 0.15**	**3.53 ± 1.17**	**6.09 ± 1.74**	70.5 ± 6.5	90.6 ± 7.5
Unglaciated	Control	4.68 ± 0.11	4.76 ± 0.55	0.46 ± 0.03	30.4 ± 3.0	39.9 ± 3.3
	Elevated pH	**5.87 ± 0.28**	**1.52 ± 0.59**	0.48 ± 0.04	**19.1 ± 1.2**	42.5 ± 4.6
	Elevated P	**5.27 ± 0.18**	**2.08 ± 0.61**	**5.56 ± 1.03**	26.8 ± 3.7	**54.1 ± 5.3**
	Elevated pH + P	**5.67 ± 0.13**	**0.97 ± 0.27**	**3.72 ± 0.65**	26.5 ± 4.4	**59.1 ± 9.7**

Bold values indicates a significant difference from the mean LME model ($n = 9$)

approximately tenfold, but to a lesser extent in the pH + P treatment (Table 3). The elevated P treatment did not influence the bicarb P fraction, but the elevated pH treatment significantly reduced this fraction for both regions (Table 3). Recalcitrant, but available, P (i.e., hydroxide P) was only significantly increased by P addition in the unglaciated forests. Among the other measured soil chemical properties, only the elevated pH treatment significantly ($P = 0.02$) increased soil nitrate by 47% (Table 4). This treatment also increased net mineralization three-fold (0.8–2.4 mg N kg^{-1} day^{-1}) and nitrification 12-fold (0.2–2.4 mg N kg^{-1} day^{-1}; Table 4). Overall, nitrification strongly correlated ($P < 0.01$; $r = 0.77$) with pH in the unglaciated sites, but not in the glaciated sites ($P = 0.80$; $r = -0.04$; Fig. 2).

Table 4 The main effect of region and treatments on soil properties for all 72 plots

Variable	*P* value			Difference of least squares means *P* value					
				Glaciated			Unglaciated		
	Region	Trt	Region × Trt	Elevated pH	Elevated P	Elevated pH + P	Elevated pH	Elevated P	Elevated pH + P
Water content	0.23	0.58	0.63	0.16	0.37	0.27	0.98	0.51	0.76
Total N	0.02	0.74	0.23	0.08	0.14	0.26	0.36	0.71	0.49
Total C	0.01	0.45	0.44	0.13	0.35	0.48	0.81	0.70	0.21
Molar C:N	0.30	0.62	0.52	0.64	0.46	0.58	0.57	0.46	0.52
NH_4^+	0.61	0.62	0.81	0.43	0.69	0.15	0.58	0.50	0.51
NO_3^-	<0.01	0.91	0.36	0.58	0.43	0.86	0.02	0.16	0.42
Net N mineralization	0.09	0.64	0.18	0.58	0.36	0.82	0.01	0.21	0.35
Nitrification	0.22	0.55	0.10	0.55	0.42	0.89	<0.01	0.09	0.17
β-1,4-Glucosidase	0.07	0.06	0.18	0.03	0.40	0.81	0.45	0.42	0.79
Cellobiohydrolase	<0.01	0.85	0.41	0.96	0.45	0.69	0.25	0.39	0.71
Chitinase	<0.01	0.02	0.02	0.60	0.54	0.99	0.21	0.05	<0.01
Leucine aminopeptidase	0.04	<0.01	<0.01	0.08	0.75	0.02	<0.01	0.95	0.30
Phosphomonoesterase	<0.01	<0.01	<0.01	0.90	0.24	0.49	0.28	0.03	<0.01
Phosphodiesterase	0.03	<0.01	<0.01	0.81	0.11	0.35	0.10	<0.01	<0.01
PLFA biomass	0.08	0.33	0.12	0.98	0.61	0.94	0.36	0.13	0.23
Fungal bacterial ratio	<0.01	0.69	0.30	0.66	0.64	0.99	0.76	0.54	0.14

The difference of least square mean of treatment effects, in comparison to controls, for measured soil properties from a linear mixed effects model where forest blocks are the random effect ($n = 9$)

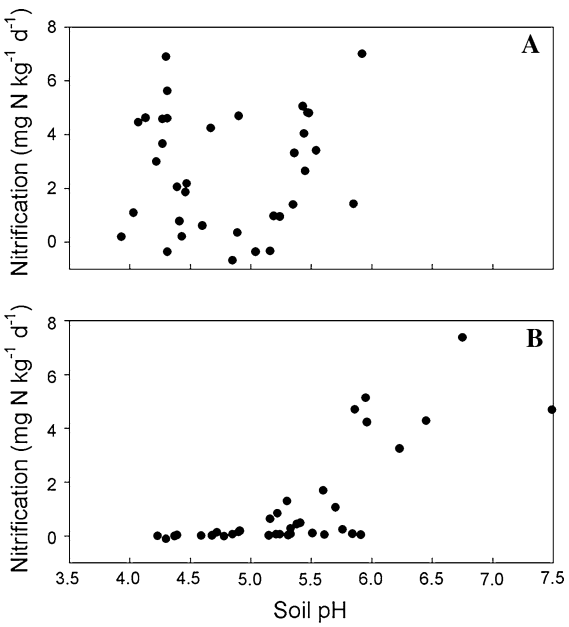

Fig. 2 The relationship between mineral soil pH and nitrification in glaciated (**a**) and unglaciated (**b**) forests ($n = 36$)

Enzymes responsible for C hydrolysis were at least 50% greater in glaciated soils when compared to unglaciated soils. For N hydrolyzing enzymes; chitinase activity was 300% higher and LAP was 33% higher in the unglaciated soils, when compared to glaciated soils. The phosphatase enzymes were at least two times higher in the unglaciated soils when compared to the glaciated soils (Fig. 3). Extracellular enzyme activity in glaciated soils was generally unaffected by the treatments, but we observed several significant treatment effects in the unglaciated region (Table 4). We observed mixed treatment response for enzymes responsible for N hydrolysis where chitinase activity was suppressed by 41% and 65% for the elevated P and pH + P treatments, respectively, but LAP activity increased by 77% in the elevated pH treatment (Table 4). Both phosphatase enzymes were significantly suppressed in P amended treatments (Fig. 3).

Microbial community

Soil microbial community composition (% mol) was significantly ($P < 0.01$, $n = 36$) different between regions (glaciated $\delta = 18.2$, unglaciated $\delta = 21.6$) using MRPP. The chance corrected within-group agreement (A) was 0.16, which gives a fair degree of confidence that microbial communities are different between regions (McCune and Grace 2002). For unglaciated soil, treatments significantly influenced microbial communities ($P = 0.02$, $n = 9$), but had a poor within-group agreement ($A = 0.06$) where the elevated P ($\delta = 22.4$) and pH + P ($\delta = 24.0$) were separated from the control ($\delta = 18.6$) or the elevated pH ($\delta = 21.0$) treatments. For glaciated soil, treatments significantly influenced microbial communities ($P = 0.04$, $n = 9$) and had a poor within-group agreement ($A = 0.03$) where the control ($\delta = 20.3$), elevated pH ($\delta = 20.1$), and elevated P ($\delta = 19.0$) were separated from the elevated pH + P ($\delta = 15.6$).

Microbial PLFA biomarkers in glaciated soils significantly correlated with a different set of soil properties than found in unglaciated soils (Fig. 4). The top four variables in glaciated soils that significantly correlated with biomarkers were: total N ($r = 0.42$), total C ($r = 0.40$), bicarb P ($r = 0.31$), and hydroxide P ($r = 0.30$). The top four variables in unglaciated soils were: hydroxide P ($r = 0.53$), N mineralization ($r = 0.42$), bicarb P ($r = 0.42$), and pH ($r = 0.36$). Only in the unglaciated region did we observe a treatment effect, where adding P significantly ($P = 0.05$; $r = 0.16$) correlated with biomarkers. Further investigation of the vectors indicated the strongest correlate with biomarkers in glaciated soils, total N, also strongly correlates ($r < 0.7$) with total C, bicarb P and to a lesser extent hydroxide P ($r = 0.52$), but not with chitinase ($r = -0.10$). For unglaciated soils, hydroxide P strongly correlated with total N ($r = 0.63$) and resin P ($r = 0.60$). N mineralization in unglaciated soils strongly correlated with bicarb P ($r = -0.71$) and pH ($r = 0.77$). Phosphatase enzyme activities had an insignificant ($P > 0.40$) and weak correlation with microbial community in glaciated and unglaciated sites. Solution NMDS stress for glaciated ordination was 4.82 and unglaciated ordination was 2.32.

Discussion

Microbial response to elevated P

Increasing P availability resulted in a suppression of the two phosphatase enzymes, which suggests P amended treatments have shifted P acquisition strategies from reliance on organic forms of P to

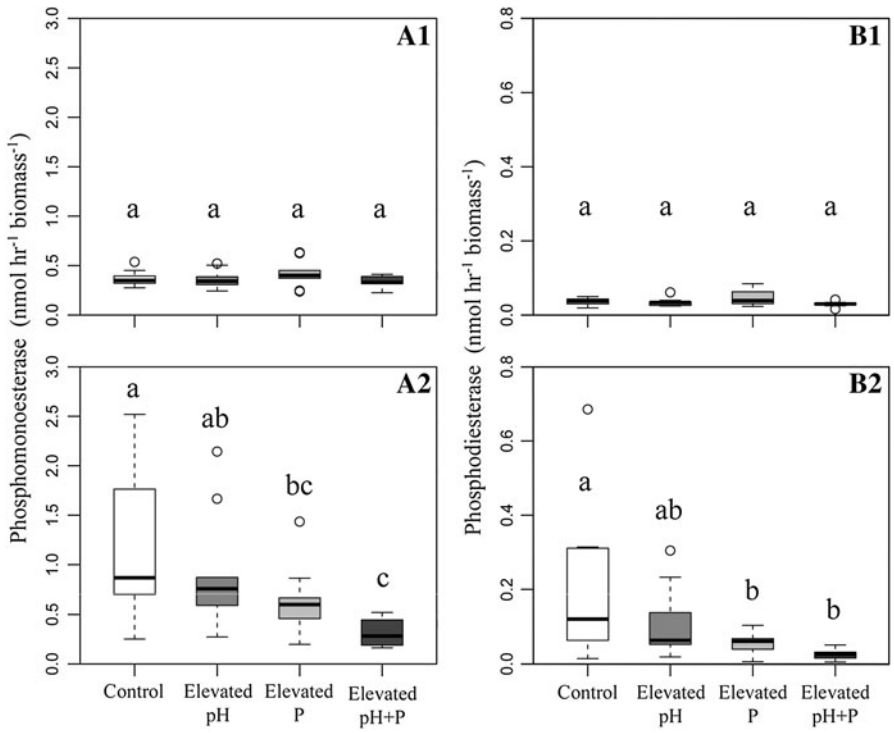

Fig. 3 The enzyme activity of phosphomonoesterase (*left panel*, **A1** and **A2**) and phosphodiesterase (*right panel*, **B1** and **B2**) in response to the four treatments: control, lime, P, and both lime + P. *Upper panels* (**A1** and **B1**) are from glaciated soils, whereas the *lower panels* (**A2** and **B2**) are from unglaciated soils. *Letters* indicated significant difference ($n = 9$)

utilization of inorganic P (McGill and Cole 1981; Olander and Vitousek 2000). This supports our hypothesis that potential P limitation can be overcome via phosphatase enzymes. However, this treatment response was observed in the unglaciated soils, not in the glaciated soils. It is possible that these patterns are related to large-scale patterns of landscape development and the emergence of low P availability in relation to the balance between disturbance (e.g. glaciation), erosional processes and mineral weathering (Porder et al. 2007), but our current dataset does not provide the proper resolution to address such mechanisms. Our results do suggest that soil microbes are more dependent on organic P, and perhaps have a greater P demand, in the unglaciated region. Soil microorganisms in the glaciated region may have access to a larger pool of inorganic P. This is supported by our finding that phosphatase enzyme activities in unglaciated soils amended with P were similar to the control treatments in glaciated soils. We reason that the unglaciated sites have crossed a threshold where inorganic P does not meet demand and greater metabolic effort is made to access organic P. The glaciated sites had ambient inorganic P levels that were two times as high as the unglaciated region, and these levels of inorganic P may have lessened the importance of organic P sources to the overall microbial community. Total growing season available P (resin P and bicarb P; Johnson et al. 2003) in the glaciated sites was around 84 mg kg^{-1}, whereas the unglaciated sites was 30 mg P kg^{-1}, so it is possible that an energetic threshold exists somewhere in between P demand and available P levels where it becomes necessary to exert more effort to acquire organic forms of P. However, an exact P threshold is likely mediated by metabolic stoichiometry (Anderson et al. 2005). These results might help explain why P fertilization in many studies might not necessarily elicit a response in certain soils above this putative P demand threshold (Elser et al. 2007).

An alternative explanation for the lack of phosphatase suppression in response to P addition in the glaciated soils is that there is a greater N and/or C demand in these soils than the unglaciated soils and microbes spend more of their limited resources accessing these resources. However, N mineralization rates were as much as 6 times greater ($P = 0.08$), and N acquisition enzyme activities were lower ($P < 0.04$) in glaciated sites than unglaciated, suggesting that the glaciated forest soils are N-rich and microorganisms have a sufficient supply of N

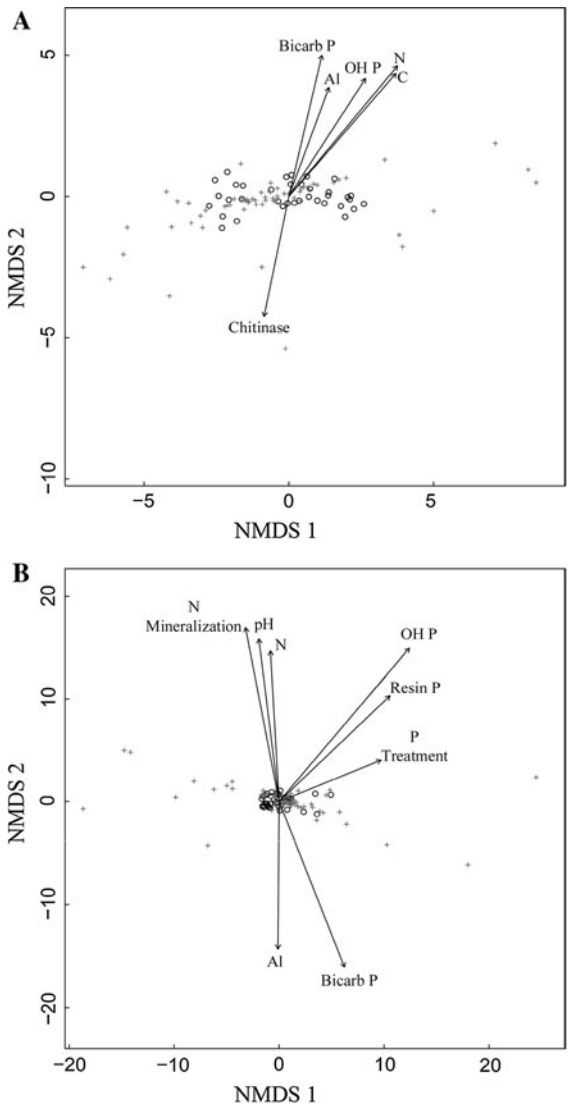

Fig. 4 Nonmetric multidimensional scaling (NDS) ordination joint plot of phospholipid fatty acid (PLFA) biomarkers with soil properties of glaciated (**a**) and unglaciated (**b**) sites. *Open circles* represent plots and crosses are individual biomarkers. The length and position of the *arrows* indicates correlation strength and direction of the relationship with the microbial community. Only significant vectors were displayed ($P > 0.05$). Solution NMDS stress for glaciated ordination was 4.82 and unglaciated ordination was 2.32

(Robertson and Groffman 2007). Consequently, the glaciated sites could be more C limited than the unglaciated sites; this is supported by greater C acquiring enzyme activity in the glaciated soils, despite greater total soil C. Regardless, our results strongly indicate that, for the short term, unglaciated forest soils are more sensitive to P additions than our glaciated forests soils.

We expected higher phosphatase activities in the non-treated plots where acidic conditions should have created a low readily bioavailable P and a greater reliance on organic P by soil microbes. For the unglaciated sites, we found evidence for a greater reliance on organic P, with suppressed phosphatase activity in the P amended plots, but we found no significant effects of P treatments on microbial PLFA biomass (Table 4), even though biomass did increase by 23% for both the elevated P and pH + P treatments. This result raises an important question: if microorganisms are using less energy acquiring P, then how are they re-allocating their resources in response to greater P availability? Contrary to our hypothesis, we have no evidence that the microbial community is investing more in acquiring C because β-glucosidase and cellobiohydrolase activities were similar among the treatments. Peng and Thomas (2010) reported that P amendment reduced soil respiration and fine root production in a northern hardwood forest, suggesting that removing any potential P co-limitation can reduce overall soil activity. Organic P acquisition may be a generalized soil function, but it is possible that significant organic P cycling is carried out by microbial specialists and resource partitioning for P takes place (Turner 2008). If some soil microbes specialize in acquiring more recalcitrant organic P and thrive in low P environments, then adding P should be detrimental to this microbial guild. We saw some evidence to suggest that P addition affected some microbial groups based on changes in enzyme activity. Phosphate additions had a greater suppression of phosphodiesterase as compared to phosphomonoesterase activity, indicating that microbial functional guilds in soil may have been affected. In addition, the amended P treatments did significantly influence microbial community composition in unglaciated soils (Fig. 4). Due to the poor resolution of PLFA analysis, it is unclear if this change was caused by changes in organic P specialists, or in organisms that respond to high levels of readily available P. Such changes may still not affect overall microbial biomass if microbes that are sensitive to available P represent a small portion of the microbial community.

Because resource availability in forests is temporally and spatially patchy, we might expect soil to

contain guilds of soil microbes that specialize in utilizing different soil resources. Litter fall in autumn, or during the senescence of herbaceous plants in late spring, could temporarily stimulate microbes specialized for organic P, N, and C acquisition, and this stimulation may or may not impact overall microbial biomass. Our sampling and analysis of these microbial communities and their function was carried out less than a year after P addition, and our results may not reflect long-term changes in microbial communities associated with elevated P, but rather short-term physiological changes in response to our P treatments. Additional work will be needed to examine the long-term response of soil microbes to an abundance of readily available P and whether these relationships are influenced by seasonal changes in resource availability.

The strong correlation between microbial composition and P fractions highlights the importance of soil P in both study regions and emphasizes the importance of microbial specialization in acquisition of certain P pools. This strong correlation with soil P has been previously reported in an old growth forest on unglaciated soils (DeForest and Scott 2010). In fact, hydroxide P was the single most important environmental correlate of microbial community composition in the unglaciated sites and fourth in the glaciated sites. Hydroxide P is considered the fraction associated with secondary minerals like mobilized Al and Fe (Cross and Schlesinger 1995), though it remains unclear why this fraction appears more important than more available fractions. It is possible our strong response in P amended treatments significantly increased hydroxide P in the unglaciated soils, but this does not explain the response in glaciated soils were treatments had no significant effect on this fraction. Hydroxide P did decline by 23% in the elevated pH plots in the glaciated forests and may have allowed microbes to access this otherwise unavailable P pool (Liptzin and Silver 2009). Additional sampling is needed to assess whether these differences will become significant over time. Nitrogen had an important influence on microbial communities from both sites while soil C was only important in the glaciated sites, again suggesting that microbial communities in these soils may be C limited and therefore unable to respond to changes in inorganic P availability (Ehlers et al. 2010).

Response to elevated pH

While pH was a significant correlate with PLFA composition in the unglaciated soils, it surprisingly did not significantly correlate with composition in the glaciated soils. This was unexpected because pH has a strong effect on microbial communities over a wide range of values (Bååth and Anderson 2003; DeForest and Scott 2010; Rousk et al. 2010). However, apparent pH response may be symptomatic of environmental conditions rather than being the proximal cause of those conditions. If microbial communities are C limited in the glaciated sites, and not primarily co-limited by N or P, then they may not exhibit a demonstrable pH response.

Given the importance of pH in shaping microbial community composition (Fierer and Jackson 2006), we also expected soil pH to have a greater influence on microbial function (i.e. enzyme activity) than we observed with our sampling (Table 4). These results are consistent with research on Ca additions in northern hardwood forests (Groffman et al. 2006; Minick et al. 2011). However, unlike Fiorentino et al. (2003), elevated pH treatment did not appear to increase available P pools and actually significantly decreased bicarb P for both regions. Furthermore, it is likely that raising the pH increased the utilization of the bicarb fraction, but it appears that raising both pH and P availability prevented this utilization (Table 3). Although extractable pools decreased, the synergistic suppression of phosphatase activity for the elevated pH + P suggests more P was bioavailable. Furthermore, our results support the Groffman et al. (2006) hypothesis that the apparent insensitivity to pH is caused by low P availability. Our elevated pH + P treatment did result in greater and more changes than the P treatment alone (Table 4). Whether this was caused by an additive effect of elevated P and pH or that elevated pH increased bioavailable P is unclear. Because our sampling was conducted less than one year after treatment initiation, microbial community treatment responses might increasingly develop over time or vary with different stages of the growing season. We sampled in mid-summer, and microbial responses might be greater during spring when plant uptake of P is higher or during autumn when leaf fall represents a large ecosystem resource input.

Soil pH and N cycling

Net mineralization and nitrification were insensitive to the lime treatment, unless soil pH exceeded 6.0 (Fig. 2). This explains the main treatment effect for NO_3^- concentration, net N mineralization, and nitrification increase with pH in the unglaciated soils (Table 4). This is contrary to Minick et al. (2011) where increased pH reduced net N mineralization in the Oa horizon. It is difficult to determine if pH will dramatically increase nitrification in our glaciated soils because only one plot exceeded a pH of 6.0. While it is recognized that N transformation gradually changes with soil pH (Minick et al. 2011; Bramley and White 1991), our results suggest more of a threshold response in mineral soil. While not explicitly discussed, such a response has been reported at a similar soil pH in boreal forests (Giesler et al. 1998). However, it is unclear if this is simply a pH effect because we found that increased net N mineralization correlated strongly with decreases in bicarb P, which suggests a coupling between N cycling and this labile P fraction. In other words, elevated soil pH may remove biochemical constraints on N transformations and thereby increase P demand.

Conclusions

A more robust understanding of P biogeochemistry and the mechanisms controlling P availability is essential for predicting how terrestrial ecosystems will respond to atmospheric deposition of anthropogenic N and acidity. Nitrogen saturation and soil acidification both point to potential P limitation due to either increased demand and/or decreased P availability. The results presented here provide evidence to suggest ecosystem responses are likely to be specific and dependent on initial state factors. Although we demonstrate that soil microbes can compensate for low inorganic P availability, the effect of P amendment was overall greater in unglaciated soils than in glaciated soils, despite substantially low ambient soil pH in the glaciated sites. Surprisingly, microbial communities and processes were fairly insensitive to a whole unit increase in soil pH, except when pH exceeded six in unglaciated plots which caused large increases in N mineralization and nitrification. It is plausible that temperate forest soil microbial communities are more buffered against soil pH changes than changes in nutrient availability, or that more time is necessary to observe a response. We suggest that our general understanding of the relationship between available P and microbial community dynamics is complex and incomplete. Future work needs to specifically address (1) if these responses are sustainable; (2) the role of microbial specialization in determining ecosystem level responses; (3) determine if changes in soil P cycling have a noticeable influence on plant productivity at either the community or species level; (4) and unraveling the influence of acquired versus inherited soil properties on P biogeochemistry and microbial dynamics.

Acknowledgments This research was supported by a grant from the National Science Foundation (DEB 0918681 and DEB 0918167). We thank Charlotte Hewins, our undergraduate research assistants Alanna Shaw and Natalie Romito, and our high school intern, Adealiah Bennett, for help with field sampling and/or laboratory analysis. We also thank Charlotte Hewins, Gary Tobaj, Matthew Lurch, Keith Gilland, Lindsay Scott, Ryan Homsher, Clinton Calhoun, and Scott Fisher for help with establishing our treatments. Special thanks to Maria Farinacci for spreading nearly seven tons of lime herself. We thank Douglas Sturtz of USDA-ARS (University of Toledo) for running samples on the ICP-MS.

References

Aber JD, Magill A, Boone R, Melillo JM, Steudler P, Bowden R (1993) Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecol Appl 3(1):156–166

Anderson TR, Hessen DO, Elser JJ, Urabe J (2005) Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. Am Nat 165(1):1–15

Attiwill P, Adams M (1993) Nutrient cycling in forests. New Phytol 124(4):561–582

Bååth E, Anderson TH (2003) Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol Biochem 35(7):955–963

Bramley RGV, White RE (1991) An analysis of variability in the activity of nitrifiers in a soils under pasture. Aust J Soil Res 29(1):95–108

Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems 5(7):680–691

Crews TE, Kitayama K, Fownes JH, Riley RH, Herbert DA, Muellerdombois D, Vitousek PM (1995) Changes in soil-phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. Ecology 76(5):1407–1424

Cross AF, Schlesinger WH (1995) A literature-review and evaluation of the Hedley fractionation-applications to the biogeochemical cycle of soil-phosphorus in natural ecosystems. Geoderma 64(3–4):197–214

Cruz AF, Hamel C, Hanson K, Selles F, Zentner RP (2009) Thirty-seven years of soil nitrogen and phosphorus fertility management shapes the structure and function of the soil microbial community in a Brown Chernozem. Plant Soil 315(1–2):173–184

DeForest JL (2009) The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biol Biochem 41(6):1180–1186

DeForest JL, Scott LG (2010) Available organic soil phosphorus has an important influence on microbial community composition. Soil Sci Soc Am J 74(6):2059–2066

DeForest JL, Zak DR, Pregitzer KS, Burton AJ (2004) Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biol Biochem 36(6):965–971

Demetz M, Insam H (1999) Phosphorus availability in a forest soil determined with a respiratory assay compared to chemical methods. Geoderma 89(3–4):259–271

Duah-Yentumi S, Ronn R, Christensen S (1998) Nutrients limiting microbial growth in a tropical forest soil of Ghana under different management. Appl Soil Ecol 8(1–3):19–24

Ehlers K, Bakken LR, Frostegård Å, Frossard E, Bünemann EK (2010) Phosphorus limitation in a Ferralsol: impact on microbial activity and cell internal P pools. Soil Biol Biochem 42(4):558–566

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10(12):1135–1142

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103(3):626–631

Finzi A (2009) Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. Biogeochemistry 92:217–229

Fiorentino I, Fahey TJ, Groffman PM, Driscoll CT, Eagar C, Siccama TG (2003) Initial responses of phosphorus biogeochemistry to calcium addition in a northern hardwood forest ecosystem. Can J For Res-Rev Can Rech For 33(10):1864–1873

Gallardo A, Schlesinger WH (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biol Biochem 26(10):1409–1415

Giesler R, Högberg M, Högberg P (1998) Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. Ecology 79(1):119–137

Giesler R, Petersson T, Högberg P (2002) Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. Ecosystems 5(3):300–314

Goldberg S, Davis JA, Hem JD (1996) The surface chemistry of aluminum oxides and hydroxides. In: Sposito G (ed) The environmental chemistry of aluminum, 2nd edn. CRC Lewis Publishers, Boca Raton, FL, pp 271–332

Gress SE, Nichols TD, Northcraft CC, Peterjohn WT (2007) Nutrient limitation in soils exhibiting differing nitrogen availabilities: What lies beyond nitrogen saturation? Ecology 88(1):119–130

Groffman PM, Fisk MC, Driscoll CT, Likens GE, Fahey TJ, Eagar C, Pardo LH (2006) Calcium additions and microbial nitrogen cycle processes in a northern hardwood forest. Ecosystems 9(8):1289–1305

Guckert J, Antworth C, Nichols P, White D (1985) Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microb Ecol 31:147–158

Johnson AH, Frizano J, Vann DR (2003) Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. Oecologia 135(4):487–499

Kempers AJ, Zweers A (1986) Ammonium determination in soil extracts by the salicylate method. Commun Soil Sci Plant Anal 17(7):715–723

Krashevska V, Maraun M, Ruess L, Scheu S (2010) Carbon and nutrient limitation of soil microorganisms and microbial grazers in a tropical montane rain forest. Oikos 119(6):1020–1028

Kuo S (1996) Phosphorus. In: Sparks DL (ed) Methods of soil analysis. Part 3. Chemical methods, vol 5. ASA and SSSA, Madison, WI, pp 869–919

Lagerström A, Esberg C, Wardle DA, Giesler R (2009) Soil phosphorus and microbial response to a long-term wildfire chronosequence in northern Sweden. Biogeochemistry 95(2–3):199–213

Lajtha K, Driscoll C, Jarrell W, Elliott E (1999) Soil phosphorus: characterization and total elemental analysis. In: Robertson G, Coleman D, Bledsoe C, Sollins P (eds) Standard soil methods for long-term ecological research. Oxford University Press, New York, pp 124–127

Liptzin D, Silver WL (2009) Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. Soil Biol Biochem 41(8):1696–1702

McCune B, Grace J (2002) Analysis of ecological communities. MJM Software Design, Gleneden Beach, OR

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic-matter. Geoderma 26(4):267–286

Minick KJ, Fisk MC, Groffman PM (2011) Calcium and phosphorus interact to reduce mid-growing season net nitrogen mineralization potential in organic horizons in a northern hardwood forest. Soil Biol Biochem 43(2):271–279

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide-Biol Chem 5(1):62–71

Mitchell CC, Delaney DP, Balkcom KS (2008) A historical summary of Alabama's Old Rotation (circa 1896): the world's oldest, continuous cotton experiment. Agron J 100(5):1493–1498

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49(2):175–190

Peng YY, Thomas SC (2010) Influence of non-nitrogenous soil amendments on soil CO_2 efflux and fine root production in an N-saturated northern hardwood forest. Ecosystems 13(8):1145–1156

Phoenix GK, Booth RE, Leake JR, Read DJ, Grime JP, Lee JA (2004) Simulated pollutant nitrogen deposition increases P demand and enhances root-surface phosphatase activities of three plant functional types in a calcareous grassland. New Phytol 161(1):279–289

Porder S, Vitousek PM, Chadwick OA, Chamberlain CP, Hilley GE (2007) Uplift, erosion, and phosphorus limitation in terrestrial ecosystems. Ecosystems 10(1):158–170

R-Project (2010) R: a language and environment for statistical computing. v.2.12.0. The R Foundation for Statistical Computing, Vienna. URL:http://www.R-project.org

Robertson G, Groffman P (2007) Nitrogen transformations. In: EA P (ed) Soil microbiology, ecology, and biochemistry, vol 3. Academic Press, Ney York, NY, pp 341–364

Robertson G, Sollins P, Ellis B, Lajtha K (1999) Exchangeable ions, pH, and cation exchange capacity. In: Robertson G (ed) Standard soil methods for long-term ecological research. Oxford University Press, New York

Rojo M, Carcedo S, Mateos M (1990) Distribution and characterization of phosphatase and organic phosphorus in soil fractions. Soil Biol Biochem 22:169–174

Rousk J, Brookes PC, Bååth E (2010) The microbial PLFA composition as affected by pH in an arable soil. Soil Biol Biochem 42(3):516–520

Sims JT (1996) Lime requirement. In: Sparks DL (ed) Methods of soil analysis. Part 3. Chemical methods, vol 5. ASA and SSSA, Madison, WI, pp 491–515

Tanner EVJ (1981) The decomposition of leaf litter in Jamaican montane rain forests. J Ecol 69(1):263–275

Thomas G, Hargrove W (1984) The chemistry of soil acidity. In: Adams F (ed) Soil acidity and liming. American Society of Agronomy, Madison, WI

Tiessen H, Frossard E, Mermut AR, Nyamekye AL (1991) Phosphorus sorption and properties of ferruginous nodules from semiarid soils from Ghana and Brazil. Geoderma 48(3–4):373–389

Townsend AR, Asner GP, Cleveland CC, Lefer ME, Bustamante MMC (2002) Unexpected changes in soil phosphorus dynamics along pasture chronosequences in the humid tropics. J Geophys Res-Atmos 107(D20):9

Turner BL (2005) Storage-induced changes in phosphorus solubility of air-dried soils. Soil Sci Soc Am J 69(3): 630–633

Turner BL (2008) Resource partitioning for soil phosphorus: a hypothesis. J Ecol 96(4):698–702

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157(3): 423–447

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea—how can it occur. Biogeochemistry 13(2):87–115

Vitousek PM, Sanford RL (1986) Nutrient cycling in moist tropical forests. Annu Rev Ecol Syst 17:137–167

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20(1):5–15

Walker L, Syers J (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19

Weand MP, Arthur MA, Lovett GM, Sikora F, Weathers KC (2010) The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization. Biogeochemistry 97(2–3):159–181

White D, Davis W, Nickels J, King J, Bobbie R (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40:51–62