Biogeochemistry (2012) 108:485–499
DOI 10.1007/s10533-011-9612-0

Changes in organic phosphorus composition in boreal forest humus soils: the role of iron and aluminium

Andrea G. Vincent · Jürgen Schleucher · Gerhard Gröbner · Johan Vestergren · Per Persson · Mats Jansson · Reiner Giesler

Received: 7 October 2010 / Accepted: 26 May 2011 / Published online: 12 June 2011

Abstract Organic phosphorus (P) is an important component of boreal forest humus soils, and its concentration has been found to be closely related to the concentration of iron (Fe) and aluminium (Al). We used solution and solid state ^{31}P NMR spectroscopy on humus soils to characterize organic P along two groundwater recharge and discharge gradients in Fennoscandian boreal forest, which are also P sorption gradients due to differences in aluminium (Al) and iron (Fe) concentration in the humus. The composition of organic P changed sharply along the gradients. Phosphate diesters and their degradation products, as well as polyphosphates, were proportionally more abundant in low Al and Fe sites, whereas phosphate monoesters such as *myo*-, *scyllo*- and unknown inositol phosphates dominated in high Al and Fe soils. The concentration of inositol phosphates, but not that of diesters, was positively related to Al and Fe concentration in the humus soil. Overall, in high Al and Fe sites the composition of organic P seemed to be closely associated with stabilization processes, whereas in low Al and Fe sites it more closely reflected inputs of organic P, given the dominance of diesters which are generally assumed to constitute the bulk of organic P inputs to the soil. These gradients encompass the broad variation in soil properties detected in the wider Fennoscandian boreal forest landscape, as such our findings provide insight into the factors controlling P biogeochemistry in the region but should be of relevance to boreal forests elsewhere.

Keywords Solution ^{31}P NMR spectroscopy · Solid state ^{31}P NMR · Sorption · Groundwater discharge · Groundwater recharge · Betsele

Electronic supplementary material The online version of this article (doi:10.1007/s10533-011-9612-0) contains supplementary material, which is available to authorized users.

A. G. Vincent (✉) · M. Jansson
Department of Ecology and Environmental Sciences, Umeå University, 901 87 Umeå, Sweden
e-mail: andrea.vincent@emg.umu.se

J. Schleucher
Department of Medical Biochemistry and Biophysics, Umeå University, 901 87 Umeå, Sweden

G. Gröbner · J. Vestergren · P. Persson
Department of Chemistry, Umeå University, 901 87 Umeå, Sweden

R. Giesler
Climate Impacts Research Centre, Department of Ecology and Environmental Sciences, Umeå University, 981 07 Abisko, Sweden

Introduction

The boreal forest is the second largest biome in the world, holding 33% of the Earth's forest cover (FAO 2001). Although nitrogen is considered to be the main limiting nutrient in boreal forests (Pettersson 1994)

and has received the most attention in the past, phosphorus (P) is also important, influencing and sometimes limiting plant growth (Giesler et al. 1998, 2002; Wardle et al. 2004; Thelin 2006; Månsson 2005) and affecting microbial growth (Giesler et al. 2004). Furthermore, P limitation is predicted to increase in boreal forests where rates of atmospheric nitrogen deposition are high (Akselsson et al. 2008). Given that P occurs as numerous types of compounds which differ greatly in their bioavailability, mobility, and vulnerability to sorption (Condron et al. 2005), detailed characterization of these compounds and their behaviour is necessary to understand P biogeochemistry, the role of P in key ecosystem processes such as plant productivity and microbial decomposition, and how they might respond to global environmental change.

Soil P occurs in organic and inorganic forms, and organic P represents a large fraction of the total soil P in boreal forests (Cross and Schlesinger 1995). In general, soil organic P is composed of phosphate monoesters, diesters, and phosphonates, which require hydrolysis before they can be assimilated by plants and microorganisms. Polyphosphates and pyrophosphate are condensed inorganic phosphates that also require hydrolysis prior to biological utilization. The composition of soil organic P at a given site is the product of several interrelated factors, such as the types of P inputs to the soil, biological P utilization, and stabilization via adsorption to soil minerals or precipitation reactions (Celi and Barberis 2005). Most organic P inputs to the soil are in diester form (Magid et al. 1996), however soil organic P is usually dominated by phosphate monoesters (Condron et al. 2005), suggesting that biological utilization and stabilization are more important controls on composition (Magid et al. 1996). Biological utilization of organic P depends to a large extent on stabilization, because stabilization protects organic P from microbial degradation (Lung and Lim 2006). Therefore, factors associated with phosphate stabilization, such as soil sorption capacity, the content of amorphous aluminium and iron (Turner 2007), as well as the inherent propensity for sorption of different organic P compounds, are all crucial controls on soil organic P composition.

Different organic P compounds vary widely in their vulnerability to sorption, and studies mostly in controlled conditions show that the tendency for sorption increases with the number of P groups on a given compound (Turner et al. 2002). For example inositol phosphates tend to accumulate in soil (Turner et al. 2002), have a high anionic charge and can complex polyvalent cations in soils. They can also sorb onto the surfaces of ferric oxides (De Groot and Golterman 1993), and compete for the same surface sites as inorganic orthophosphate (Ognalaga et al. 1994). Conversely, phosphate diesters such as DNA, RNA, and phospholipids have a lower propensity to sorption because they have lower charge densities and their phosphate groups are sterically shielded from ionic interactions, leaving them vulnerable to microbial attack. As a result, diesters are considered to be much more bioavailable than monoesters (Celi and Barberis 2005).

In natural soils, inorganic and organic phosphorus sorption is connected with the presence of reactive surfaces in mineral horizons and is well described in the literature. However in organic horizons, aluminium (Al) and iron (Fe) precipitates or organically bound Fe and Al are also strong sorption sites for phosphorus (Giesler et al. 2005; Dell'Olio et al. 2008), albeit less well characterized. For example, Al and Fe accumulate in the humus soils of groundwater discharge (GDI) areas (Mulder et al. 1991; Norrström 1993, 1995; Giesler et al. 1998), i.e., 'flushed' areas *sensu* Gorham (1953), conferring them P sorbing properties (Giesler et al. 2005). Detailed studies of some groundwater recharge (GRE) and GDI areas in boreal Sweden revealed relationships between soil pH, N concentration and plant productivity that are remarkably similar to those reported in a regional survey of the wider Fennoscandian boreal forest region (Dahl et al. 1967; Lahti and Vaisanen 1987; Giesler et al. 1998), suggesting that GRE/GDI gradients encompass the variation in soil and plant properties detected at the landscape level (Giesler et al. 1998). The studies mentioned above also show co-variation between soil pH, N, and plant productivity with Al and Fe concentration in the humus layer (Giesler et al. 2002, 2005).

Given that hydrology underlies the variation in soil properties mentioned above, GRE and GDI gradients are also likely to encompass the natural variation in P availability at larger scales (Giesler et al. 2002, 2004). Therefore, we used two GRE/GDI gradients together with solution and solid state ^{31}P NMR spectroscopy to characterise soil organic P

composition in boreal forest humus soils and address the following questions: (1) Is soil organic P in highly phosphate sorbing GDI soils dominated by compounds with a propensity for sorption, such as inositol phosphates? (2) Do compounds with a lower propensity for sorption dominate in low sorbing, GRE soils? Our ultimate aim was to gain a better understanding of the factors controlling P composition and dynamics in boreal forest soils.

Methods

Study sites

Betsele is a 90 m productivity gradient which has been well described in terms of soil–plant relationships (Giesler et al. 1998; Högberg 2001; Nordin et al. 2001) and plant–microbe–soil interactions (e.g., Högberg et al. 2003; Nilsson et al. 2005). The gradient is located at the bottom of the Umeå river valley northwest of Betsele, northern Sweden (64°39'N, 18°30'E; 235 m above sea level), and has a fairly constant slope of 2%. The mean annual temperature is 1°C and mean annual precipitation is 570 mm. The site is covered by snow approximately from late October to early May. During spring snowmelt the GDI area is flooded by groundwater, this generally lasts a few weeks. Flooding can also take place following heavy rains during summer and autumn.

At the unproductive end of the transect (0 m, GRE) there is an open Scots pine (*Pinus sylvestris* L.) forest whereas at the productive end (90 m, GDI) there is a closed Norway spruce (*Picea abies* Karst.) forest (Giesler et al. 1998). There are three main types of field layer vegetation along the Betsele transect; ericaceous dwarf-shrubs dominate between 0 and 40 m, short-herb plants are intermingled with ericaceous dwarf shrubs between 50 and 80 m, and tall herbs dominate at 90 m (Giesler et al. 1998). Soils along the gradient are loamy, sandy tills with many boulders, classified as Typic Haplocryods in the GRE and as Aquic or Oxyaquic Haplocryods in the GDI areas (Soil Survey Staff 1992), because water saturation to within 100 cm of the soil surface probably takes place during part of the year. The O horizons in the GRE sites are usually intermediate in their degree of decomposition (Oe), whereas humus material from the GDI areas are highly decomposed (Oa). Soil physical and chemical conditions show marked differences along this gradient (Table 1). The pH in the soil solution of the humus soil varies from 3.5 in the recharge (0 m) area to 6.4 in the discharge (90 m) area (Giesler et al. 1998).

The Flakastugan gradient is located close to Vindeln (64°25'N, 19°25'E; 225 m above sea level), and was sampled at two points at the GRE and adjacent GDI site (Giesler et al. 2002). The forest is dominated by *Picea abies* intermingled with *Pinus sylvestris* and the field layer vegetation at the GRE site is also dominated by ericaceous dwarf shrubs and interspersed with 'short herb' plants whereas the GDI site is dominated by 'tall herb' species. The soils are similar to those in Betsele (Giesler et al. 2002).

Table 1 Selected soil characteristics along two groundwater recharge (*GRE*) and discharge (*GDI*) gradients in Betsele and Flakastugan, Northern Sweden

Site	Position (m)	GRE/GDI	Field layer vegetation	C/N[a]	C/P[a]	LOI (%)	pH	Al[b] (mg kg^{-1})	Fe[b] (mg kg^{-1})
Betsele	0	GRE	DS	nd	nd	94	4.3	43	1162
	50	GRE	SH	nd	nd	77	4.4	67	2463
	70	GRE	SH	22	326	34	4.8	77	2168
	90	GDI	TH	17	148	84	6.1	266	20729
Flakastugan		GRE	SH	33	445	87	4.3	103	5063
		GDI	TH	28	247	40	4.9	362	10494

DS dwarf shrub, *SH* short herb, *TH* tall herb, *nd* not determined

[a] Values reported in Giesler et al. (2004)

[b] 'Acid-digestible' Al and Fe, determined by ICP-OES after microwave digestion with HNO_3 and H_2O_2

Soil sampling

Samples from the entire humus soil (O horizon) were taken on 13 November 2009 at four sites along the Betsele productivity gradient (GRE at 0, 50, and 70 m, GDI at 90 m) and at two sites in Flakastugan (GRE and GDI), for a total of 6 sampling sites. The O horizon was 5–15 cm thick across all sites. In each site, 5–10 cores were taken within an area 20–50 m in radius; cores were taken with a soil auger (0.10 m diameter) at randomly selected locations, and always $\geq$1 m apart. Each sample was kept separately in a polyethylene bag, and stored at −20°C on the same day of collection. Samples were kept at −20°C for 3 months, after which they were thawed at 5°C. Soils were immediately sieved through a 5 mm mesh, and a subsample of approximately 25 g taken for determination of water content (105°C, 24 h).The remaining soil was set to dry in an incubator at 35°C for 10 days. Dry soils were milled by hand in a mortar for ^{31}P NMR analysis. A further portion was set aside for determination of acid digestible P, Al, and Fe, as well as organic matter by loss on ignition (LOI).

Solution ^{31}P NMR

NaOH-EDTA extraction

Phosphorus was extracted by shaking 10 g of dry and ground soil in 200 ml of a solution containing 250 mM NaOH and 50 mM Na_2EDTA (ethylenediaminetetraacetate) for 16 h (Cade-Menun and Preston 1996). The extracts were centrifuged (30 min, 14,000×*g*), and the supernatant (~150 ml) was spiked with 1 ml of 50 μg P ml^{-1} methylene diphosphonic acid (MDPA) solution as an internal standard. Spiked extracts were then frozen at −80°C and lyophilized. The total amount of lyophilized material produced was ~5 g, but we only used ~0.5 g for NMR analysis.

Spectra acquisition

A portion of each lyophilized extract (~500 mg of NaOH/EDTA extracts, ~10% of the total lyophilized extract produced) was re-dissolved in 2.0 ml of deuterium oxide, and then transferred to a 10-mm NMR tube. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer (Bruker, Germany) operating at 202.456 MHz for ^{31}P. NaOH–EDTA extracts were analyzed using a 12 μs pulse (45°), a delay time of 1.3 s, an acquisition time of 0.2 s, and broadband proton decoupling; between 12 and 43 K scans were acquired. Spectra were plotted with a line broadening of 2 Hz, and chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% orthophosphoric acid (H_3PO_4).

To confirm the presence of *myo*-inositol hexakisphosphate in our spectra we spiked a NaOH–EDTA soil extracts (2 ml) from Betsele 0 m (GRE) with 40 μl of *myo*-inositol hexakisphosphate (dipotassium salt; Sigma P 5681) solution (33 g l^{-1}). Furthermore, it has been claimed (Doolette et al. 2010) that a broad signal due to P compounds within large, complex humic compounds lies under the monoester region, which could lead to the overestimation of inositol phosphates, and which therefore should be subtracted before their quantification. To investigate the possible presence of a broad signal under the monoester region, we analyzed $T_{1\rho}$ relaxation times (transverse relaxation of NMR signals, related to their true linewidths) on the Betsele 90 m (GDI) soil. In these experiments, P signals are allowed to relax according to their transverse relaxation times, which correspond to their line widths, during a spin lock of variable duration. The duration of the spin lock of 3000 Hz field strength was varied between 100 μs and 32 ms. In this experiment, the signal of any broad component should decay within a few milliseconds during the spin lock, while the sharper signal of other monoesters should take tens of milliseconds to decay. Hence, the presence of a broad signal should be visible as bi-exponential decay of the signal, and the spectra acquired with a spin lock of a few milliseconds should show the true integrals of inositol phosphates and other monoesters ("T_2 filtering"). Longitudinal ^{31}P relaxation times were derived from an inversion recovery experiment, varying the delay between the inversion and the excitation pulse between 2 and 700 ms.

Identification of the main P classes and of individual compounds was based on literature reports of both model compounds and natural soils spiked in NaOH–EDTA soil extracts (Table 2). Peaks were first 'picked' using an automatic peak fitting procedure, and peaks not 'picked up' by the program but clearly visible, were manually defined. Deconvolution was used to integrate peak areas (Fig. 1). Because we only

Table 2 Chemical shifts of different phosphorus compounds detected in NaOH–EDTA extracts of soils

Phosphorus class	Chemical shift (ppm)	References
Phosphonates	12 to 23	Turner et al. (2003)
Methylene diphosphonic acid	17.6	Turner (2008)
Inorganic orthophosphate	5.7 to 6.2	Turner et al. (2003), Doolette et al. (2009)
Monoesters	2.5 to 7[a]	Turner et al. (2003)
Myo-inositol hexakisphosphate	5.9	Turner et al. (2003)
	4.9	
	4.6	
	4.4	
Scyllo-inositol hexa*kis*phosphate	4.2	Turner and Richardson (2004)
Unidentified inositol phosphates	6.6 to 6.8	Turner and Richardson (2004)
α-Glycerophosphate	4.96 to 4.97	Doolette et al. (2009)
β-Glycerophosphate	4.63	Doolette et al. (2009)
	4.80	Turner et al. (2003)
Diesters	−1 to 2.0	Turner et al. (2003)
DNA	−0.37 to 0	Makarov et al. (2002), Turner et al. (2003)
RNA	0.3 to 0.54	Makarov et al. (2002), Turner et al. (2003)
RNA degradation products	4 to 5	Makarov et al. (2002), Turner et al. (2003)
Adenosine 2′ and 3′ monophosphate	4.48 and 4.78	Turner et al. (2003)
Adenosine 5′ monophosphate	4.65	Turner et al. (2003)
Phospholipids	0.6 to 1.75	Makarov et al. (2002), Turner et al. (2003)
Pyrophosphate	−4.4	Turner et al. (2003)
Inorganic polyphosphate (end groups)	−4.0	Turner et al. (2003)
Inorganic polyphosphate (penultimate and mid chain groups)	−17 to −21	Turner et al. (2003)

[a] Excluding the main inorganic orthophosphate signal between 6.3 and 6.4 ppm

used 10% of the lyophilized material, the MDPA spike was too small for accurate quantification. Therefore we added supplementary MDPA to each NMR sample, ran a second set of spectra with fewer scans, and calculated concentrations in the original spectra by comparing absolute intensities of both sets of NMR spectra. Concentrations of P compounds are given as mg P kg^{-1} soil. All spectral processing was done using the program TopSpin 2.0 (Bruker, Germany).

Solid state ^{31}P MAS NMR

Dried soils were ground with pestle and mortar, and then loaded without further modifications into a 7.5 mm MAS sample rotor (Chemagnetics/Varian Fort Collins, CO, USA). NMR Experiments were carried out at a 400 MHz (proton frequency) Infinity

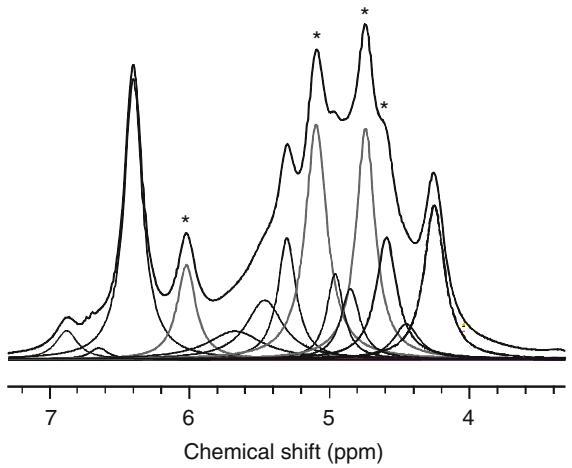

Fig. 1 Main monoester signals in a NaOH–EDTA extract of the Betsele 0 m soil, as determined by ^{31}P NMR and spectral deconvolution. The four *myo*-inositol hexakisphosphate peaks are denoted by *asterisks*

NMR spectrometer (Chemagnetics/Varian Fort Collins, CO, USA) equipped with a 7.5 mm double resonance MAS NMR probe and at a ^{31}P frequency of 162 MHz. ^{31}P MAS NMR spectra were acquired at a sample spinning speed of 4 kHz at 293 K. Since the sample contained paramagnetic impurities, a single pulse excitation sequence was used with simultaneous proton decoupling, as described by Shand et al. (1999). The excitation pulse duration was 3 μs (90°), dwell time was 20 μs, pulse and acquisition delay 1.5 s and 10 μs, respectively, acquisition time 20.4 ms. We acquired 9–62 K scans per spectrum. Spectra were referenced using solid KH_2PO_4 as an external standard, set to 6 ppm (Hupfer et al. 2004).

Soil chemical analyses

Acid-digestible P, Al, and Fe concentration was determined on 200 mg of dry and ground soils, using concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in a microwave digestor (Mars XPress, CEM, Germany). Total P in digests was determined with automated molybdate colorimetry (FIAstar, Tecator, Höganäs, Sweden), whereas Al and Fe were determined with ICP-OES (Perkin Elmer). Soil pH was measured on moist soils that had been stored for 3 months at −20°C immediately after collection, and then thawed at 5°C. The soil/water slurry (1:10 soil to solution ratio, 2 g moist soil and 20 ml H_2O) was shaken overnight, left to settle for 1 h and pH measured using a glass electrode. Total organic matter content was determined by loss on ignition in a furnace (4 h, 550°C). Strongly bound P was estimated as the difference between acid-digestible and NaOH–EDTA extractable P.

Statistical analyses

Linear regression was used to test the influence of Al and Fe concentrations on those of different P classes, after checking that data met normality and homogeneity of variances assumptions. Regressions with Fe concentration were performed after a transformation (natural logarithm) owing to the large variation in Fe values across sites. Significant differences refer to the $P < 0.05$ level. Analyses were done with R version 2.2.0 (www.r-project.org).

Results

Characterization of P composition by solution ^{31}P NMR

NaOH-EDTA extracted 36–73% of the total soil P (Table 3). Organic P concentration was two to three times higher in GDI than in GRE sites, whereas inorganic P showed no clear trend (Table 3). The proportion of strongly bound P decreased along both

Table 3 Concentration (mg P kg^{-1} soil) of phosphorus fractions in soils (O-horizon) collected in two groundwater recharge (*GRE*) and discharge (*GDI*) gradients in Betsele and Flakastugan, Northern Sweden

Site	Position (m)	GRE/GDI	Total P[a]	Strongly bound P[b]	NaOH–EDTA P[c]	Organic P[c,d]	Inorganic P[c,e]
Betsele	0	GRE	927	593 (64)	334 (36)	175 (19)	160 (17)
	50	GRE	1301	708 (54)	593 (46)	322 (25)	271 (21)
	70	GRE	1237	690 (56)	547 (44)	327 (26)	219 (18)
	90	GDI	1433	734 (51)	699 (49)	514 (36)	183 (13)
Flakastugan		GRE	1123	654 (58)	469 (42)	355 (32)	113 (10)
		GDI	1052	283 (27)	769 (73)	651 (62)	119 (11)

Values in parenthesis are proportion (%) of total soil phosphorus

[a] Determined in soils with molybdate colorimetry after nitric acid and hydrogen peroxide digestion. We consider this extraction to represent total P in soil, but are aware that this procedure is unlikely to dissolve all of the P associated with mineral grains

[b] Difference between total soil P and NaOH–EDTA P

[c] Determined by solution ^{31}P NMR spectroscopy

[d] Sum of orthophosphate monesters, diesters and phosphonates (see Table 4)

[e] Sum of inorganic orthophosphate, pyrophosphate and polyphosphate (see Table 4)

gradients, but its concentration did not follow any clear patterns.

Solution ^{31}P NMR spectra of NaOH–EDTA extracts are shown in Fig. 2. Aside from the internal standard (MDPA) at 17.5 ppm, the main signals were assigned to broad P classes following the chemical shifts in Table 2. Phosphate monoesters (30–69% of NaOH–EDTA extractable P) were in general the most abundant P class detected, followed by inorganic orthophosphate, thereafter called 'phosphate' (13–30%), diesters (9–27%), inorganic polyphosphate (0–16%), phosphonates (2–7%), pyrophosphate (1–5%), and unidentified compounds (0–5%) (Table 4). All polyphosphates detected were considered to be inorganic, as we did not detect signals between −9 and −10 ppm which are indicative of organic polyphosphates (Turner et al. 2003a).

Deconvolution analysis of the monoester region and $T_{1\rho}$ relaxation

The monoester region consisted of many overlapping peaks (Fig. 3), which were quantified by deconvolution. *Myo*-inositol hexakisphosphate was identified as a set of four signals at 6.02, 5.11, 4.75 and 4.59 ppm and in approximately a 1:2:2:1 ratio (Turner et al. 2003a; Doolette et al. 2009). These signals closely matched the four *myo*-inositol hexakisphosphate resonances obtained by spiking one of our soil extracts with this compound (Fig. S1). As has been observed before (Turner et al. 2007), the signal intensity of the C2 phosphate at approximately 6.02 ppm appears to deviate from the expected 1:2:2:1 ratio, but this is predictable given the strong overlap in the monoester region. The presence of *scyllo*-inositol hexakisphosphate was indicated by a strong signal at 4.25 ppm, and that of unknown inositol phosphates by a set of signals at 6.7–6.9 ppm (Turner and Richardson 2004).

In addition to the inositol phosphate signals above, the monoester region contained several other strong signals at 5.32, 4.97, 4.83, 4.53 and 4.42 ppm (Fig. 3; Table 5). It is likely that some if not most of these belong to the degradation products of diesters in alkaline solution. For example in alkaline soil (Doolette et al. 2009) and soil microbial tissue (Bünemann et al. 2008) extracts, prominent resonances at 4.96–4.97 and 4.63 ppm were assigned to α- and

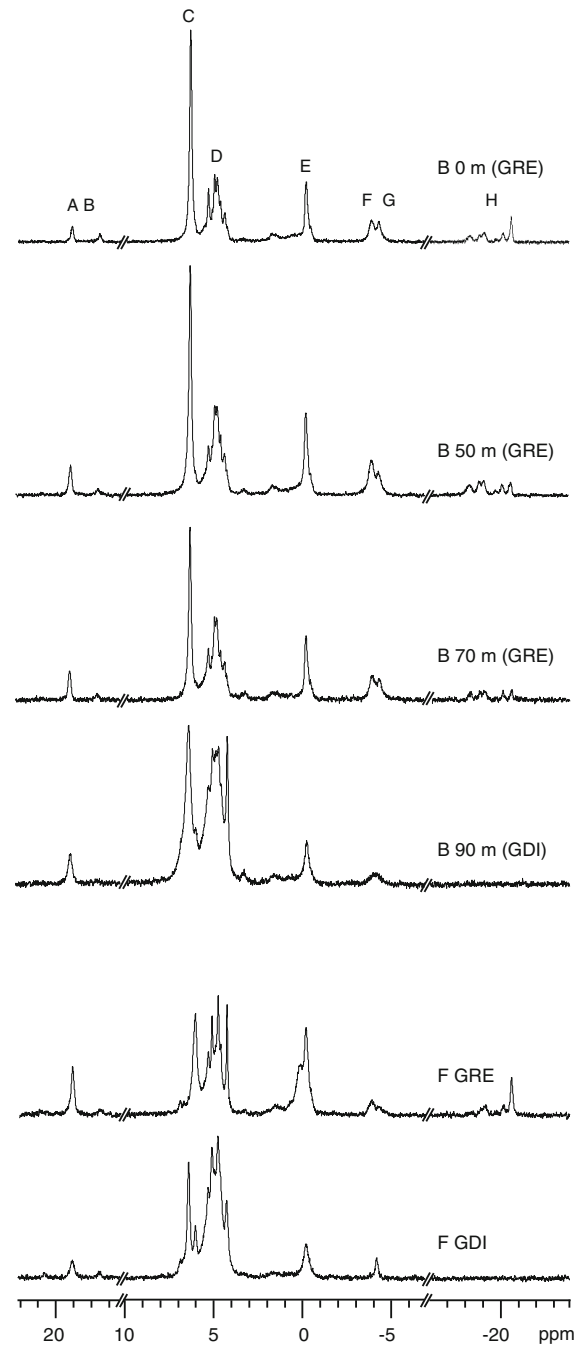

Fig. 2 Solution ^{31}P NMR spectra of NaOH–EDTA extracts of boreal forest soils from groundwater recharge (*GRE*) and discharge (*GDI*) areas in Betsele (*B*) and Flakastugan (*F*), Northern Sweden. Spectra scaled according to number of scans. *A* Phosphonates, *B* methylene diphosphonic acid spike, *C* inorganic orthophosphate, *D* monoesters, *E* diesters, *F* polyphosphate (terminal P groups); *G* pyrophosphate, *H* polyphosphate (penultimate and mid-chain P groups)

Table 4 Concentration of phosphorus compounds in soils (O-horizon) from two groundwater recharge (*GRE*) and discharge (*GDI*) gradients in Betsele and Flakastugan, Northern Sweden, as determined in NaOH–EDTA extracts by solution ^{31}P NMR spectroscopy

Site	Position (m)	GRE/ GDI	Inorganic P (mg kg^{-1})			Organic P (mg kg^{-1})				Monoester: diester
			Phosphate	Pyro-phosphate	Poly-phosphate	Monoesters	Diesters	Phos-phonates	Unknown	
Betsele	0	GRE	100 (30)	11 (4)	45 (14)	102 (30)	65 (20)	8 (2)	0 (0)	1.6
	50	GRE	156 (26)	20 (3)	94 (16)	189 (32)	102 (17)	21 (4)	10 (2)	1.9
	70	GRE	134 (24)	28 (5)	57 (10)	214 (39)	86 (16)	24 (4)	3 (1)	1.6
	90	GDI	164 (23)	19 (3)	0 (0)	387 (55)	65 (9)	29 (4)	33 (5)	6.0
Flakastugan		GRE	66 (13)	7 (1)	41 (9)	189 (42)	126 (27)	33 (7)	7 (1)	1.6
		GDI	100 (13)	19 (3)	0 (0)	533 (69)	75 (10)	25 (3)	18 (2)	7.1

Note Values in parenthesis are the proportion (%) of the total phosphorus extracted by NaOH–EDTA (see Table 3 for NaOH–EDTA *P* values)

β-glycerophosphate, respectively, both of which are products of the alkaline hydrolysis of phospholipids (Baer and Kates 1950). In the same spectral region, Turner et al. (2003a) assign a resonance at 4.80 ppm to β-glycerophosphate, and Turner et al. (2003a) and Bünemann et al. (2008) assign signals at/or between 4.48 and 4.78 ppm to adenosine monophosphates, from the alkaline degradation of RNA. Makarov et al. (2002) also report the presence of mononucleotides in the region between 4.1 and 5.3 ppm, in alkaline extracts of microbial cells.

In the $T_{1\rho}$ relaxation experiment, all signals of P monoesters decayed as single exponentials with $T_{1\rho}$ relaxation times of 10–30 ms, corresponding to linewidths between 9 and 24 Hz (orthophosphate 38 Hz), without any detectable fast-relaxing component (Fig. S2).

Changes in P composition along the gradients

We observed a shift in P composition across sites, as revealed by solution ^{31}P NMR of NaOH–EDTA extracts of humus soils. Diesters and their monoester degradation products (see previous section), as well as polyphosphates, represented a larger fraction of extractable P at Betsele GRE sites, whereas monoesters such as *myo*-, *scyllo*- and unknown inositol phosphates dominated in both GDI sites and Flakastugan GRE (Fig. 4). The relative abundance of inorganic orthophosphate decreased along Betsele and showed no clear trend in Flakastugan, whereas that of pyrophosphate and unknown compounds showed no clear trend in either site (Fig. 4). The proportion of diesters decreased two to three-fold, whereas that of monoesters increased three to four-fold, leading to higher monoester to diester ratios in GDI sites (Table 4).

The concentration of acid-digestible Al was strongly and positively correlated to the concentration of monoesters such as *myo*- ($r^2 = 0.92$, $P = 0.003$) and *scyllo*- ($r^2 = 0.95$, $P < 0.001$) inositol phosphates, but not to that of diesters and their monoester degradation products ($r^2 = 0.14$, $P = 0.471$) (Fig. 5). None of the other P classes were significantly correlated to Al concentration. Similar patterns were observed with Fe, the natural logarithm of the concentration of acid-digestible Fe was significantly correlated with the concentration of *myo*- ($r^2 = 0.66$, $P = 0.049$) and *scyllo*-inositol phosphates ($r^2 = 0.86$, $P < 0.007$) but not with diester concentration ($r^2 = 0.02$, $P = 0.794$) (Fig. 5).

Solid state ^{31}P NMR

Solid state ^{31}P MAS NMR spectra showed one single broad peak centered at approximately 0 ppm, ranging approximately from 22 to −22 ppm (Fig. 6). Spinning sidebands, caused by sample rotation in the spectrometer's magnetic field, occurred at 25 and −25 ppm and at multiple distances from the central peak (Fig. 6). The central peak was asymmetrical and broad, but close inspection revealed soil-wise variations in lineshape and sideband intensities, which probably reflect changes in total P composition along the gradients. Given the poor signal-to-noise ratio and broad linewidths, we do not attempt to quantitatively determine P composition with solid state ^{31}P NMR.

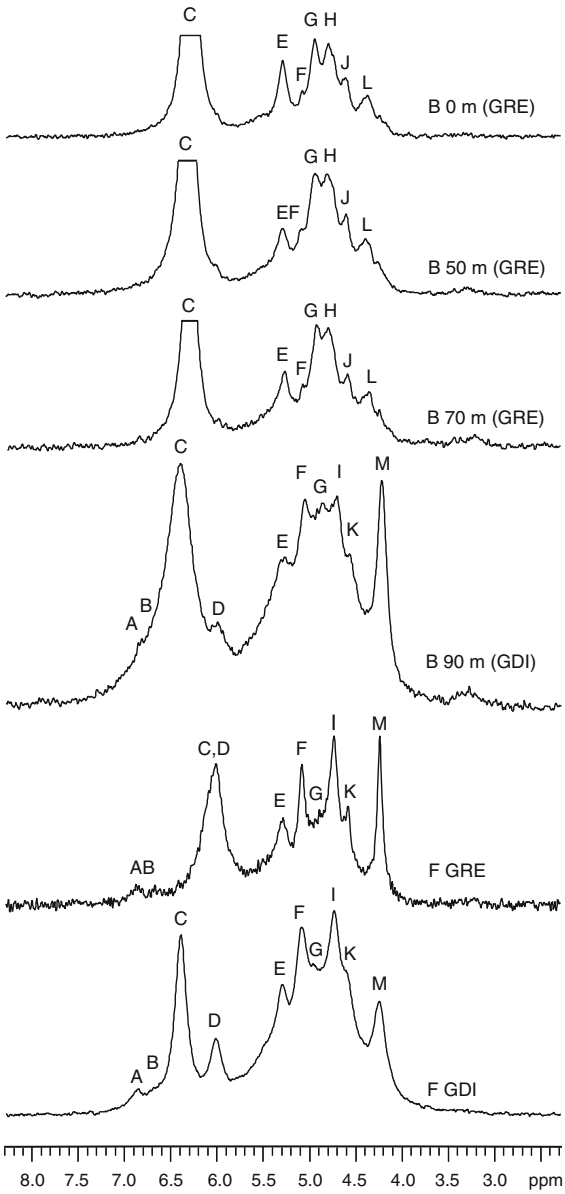

Fig. 3 Solution ^{31}P NMR spectra of the monoester region in NaOH–EDTA extracts of boreal forest soils in groundwater recharge (*GRE*) and discharge (*GDI*) sites in Betsele and Flakastugan, Northern Sweden. Spectra scaled according to number of scans. *A*, *B* unidentified inositol phosphates; *C* inorganic orthophosphate, truncated; *D*, *F*, *I*, *K* *myo*-inositol hexakisphosphate; *E*, *G*, *H*, *J*, *L* tentatively assigned to diester degradation products; *M* *scyllo*-inositol hexakisphosphate. *Letters* equivalent to those in Table 5

Discussion

Solution ^{31}P NMR analyses of the soluble P fraction revealed a clear shift in P composition across sites. Monoesters such as *myo*-, *scyllo*- and unidentified inositol phosphates dominated in humus soils with a higher concentration of Al and Fe (both GDI sites and Flakastugan GRE), whereas diesters and their degradation products, as well as polyphosphates, were proportionally more abundant in sites with lower concentrations of Al and Fe (Betsele GRE). In general, the large variety of organic and inorganic P compounds detected was characteristic of acidic soils in cool and moist conditions (Turner et al. 2004 and references therein), as well as of the humic acids extracted from high organic matter soils (Bedrock et al. 1994; Makarov et al. 1997). The abundance and diversity of soil organic P is thought to be greater under these conditions because low microbial mineralization rates lead to the accumulation of relatively labile compounds such as diesters, phosphonates and polyphosphates (Tate and Newman 1982; Makarov et al. 1997).

Organic P composition in highly sorbing soils

We found strong relationships between inositol phosphate concentration and those of Al and Fe in the humus layer (Fig. 5), although the relationships involving Fe were less strong than with Al. This is the first time that inositol phosphate concentrations are reported to change so sharply over distances as short as 90 m (Betsele gradient), and in relation to sorption sites in the humus. The fact that *myo*-inositol hexakisphosphate, the most abundant inositol phosphate detected here, was related with Al and Fe is consistent with other studies which find correlations between monoesters and oxalate-extractable Al and Fe (e.g., Turner et al. 2003b) and the fact that the abundance of inositol phosphates in a wide range of soils is often more strongly correlated with factors associated with phosphate stabilization, such as soil sorption capacity, than with other commonly measured variables such as organic carbon, nitrogen, or microbial biomass (Turner 2007 and references therein).

The fact that the relationship between inositol phosphates and Fe was weaker than with Al could be due to acid-digestible Fe not accurately reflecting the sorption properties of Fe, as suggested by a study using extended X-ray absorption fine structure spectroscopy (XAFS) on humus soils from both Betsele and Flakastugan GDI sites (Karlsson et al. 2008).

Table 5 Relative abundance (% of the monoester region) of monoester signals in NaOH–EDTA extracts of boreal forest soils in groundwater recharge (*GRE*) and discharge (*GDI*) sites in Betsele and Flakastugan, Northern Sweden, determined with solution ^{31}P NMR spectroscopy and spectral deconvolution

Site	Position (m)	GRE/GDI	A	B	D	E	F	G	H	I	J	K	L	M	Total myo-IHP	Total IP	Total DDP
Chemical shift (ppm)			6.89	6.68	6.02	5.32	5.11	4.97	4.83	4.75	4.63	4.59	4.42	4.25			
Betsele	0	GRE	0.0	0.0	0.9	19	3.2	21	19	4.6	11	0.0	12	1.7	9	10	82
	50	GRE	0.0	0.0	0.2	13	3.4	21	23	3.0	8.2	0.0	13	1.8	7	8	79
	70	GRE	0.0	0.0	0.5	14	3.2	18	27	0.0	7.4	0.0	13	2.3	4	6	79
	90	GDI	2.3	3.5	2.6	3.7	8.9	4.8	1.7	8.7	0.0	9.2	3.9	13	29	42	14
Flakastugan		GRE	2.0	0.7	15[a]	15	9.5	1.9	1.7	17	3.0	2.5	5.6	12	44	56	27
		GDI	2.2	0.8	6.3	7.8	18	5.0	4.8	16	9.1	0.7	3.2	11	41	52	30
Assignment			Unidentified IP's		*myo*-IHP	DDP	*myo*-IHP	DDP	DDP	*myo*-IHP	DDP	*myo*-IHP	DDP	*scyllo*-IHP			

Letters correspond to signals in Fig. 3; *DDP* degradation product of diesters in alkaline conditions, tentative assignment, *IHP* inositol hexakisphosphate, *IP* inositol phosphate

Note Tabulated chemical shifts are the means of shifts observed across samples; the maximum standard deviation is 0.06 ppm

[a] This peak is a combination of *myo*-IHP and the inorganic orthophosphate signal

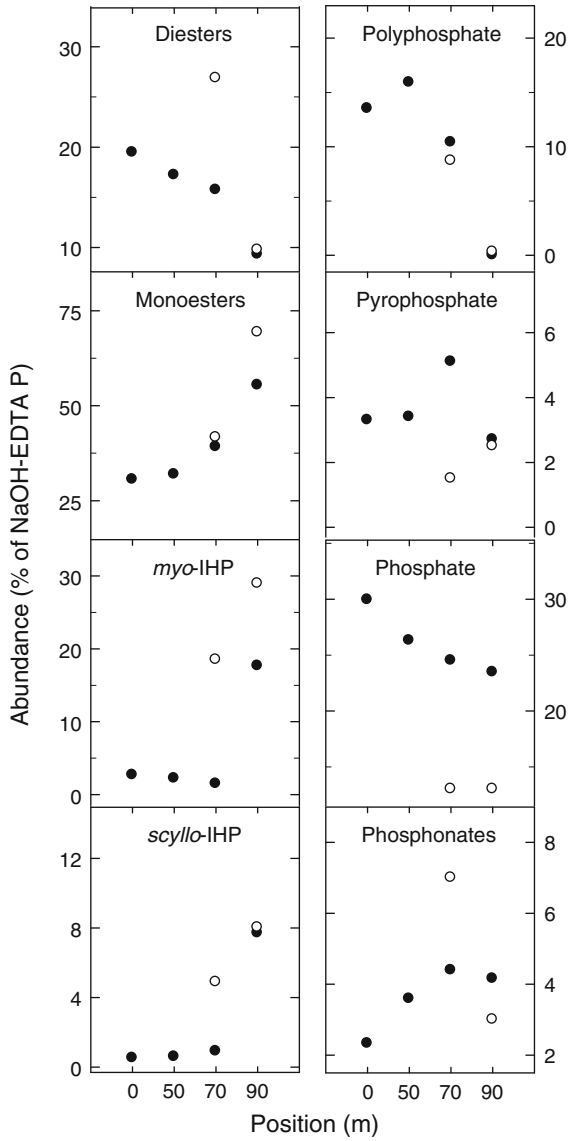

Fig. 4 Changes in the relative abundance (% of NaOH–EDTA extractable P) of different phosphorus classes determined by solution ^{31}P NMR in soils along two groundwater recharge (*GRE*) and discharge (*GDI*) gradients in Betsele (*solid circles*) and Flakastugan (*open circles*), Northern Sweden; *IHP* inositol hexakisphosphate. Position (m) applies to the Betsele gradient only; 0, 50, and 70 m are GRE sites, 90 m is a GDI site. The Flakastugan GRE site is roughly equivalent to Betsele 70 m, and the Flakastugan GDI site to Betsele 90 m

Karlsson et al. (2008) showed the presence of two dominating Fe species probably with different reactivities, which were not reflected by wet-chemical methods for Fe speciation. Therefore a more detailed characterization of Fe species would have been

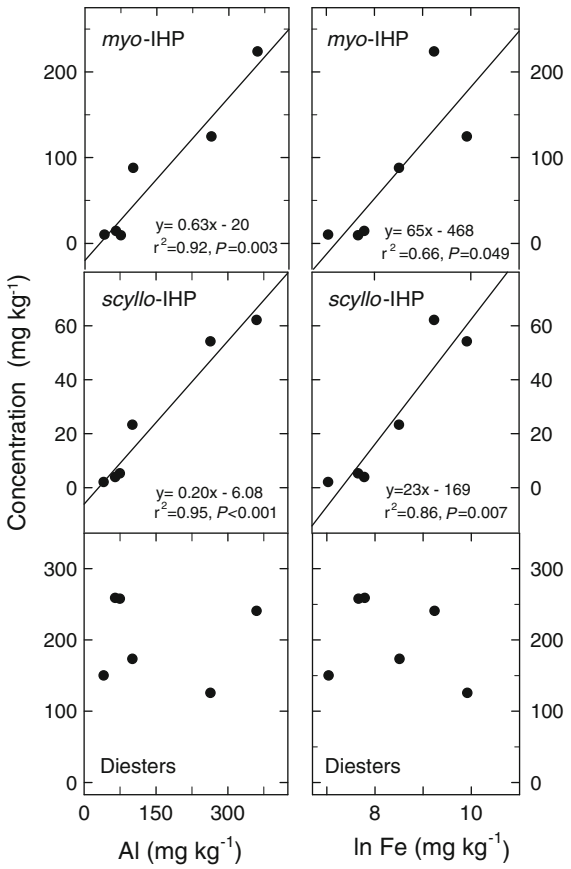

Fig. 5 Relationship between the concentrations of aluminium (Al) and iron (Fe) with those of inositol hexakisphosphates (*IHP*) and diesters (diester category includes diester degradation products, see Table 5) determined by solution ^{31}P NMR spectroscopy of NaOH–EDTA extracts of soils from two groundwater recharge and discharge gradients in Betsele and Flakastugan, Northern Sweden

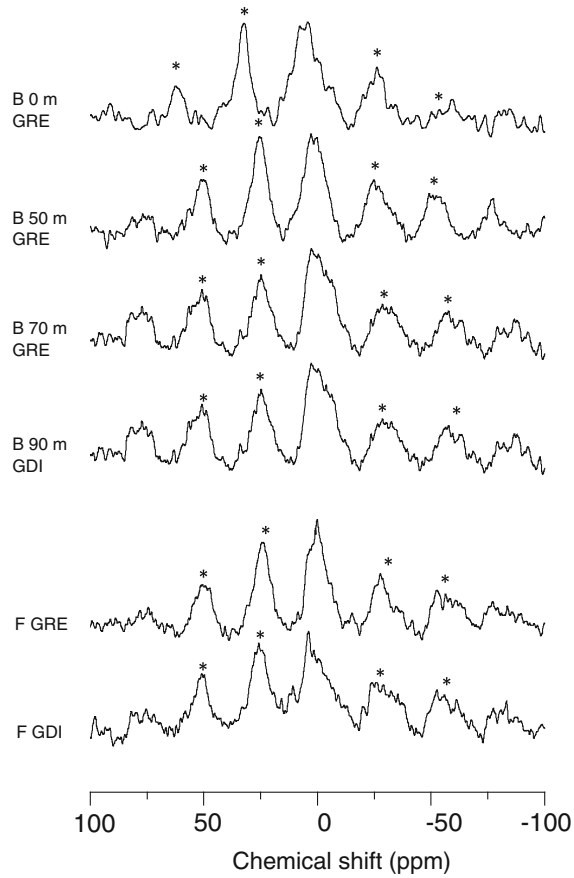

Fig. 6 Solid state ^{31}P MAS NMR spectra of soils from groundwater recharge (*GRE*) and discharge (*GDI*) sites in Betsele and Flakastugan, northern Sweden. Spectrum for Betsele 0 m (*GRE*) acquired with a spinning rate of 4.5 kHz, the remaining spectra at 4 kHz. Spinning sidebands are marked with *asterisks*. Line broadening for Betsele 0 m (GRE) is 270 Hz, and for the remaining spectra is 150 Hz

advantageous in this study to further enhance our understanding of its relationship with inositol phosphates. We cannot exclude the possibility that the differences in inositol phosphate concentration observed here were due to differences in P inputs across the sites, however, this is unlikely to fully explain the large differences in concentration observed, because *myo*-inositol hexakisphosphate is ubiquitous in eukariotic organisms (Raboy 2003), and as such it is improbable that the plant and microbial assemblages in low P sorption sites do not contain this compound.

We hypothesized that inositol phosphates would increase with increasing soil sorption capacity, but their near absence in low P sorption sites (Betsele GRE) was somewhat unexpected, because these compounds are considered to be recalcitrant and they accumulate in many soils (Turner et al. 2002). The absence of inositol phosphates has also been reported in places where in theory they should accumulate, either due to cold, acidic conditions with low levels of microbial activity (Turner et al. 2004) or high soil sorption capacity (Vincent et al. 2010). It is possible that conditions in Betsele GRE sites are more conducive to inositol phosphate mineralization. The microbial community in Betsele GRE soils is dominated by ecto- and ericoid mycorrhizal fungi, whereas in the Betsele GDI site bacteria become more abundant (Högberg et al. 2003; Nilsson et al. 2005). The mineralization of inositol phosphates

requires the specific action of phytases acting on soluble compounds (Lung and Lim 2006), and phytase activity has been detected in the 20 or so ectomycorrhizal species so far tested (Leake 2002), thus leading some to propose that ectomycorrhizal fungi might be especially important in the mineralization of inositol phosphates (Turner et al. 2002). Phytases are also produced by bacteria (Hill and Richardson 2007), but the relative importance of bacteria vs. fungi in mineralizing soil phytate is currently unknown. Fungi are the major decomposers of forest litter and are usually more efficient at degrading complex organic substrates than bacteria (e.g., Strickland et al. 2009). Furthermore, filamentous fungi in particular have been used extensively for their high phytase production efficacy (Hill and Richardson 2007) and fungi have been found to be more effective at solubilising phytate than bacteria (Oliveira et al. 2009). We hypothesize that low sorption capacity, combined low soil pH as well as a dominance of ecto-and ericoid mycorrhizae in our low Al and Fe sites create conditions more conducive to inositol phosphate mineralization.

Organic P composition in low sorbing soils

The concentration of diesters did not change appreciably across the six sites tested. This occurred despite large differences in the concentration of Al and Fe in the humus (Giesler et al. 2005; this study), microbial and plant community composition, pH, and forest productivity (Giesler et al. 1998), and is consistent with the view that diesters have a low propensity for sorption. However, we expected diesters to be proportionally more abundant than other organic P compounds in low sorption sites. Deconvolution analysis suggests that this was the case, because most monoester signals detected in extracts from low sorption soils (Betsele GRE) appeared to be degradation products of diesters, such as mononucleotides and α- and β-glycerophosphate. For example, 79–82% of the total monoester region of soil extracts from these sites potentially belonged to diester degradation products (Table 5) and including these signals in the diester category resulted in monoester to diester ratios <1 in low- and >1 in high-Al and Fe sites. Therefore, soil organic P in low sorbing soils closely reflects the inputs of organic P, usually dominated by diesters. Nucleic acids are important microbial inputs of organic P, adding up to approximately 60% of the intracellular P in fungi, bacteria and mycorrhizal mycelium (Gianfrancesco and Leake 2002). Phospholipids can constitute 5–30% of the organic P in microbes (Magid et al. 1996), and α- and β-glycerophosphates, both phospholipid degradation products, were found to be the dominant signals in ^{31}P NMR spectra of extracts from fungal and bacterial tissues (Bünemann et al. 2008). Phospholipids also constitute a large proportion of organic P in fresh plant tissue (Bieleski 1973).

Polyphosphate concentrations decreased sharply along both GRE/GDI gradients. This probably reflects a decline in the fungal contribution to soil organic P, because fungal to bacterial ratios decrease along the Betsele gradient (Högberg et al. 2003) and ecto-mycorrhizal mycelial production decrease along both Betsele and Flakastugan gradients (Nilsson et al. 2005). Fungal tissues are known to contain higher proportions of polyposphate (Makarov et al. 2005; Bünemann et al. 2008) than bacteria or plants. Consequently, the presence of polyphosphates is thought to indicate fungal decomposition (Bedrock et al. 1994). Interestingly, although low pH has also been found to inhibit the activity of phosphonate degrading microbes (Tate and Newman 1982), which should lead to an accumulation of phosphonates in the sites with lower pH, we found no clear patterns of abundance of phosphonates along the gradients.

Methodological considerations

We tested the possibility that we are overestimating inositol phosphate concentrations, given the strong overlap in the monoester region. Some argue that the monoester region is composed of individual sharp signals 'sitting' on the background of an inherently broad signal, which should be subtracted before the quantification of inositol phosphates (Doolette et al. 2010). However, many overlapping sharp signals and sharp signals on a broad background can in theory yield the same shape of spectrum, and it is therefore impossible to decide from spectrum shape alone if deduction of a broad signal is warranted. As such we gathered objective evidence regarding the presence of a broad component from a $T_{1\rho}$ experiment. This experiment demonstrated that there was no broad signal under the monoester region, because the signal intensity in the monoester region decayed without

any detectable fast-relaxing component. This indicates that the monoester region was instead composed of an unknown number of individual components of comparable line widths, so that subtracting a broad baseline before quantification of the inositol phosphate or other monoester signals would be arbitrary. Deconvolution of the monoester region and quantification of individual components assumes that no other signals overlap with the signals in question. In crowded spectral regions this assumption is problematic, but it is the simplest spectral model, which should be chosen unless evidence points to the need for a more complex one.

Solid state NMR has the potential to provide information on the extractable as well as the non-extractable P fractions in soils, which is important because although the NaOH–EDTA procedure is assumed to quantitatively extract organic P from soil, this is impossible to verify as there is no procedure to determine soil organic P directly (Turner et al. 2005). Therefore, we acquired solid state ^{31}P MAS NMR spectra of untreated soils from GRE and GDI sites in Betsele and Flakastugan (Fig. 6). The single broad peak centered at 0 ppm, which was common to all our solid state ^{31}P MAS NMR spectra, is typical for solid state ^{31}P NMR spectra of soils (e.g., Lookman et al. 1997; Shand et al. 1999), and is consistent with the chemical shift range in which signals from extractable P species are detected by solution NMR (Fig. 2). In theory, the central peak should contain detailed information about the P species present and their relative abundances. However, our peaks were generally broad and therefore overlapped (Shand et al. 1999) so that signals corresponding to individual P species could not be identified. Line broadening is caused by heterogeneous micro-environments and the presence of paramagnetic ions (Fe, Mn), which may quench signals of neighboring P species. Thus, solid state NMR detects both the extractable and the non-extractable P fractions, but might not detect the extractable P species associated with paramagnetics. Conversely, solution NMR 'sees' the NaOH–EDTA extractable fraction and also P associated with paramagnetic ions. In summary, solution and solid state spectra may complement each other without agreeing very well, given that both methods detect different P fractions. It is clear that although solid state ^{31}P NMR has a great potential to yield valuable information on the total P matrix of untreated, unextracted soils, further methodological improvements are needed for quantitative analysis of P composition.

Conclusions

Our results showed a positive relationship between Al and Fe and monoesters such as *myo*- and *scyllo*-inositol phosphates. Aluminium and iron are likely to have a stabilizing effect that protects inositol phosphates from microbial degradation, explaining why we see higher concentrations of inositol phosphates in humus soils with a high Al and Fe content. The near absence of inositol phosphates in our low Al and Fe sites was somewhat unexpected given the commonly assumed recalcitrance of these compounds, and suggests that conditions in those sites are more conducive to inositol phosphate mineralization. We found no relationship between diesters and Al and Fe concentrations although the relative proportion of diesters was higher in sites with low Al and Fe, and the same was true of polyphosphates. Polyphosphate decline along both groundwater recharge/discharge gradients could be related to the fact that microbial communities shift from being fungi-dominated at the groundwater recharge end of the gradients, to being bacterial-dominated at the discharge end (Nilsson et al. 2005), and polyphosphates are known to be more abundant in fungal than bacterial tissue (Makarov et al. 2005). These gradients include the variation in soil properties detected in the wider Fennoscandian boreal forest landscape, thus our findings provide insight into the factors regulating P biogeochemistry in the region and possibly boreal forests elsewhere, but more replication is needed to confirm this.

Acknowledgments We are grateful to Dr. Tobias Sparrman for assistance with the deconvolution procedure, as well as to Maja K Sundqvist and two anonymous reviewers for helpful comments on this manuscript. Funding was provided by the Kempe Foundation (AGV), the Swedish Research Council VR (JS, GG, RG, PP, MJ), the Swedish Research Council FORMAS (JS) and the Centre for Environmental Research in Umeå (CMF) (JV).

References

Akselsson C, Westling O, Alveteg M, Thelin G, Fransson AM, Hellsten S (2008) The influence of N load and harvest intensity on the risk of P limitation in Swedish forest soils.

Sci Total Environ 404:284–289. doi:10.1016/j.scitotenv.2007.11.017

Baer E, Kates M (1950) Migration during hydrolysis of esters of glycerophosphoric acid. 2. The acid and alkaline hydrolysis of L-Alpha-Lecithins. J Biol Chem 185:615–623

Bedrock CN, Cheshire MV, Chudek JA, Goodman BA, Shand CA (1994) Use of 31 P-NMR to study the forms of phosphorus in peat soils. Sci Total Environ 152:1–8

Bieleski RI (1973) Phosphate pools, phosphate transport and phosphate availability. Ann Rev Plant Physiol 24: 225–252

Bünemann EK, Smernik RJ, Doolette AL, Marschner P, Stonor R, Wakelin SA, McNeill AM (2008) Forms of phosphorus in bacteria and fungi isolated from two Australian soils. Soil Biol Biochem 41:1908–1915. doi:10.1016/j.soilbio.2008.03.017

Cade-Menun BJ, Preston CM (1996) A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. Soil Sci 161:770–785

Celi L, Barberis E (2005) Abiotic stabilization of organic phosphorus in the environment. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. CAB International, Wallingford, pp 113–132

Condron LM, Turner BL, Cade-Menun J (2005) Chemistry and dynamics of soil organic phosphorus. In: Sims JT, Sharpley AN (eds) Phosphorus: agriculture and the environment. American Society of Agronomy, Madison, WI, pp 87–121

Cross AF, Schlesinger WF (1995) A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64:197–214. doi:10.1016/0016-7061(94)00023-4

Dahl E, Gjems O, Kjelland-Lund JJ (1967) On the vegetation of Norwegian conifer forest in relation to the chemical properties of the humus layer. Meddelende i Norske Skogsforsøksvesen 85:501–531

De Groot CJ, Golterman HL (1993) On the presence of organic phosphate in some Camargue sediments—evidence for the importance of phytate. Hydrobiologia 252:117–126

Dell'Olio LA, Maguire RO, Osmond DL (2008) Influence of Mehlich-3 extractable aluminum on phosphorus retention in organic soils. Soil Sci 173:119–129. doi:10.1097/ss.0b013e31815d8eb7

Doolette AL, Smernik RJ, Dougherty WJ (2009) Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. Soil Sci Soc Am J 73:919–927. doi:10.2136/sssaj2008.0192

Doolette AL, Smernik RJ, Dougherty WJ (2010) Rapid decomposition of phytate applied to a calcareous soil demonstrated by a solution ^{31}P NMR study. Eur J Soil Sci. doi:10.1111/j.1365-2389.2010.01259.x

FAO (2001) Production yearbook 1999. Food and Agricultural Organization of the United Nations, vol 53, Statistical series no. 156. FAO, Rome

Gianfrancesco RU, Leake JR (2002) Utilisation of phosphodiester-P by ectomycorrhizal fungi: use of DNA as a source of phosphorus and preliminary characterisation of phosphatases in *Amanita rubescens* and *Suillus bovinus*. In: Whitton BA, Hernandez I (eds) Phosphatases in the environment. Kluwer Academic, Dordrecht

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

Giesler R, Petersson T, Högberg P (2002) Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. Ecosystems 5:300–314. doi:10.1007/s10021-001-0073-5

Giesler R, Satoh F, Ilstedt U, Nordgren A (2004) Microbially available phosphorus in boreal forests: effects of aluminum and iron accumulation in the humus layer. Ecosystems 7:208–217. doi:10.1007/s10021-003-0223-z

Giesler R, Andersson T, Lovgren L, Persson P (2005) Phosphate sorption in aluminum- and iron-rich humus soils. Soil Sci Soc Am J 69:77–86

Gorham E (1953) The development of the humus layer in some woodlands of the English Lake District. J Ecol 41:123–152

Hill J, Richardson AE (2007) Isolation and assessment of organisms that utilize phytate. In: Turner BL, Richardson AE, Mullaney EJ (eds) Inositol phosphates. Linking agriculture and the environment. CAB International, Wallingford, Oxfordshire, pp 61–77

Högberg P (2001) Interactions between hillslope hydrochemistry, nitrogen dynamics, and plants in Fennoscandian boreal forest. In: Schulze ED, Heimann M, Harrison S (eds) Global biogeochemical cycles in the climate system. Academic Press, San Diego, CA, pp 237–256

Högberg MN, Bååth E, Nordgren A, Arnebrant K, Högberg P (2003) Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs—a hypothesis based on field observations in boreal forest. New Phytol 160:225–238. doi:10.1046/j.1469-8137.2003.00867.x

Hupfer M, Rübe B, Schmieder P (2004) Origin and diagenesis of polyphosphate in lake sediments: a ^{31}P-NMR study. Limnol Oceanogr 49:1–10

Karlsson T, Persson P, Skyllberg U, Mörth CM, Giesler R (2008) Characterization of Iron(III) in organic soils using extended X-ray absorption fine structure spectroscopy. Environ Sci Technol 42:5449–5454. doi:10.1021/es800322j

Lahti T, Vaisanen RA (1987) Ecological gradients of boreal forests in south Finland: an ordination test of Cajander's forest type theory. Vegetation 68:145–156

Leake JR (2002) Organic phosphorus utilisation by mycorrhizal plants and fungi: how much do we really know? In: Whitton BA, Hernandez I (eds) Phosphatases in the environment. Kluwer Academic, Dordrecht

Lookman R, Grobet P, Merckx R, Van Riemsdijk WH (1997) Application of ^{31}P and ^{27}Al MAS NMR for phosphate speciation studies in soil and aluminium hydroxides: promises and constraints. Geoderma 80:369–388

Lung SC, Lim BL (2006) Assimilation of phytate-phosphorus by the extracellular phytase activity of tobacco (*Nicotiana tabacum*) is affected by the availability of soluble phytate. Plant Soil 279:187–199. doi:10.1007/s11104-005-1009-1

Magid J, Tiessen H, Condron LM (1996) Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. In: Piccolo A (ed) Humic substances in terrestrial ecosystems. Elsevier, Amsterdam, pp 429–466

Makarov MI, Malysheva TI, Haumaier L, Alt HG, Zech W (1997) The forms of phosphorus in humic and fulvic acids

of a toposequence of alpine soils in the northern Caucasus. Geoderma 80:61–73

Makarov MI, Haumaier L, Zech W (2002) Nature of soil organic phosphorus: an assessment of peak assignments in the diester region of 31P NMR spectra. Soil Biol Biochem 34:1467–1477

Makarov MI, Haumaier L, Zech W, Marfenina OE, Lysak LV (2005) Can P-31 NMR spectroscopy be used to indicate the origins of soil organic phosphates? Soil Biol Biochem 37:15–25. doi:10.1016/j.soilbio.2004.07.022

Månsson K (2005) Plant-bacterial and plant-fungal competition for nitrogen and phosphorus. Dissertation, Lund University

Mulder J, Pijpers M, Christophersen N (1991) Water flow-paths and the spatial distribution of soils and exchangeable cations in an acid-rain impacted and a pristine catchment in Norway. Water Resour Res 27:2919–2928

Nilsson LO, Giesler R, Bååth E, Wallander H (2005) Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. New Phytol 165:613–622. doi:10.1111/j.1469-8137.2004.01223.x

Nordin A, Högberg P, Nasholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. Oecologia 129:125–132. doi:10.1007/s004420100698

Norrström AC (1993) Retention and chemistry of aluminium in groundwater discharge areas. Environ Pollut 81:269–275

Norrström AC (1995) Concentration and chemical species of iron in soils from groundwater/surface water ecotones. Hydrol Sci 40(3):319–329

Ognalaga M, Frossard E, Thomas F (1994) Glucose-1-phosphate and myo-inositol hexaphosphate adsorption mechanisms on goethite. Soil Sci Soc Am J 58:332–337

Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimaraes CT, Schaffert RE, Sa NMH (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol Biochem 41:1782–1787. doi:10.1016/j.soilbio.2008.01.012

Pettersson E (1994) Predictive functions for impact of nitrogen fertilization on growth over five years. Forest Research Institute of Sweden, Report no. 3, pp 1–56

Raboy V (2003) Myo-inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry 64:1033–1043. doi:10.1016/s0031-9422(03)00446-1

Shand CA, Cheshire MV, Bedrock CN, Chapman PJ, Fraser AR, Chudek JA (1999) Solid-phase P-31 NMR spectra of peat and mineral soils, humic acids and soil solution components: influence of iron and manganese. Plant Soil 214:153–163

Soil Survey Staff (1992) Keys to soil taxonomy. SMSS Technical Monograph, vol 19, Blacksburg (VA)

Strickland MS, Lauber C, Fierer N, Bradford MA (2009) Testing the functional significance of microbial community composition. Ecology 90:441–451

Tate KR, Newman RH (1982) Phosphorus fractions of a climosequence of soils in New Zealand tussock grassland. Soil Biol Biochem 14:191–196

Thelin G (2006) Askåterföring till gran - och bokbestånd - effekter på näring, tillväxt kvävedynamik och kolbalans. Värmeforsk, Stockholm

Turner BL (2007) Inositol phosphates in soil: amounts, forms and significance of the phosphorylated inositol stereoisomers. In: Turner BL, Richardson AE, Mullaney EJ (eds) Inositol phosphates. Linking agriculture and the environment. CAB International, Wallingford, pp 186–206

Turner BL (2008) Soil organic phosphorus in tropical forests: an assessment of the NaOH-EDTA extraction procedure for quantitative analysis by solution P-31 NMR spectroscopy. Eur J Soil Sci 59:453–466. doi:10.1111/j.1365-2389.2007.00994.x

Turner BL, Richardson AE (2004) Identification of *scyllo*-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci Soc Am J 68:802–808

Turner BL, Papházy MJ, Haygarth PM, McKelvie ID (2002) Inositol phosphates in the environment. Philos Trans R Soc Lond Ser B 357:449–469

Turner BL, Mahieu N, Condron LM (2003a) Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. Soil Sci Soc Am J 67:497–510

Turner BL, Mahieu N, Condron LM (2003b) Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution P-31 NMR spectroscopy and spectral deconvolution. Soil Sci 168(7):469–478. doi:10.1097/01.ss.0000080332.10341.ed

Turner BL, Baxter R, Mahieu N, Sjögersten S, Whitton BA (2004) Phosphorus compounds in subarctic Fennoscandian soils at the mountain birch (*Betula pubescens*)—tundra ecotone. Soil Biol Biochem 36:815–823. doi:10.1016/j.soilbio.2004.01.011

Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus. Talanta 66:294–306. doi:10.1016/j.talanta.2004.11.012

Turner BL, Condron LM, Richardson SJ, Peltzer DA, Allison VJ (2007) Soil organic phosphorus transformations during pedogenesis. Ecosystems 10:1166–1181. doi:10.1007/s10021-007-9086-z

Vincent AG, Turner BL, Tanner EVJ (2010) Soil organic phosphorus dynamics following perturbation of litter cycling in a tropical moist forest. Eur J Soil Sci 61:48–57. doi:10.1111/j.1365-2389.2009.01200.x

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513. doi:10.1126/science.1098778