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Phosphorus composition of upland soils polluted by long-term atmospheric nitrogen deposition

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Abstract. Atmospheric N deposition can enhance biological P limitation in terrestrial ecosystems and increase the importance of organic P to plants and microorganisms. We used NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy to determine the P composition of soils in the Upper Teesdale National Nature Reserve, northern England, an upland region influenced by such deposition for at least 150 years. Three characteristic soil types were sampled on three occasions during an annual cycle: blanket peat (318 mg g^{-1} total C, 607 μg g^{-1} total P, pH 3.9); acid organic soil under grassland (354 mg g^{-1} total C, 1190 μg g^{-1} total P, pH 3.7); calcareous soil under grassland (140 mg g^{-1} total C, 649 μg g^{-1} total P, pH 7.3). Between 58 and 99% of the total P in soil and litter layers was extracted by 0.25 M NaOH + 0.05 M EDTA. Extracts of all soils were dominated by organic P, mainly in the form of orthophosphate monoesters (43–69% extracted P). The two acidic soils also contained large proportions of orthophosphate diesters (6–19% extracted P) and phosphonates (7–16% extracted P), suggesting that these compounds become stabilised at low pH. However, a seasonal trend of increasing orthophosphate monoester-to-diester ratios, most evident in the calcareous grassland soil, indicated the preferential degradation of orthophosphate diesters during the growing season. Orthophosphate was the major inorganic P compound (17–34% extracted P), and all soils contained pyrophosphate (1–5% extracted P). However, orthophosphate determined in the NaOH–EDTA extracts by solution ^{31}P NMR spectroscopy was substantially greater than that determined by molybdate colourimetry, suggesting that orthophosphate occurred in complexes with humic compounds that were not detected by conventional procedures. Our results suggest that organisms able to use recalcitrant soil organic P may have a competitive advantage in environments under enhanced atmospheric N deposition.

Abbreviations: C – carbon, EDTA – ethylenediaminetetra-acetic acid, N – nitrogen, P – phosphorus, ^{31}P NMR – phosphorus-31 nuclear magnetic resonance.

Introduction

Atmospheric N deposition can cause or enhance P limitation in ecosystems (Aber et al. 1989), which can change plant and microbial communities through competi-

tive exclusion when coupled with other effects of atmospheric N deposition (Van der Eerden et al. 1991; Bobbink et al. 1998). Increased P demand under such conditions depletes soil inorganic P and increases the importance of organic P to plants and microorganisms (Johnson et al. 1998; Lee and Caporn 1998). Thus, the biological availability of soil organic P may partly regulate the effects of atmospheric N deposition.

Organic P typically represents 25 to 50% of the total soil P, but can account for up to 90% in high organic matter soils (Harrison 1987). It is probably of greatest importance to plants in natural environments where mineral P is scarce, but its role in plant nutrition remains poorly understood, partly due to the analytical difficulties associated with determining the multitude of chemical forms present in soil (Anderson 1980). Much of the soil organic P remains uncharacterised, but orthophosphate monoesters and diesters, polyphosphates and phosphonates can all be present in varying amounts (e.g., Newman and Tate (1980), Bedrock et al. (1994), Cade-Menun and Preston (1996), Guggenberger et al. (1996)). Orthophosphate monoesters dominate the organic P in most soils, mainly as plant-derived inositol phosphates that become stabilised by association with clays and humic substances (Turner et al. 2002b). Other monoesters, such as mononucleotides and sugar phosphates, are more labile, as are orthophosphate diesters, including nucleic acids and phospholipids (Bowman and Cole 1978). Phosphonates, which contain a direct C–P bond, only occur in measurable quantities in moist, cold, or acidic soils (Tate and Newman 1982).

Information on the composition of organic P in soils under atmospheric N deposition is scarce, but is fundamental to understanding and managing the effects of such pollution on plant and microbial communities. The aim of this study was to characterise over a seasonal cycle the P composition of characteristic upland soils polluted by long-term atmospheric N deposition. The Upper Teesdale National Nature Reserve was chosen for the study area, because it is subject to substantial atmospheric N deposition and contains plant communities that are sufficiently rare to be of international importance (Clapham 1978). The area has suffered a considerable loss of some species during the previous 25 years, notably reductions in bryophyte diversity and lichen abundance (Huntley et al. 1998). There are several possible reasons for this, not necessarily the same for each species, although some plant communities are strongly limited by the availability of P (Turner et al. 2001).

Methods

Site and soil description

The Upper Teesdale National Nature Reserve, County Durham, northern England, contains relict late-glacial plant assemblages and has three distinct soil types and related plant communities (Clapham 1978):

1. Blanket peat, dominated by *Calluna vulgaris*, *Erica tetralix* and *Sphagnum* spp.;
2. Acid organic soils under grassland, dominated by *Festuca ovina* and *Nardus* stricta);
3. Calcareous soils under grassland, dominated by *Kobresia simpliciuscula*, *Carex ericetorum* and *Thymus praecox* ssp. *arcticus*).

The grasslands are grazed by sheep. Soil samples were collected on Widdybank Fell (Ordnance Survey Grid Ref. NY 820 300, latitude 54°40′ N, longitude 2°15′ W, maximum height above sea level 519 m, mean annual rainfall 1560 mm), a sufficiently small area to eliminate differences in climate. Mean daily temperatures range from an average of 0.1 °C in February to 12.3 °C in July. Current annual rates of N deposition on Great Dun Fell, close to Widdybank Fell, are between 20 and 40 kg ha^{-1} depending on altitude (Hicks et al. 2000).

Soil sampling and preparation

Soils were sampled on three occasions during 2000 (January, May, September). On each occasion, 8 cores were taken to 5 cm depth from designated 5 m × 5 m areas on each soil type. On one occasion samples were also taken at 5–10 cm depth, in addition to litter samples from the blanket peat and acid grassland soil (there was no litter layer on the calcareous grassland soil). Soils were bulked and roots, stones and other macrofauna removed by hand. The soils were then dried for 10 days at 30 °C and ground to pass a 600-μm sieve.

Determination of soil properties

Soil pH was determined in a 1:2.5 ratio of soil to deionised water. Moisture content was determined gravimetrically by drying at 105 °C. Soil volume was determined by calculating the dry weight of a 5-cm^3 cube. Total soil C and N were determined simultaneously using a Carlo-Erba NA2000 analyser. Total soil P was determined by digestion with concentrated sulphuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), and selenium catalyst (Novozamsky et al. 1983). Phosphorus was detected by molybdate colourimetry at 880 nm (Murphy and Riley 1962). The statistical significance of differences in soil P fractions between sampling dates was determined using single-factor analysis of variance. Where F-values were significant ($P < 0.05$), individual differences were compared by least significant difference.

Solution ^{31}P NMR spectroscopy

Phosphorus was extracted by shaking 5-g soil with 100 ml of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 hours at 20 °C (Cade-Menun and Preston 1996). The extracts were centrifuged at 10,000 × g for 30 minutes, frozen in liquid N, then freeze-dried over several days. Inorganic orthophosphate was estimated in diluted extracts by molybdate colourimetry (Murphy and Riley 1962), and total P was determined by the same procedure following acid-persulphate digestion (Rowland and Haygarth 1997). Organic P was estimated as the difference between total

P and inorganic orthophosphate. This fraction also includes inorganic polyphosphates, but we use the term organic P for clarity. Precipitation of humic material caused problems with the analysis of extracts of the acid grassland soil (and to some extent the blanket peat) by molybdate colourimetry. This was corrected by running blank samples with reagent-free acid, although alternative techniques such as standard additions or acidification/centrifugation are also appropriate. All results are means of three replicate analyses, with standard errors (not shown) less than ± 5% of the mean value. An internal reference soil was used to monitor the reproducibility of the extraction procedure.

Freeze dried NaOH–EDTA extracts were re-dissolved in 1 ml 1 M NaOH and 0.2 ml D_2O and transferred to 5-ml NMR tubes. The addition of NaOH ensures a solution pH > 12, so that chemical shifts are consistent due to the deprotonation of compounds (Cade-Menun and Preston 1996). Solution $^{31}P\{^{1}H\}$ spectra were run on a Bruker AVANCE DPX300 FT NMR spectrometer resonating at 121.5 MHz for ^{31}P and 299.9 MHz for ^{1}H. WALTZ decoupling was used and acquisition parameters were chosen to give a quantitative relationship between the intensities of the observed resonances: PW 5.0 μs (30°); TR 1.0 s; TD 16k words; SW 14,700 Hz. Data were transformed into 8k words spectra using 25 Hz line broadening. The low concentrations of P in the extracts meant that long run times (36,000 scans, equivalent to approximately 24 hours machine time) were necessary to obtain acceptable signal to noise ratios. Chemical shifts (ppm) were measured relative to an external standard of orthophosphoric acid, and compounds were identified according to literature reports (Turner et al. 2003 (in press)). The proportions of the total P represented by each functional compound class were calculated by integration.

Results

Soil properties

Soil physical and chemical properties are presented in Table 1. The blanket peat and acid grassland soil were pH < 4, whilst the calcareous grassland soil was pH > 7. Total C and N concentrations were greater in the two acidic soils compared to the calcareous grassland soil, and decreased with depth from the litter layers to the 5–10 cm layers. Bulk density of all three soils was between 0.15 g cm^{-3} for the acid grassland soil at 5–10 cm depth to 0.57 g cm^{-3} for the calcareous grassland soil at 0–5 cm. Bulk densities of litter layers were < 0.1 g cm^{-3}.

Total P concentrations varied amongst the soils when expressed per unit mass (Table 2), but were similar when expressed per unit volume. Mean total P concentrations for the 0–5 cm layer were: blanket peat 607 μg P kg^{-1} soil (196 μg P cm^{-3} soil); acid grassland soil 1190 μg P g^{-1} soil (270 μg P cm^{-3} soil); calcareous grassland soil 649 μg P g^{-1} soil (367 μg P cm^{-3} soil). The litter layers of the blanket peat (362 μg P g^{-1}, 25 μg P cm^{-3}) and acid grassland (796 μg P g^{-1}, 51 μg P cm^{-3}) contained much lower total P concentrations than the 0–5 cm soil lay-

Table 1. Physical and chemical properties of the Widdybank Fell soils by depth. Soils were sampled during September 2000.

Soil type	Depth of sample	pH	Bulk density	Total C†	Total N	C-to-N
		(water)	g cm^{-3} soil	mg g^{-1} soil	mg g^{-1} soil	(by mass)
Blanket peat	litter	3.6	0.068	408	15.7	25.9
	0–5 cm	3.9	0.306	318	16.7	19.0
	5–10 cm	3.7	0.242	266	11.9	22.4
Acid grassland soil	litter	3.6	0.064	533	25.3	21.1
	0–5 cm	3.7	0.234	354	24.4	14.5
	5–10 cm	3.4	0.152	320	20.5	15.6
Calcareous grassland soil	0–5 cm	7.3	0.566	140	8.8	15.9
	5–10 cm	7.4	0.445	100	7.0	14.2

† Values for the calcareous soil include carbonates.

ers. Similarly, the 5–10 cm layers contained slightly smaller total P concentrations than the 0–5 cm layers. The variability in total P between the three sampling dates highlights the inherent natural spatial variation of soil properties, although the differences were not statistically significant (Table 2).

Total C-to-P ratios ranged between 206 and 515 and total N-to-P ratios between 12.9 and 27.1. The deeper soil layers tended to have lower C-to-P and N-to-P ratios than the 0–5 cm layers, whilst the litter layers contained much greater ratios than the soil layers. Total C in the calcareous soil probably included a large proportion of calcium carbonate, so for this soil the ratios will be greater than those calculated using organic C.

Phosphorus extraction by NaOH–EDTA

Mean total P recoveries in NaOH–EDTA from the 0–5 cm layers were 68% for the blanket peat, 64% for the acid grassland soil, and 77% for the calcareous grassland soil (Table 2). Greater proportions of P were recovered from litter layers of the blanket peat (97%) and the acid grassland soil (81%). This confirms the efficiency of this extractant for total soil P (Cade-Menun and Preston 1996; Dai et al. 1996), being an improvement on NaOH alone which typically extracts < 50% total soil P (e.g., Newman and Tate (1980) and Guggenberger et al. (1996)).

Most of the NaOH–EDTA extractable P was classified as organic P by molybdate colourimetry (90–99% extracted P). Inorganic orthophosphate concentrations determined by this method were small (1–10% extracted P), and it is likely that these were overestimated in the blanket peat and acid grassland soil extracts due to slight interference from precipitated humic materials.

Table 2. Phosphorus in the whole soil and in NaOH–EDTA extracts determined by molybdate colourimetry. Values are means of triplicate analyses with standard errors less than ± 5%. Total P concentrations for an individual soil with the same letters are not significantly different ($P > 0.05$).

Soil type and sampling date	Depth of sample		NaOH–EDTA extracts		
		Total soil P	Total P†	Inorganic orthophosphate‡	Organic P‡
		μg P g^{-1} soil			
Blanket peat					
January	0–5 cm	596[a]	401 (67)	40 (10)	361 (90)
May	0–5 cm	608[a]	436 (72)	15 (4)	421 (96)
September	litter	362[b]	352 (97)	5 (1)	347 (99)
	0–5 cm	617[a]	395 (64)	30 (7)	365 (93)
	5–10 cm	575[a]	373 (65)	33 (9)	340 (91)
Acid grassland					
January	0–5 cm	1137[a]	765 (67)	46 (6)	719 (94)
May	0–5 cm	1200[a]	692 (58)	69 (10)	623 (90)
September	litter	796[b]	641 (81)	76 (12)	565 (88)
	0–5 cm	1233[a]	835 (68)	53 (6)	782 (94)
	5–10 cm	1217[a]	761 (63)	49 (6)	712 (94)
Calcareous grassland					
January	0–5 cm	617[a]	484 (79)	19 (4)	465 (96)
May	0–5 cm	650[a]	423 (65)	2 (1)	421 (99)
September	0–5 cm	679[a]	589 (86)	22 (4)	565 (96)
	5–10 cm	612[a]	549 (90)	17 (3)	532 (97)

† Values in parentheses indicate the percentage of the total soil P.
‡ Values in parentheses indicate the percentage of the total P in the NaOH–EDTA extract.

Phosphorus composition of soil extracts determined by solution ^{31}P NMR spectroscopy

The soils contained orthophosphate monoesters, orthophosphate diesters, phosphonates, pyrophosphate and inorganic orthophosphate. The blanket peat and acid grassland soil contained a similar P composition compared to the calcareous grassland (Figures 1, 2 and 3). Chemical shifts were generally slightly upfield of literature reports (e.g., Turner et al. (2003) (in press)) and varied slightly amongst extracts of the different soils, almost certainly due to high concentrations of salts and paramagnetics ions (Table 3).

Signals between 3.0 and 6.0 ppm were assigned to orthophosphate monoesters. These dominated the extractable P in all soils, constituting between 43 and 69% extracted P (Table 4). The region assigned to orthophosphate monoesters generally represented a single envelope, although some clear signals were distinguishable (Table 3).

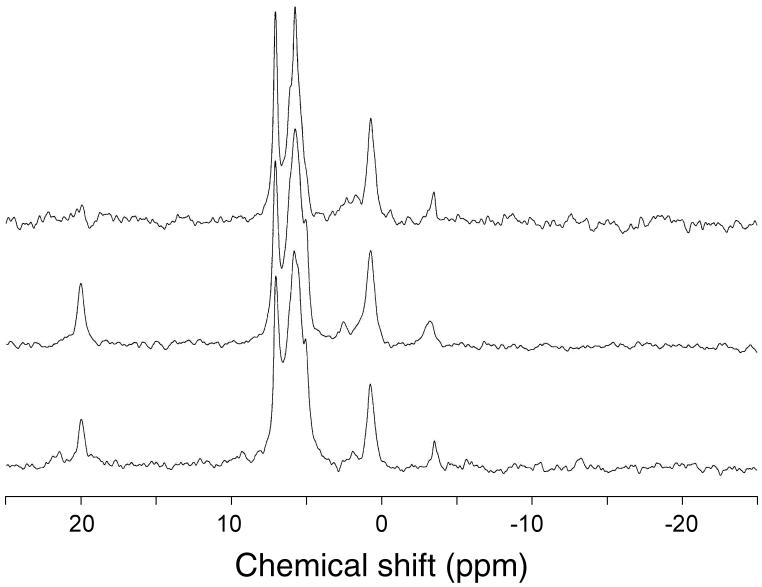

Figure 1. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of blanket peat sampled during September 2000 showing litter (top), 0–5 cm (centre) and 5–10 cm (bottom) layers.

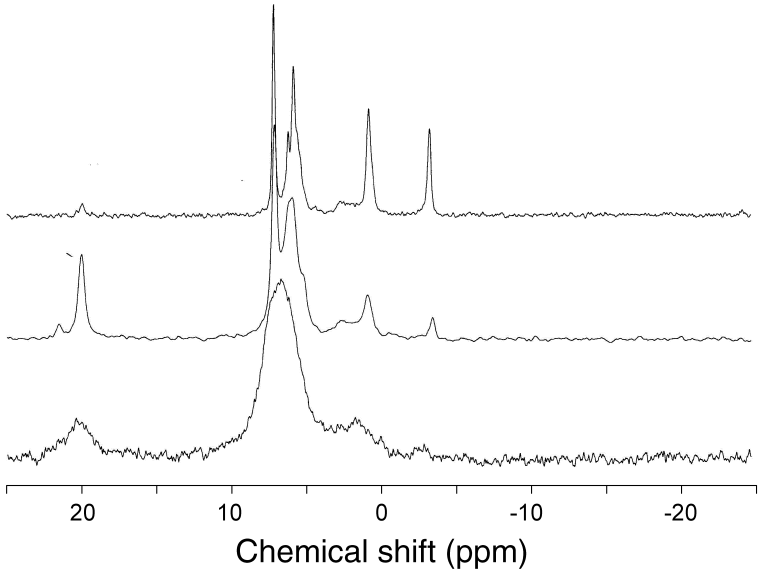

Figure 2. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of an acid grassland soil sampled during September 2000 showing litter (top), 0–5 cm (centre) and 5–10 cm (bottom) layers.

Distinct signals between 6.5 and 7.1 ppm were assigned to inorganic orthophosphate, which constituted between 17 and 34% extracted P, despite only small con-

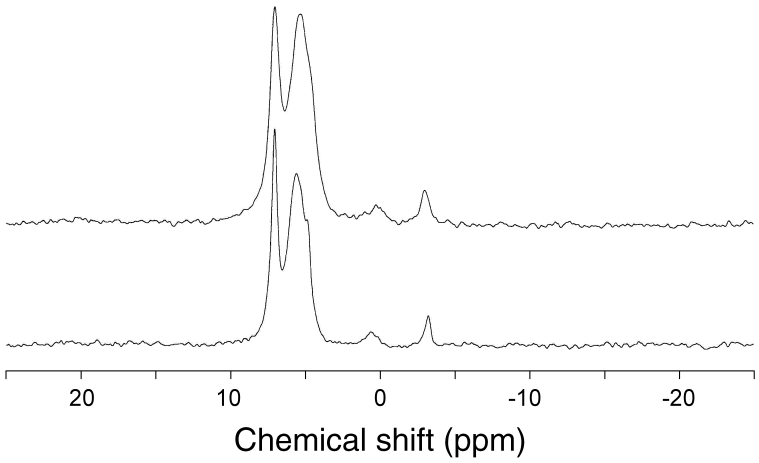

Figure 3. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of calcareous grassland soil sampled during September 2000 showing the 0–5 cm (top) and 5–10 cm (bottom) layers.

Table 3. Solution ^{31}P NMR chemical shifts and their assignments in NaOH–EDTA extracts of three soils from the Upper Teesdale National Nature Reserve, northern England. Chemical shifts are in ppm from an external orthophosphoric acid standard, and assignments are based on literature reports (Turner et al. 2002b).

Compound class	Integration range	Specific signals identified		
		Blanket peat	Acid grassland soil	Calcareous grassland soil
Phosphonates	19.0 to 21.5	19.4 to 19.6	19.4 to 19.8	19.6
		20.9 to 21.0	20.5 to 21.0	
Inorganic orthophosphate		6.5 to 6.7	6.5 to 6.6	6.7 to 7.1
Orthophosphate monoesters	3.0 to 6.0	4.5 to 4.6	4.6	4.5 to 4.7
		5.2 to 5.3	5.3	5.2 to 5.3
		5.5 to 5.6	5.5 to 5.6	5.4
Orthophosphate diesters	−1.0 to 2.0	0.2 to 0.3	0.1 to 0.3	0.1 to 0.3
		0.4 to 2.0†	0.4 to 2.0†	0.4 to 2.0†
Pyrophosphate		−3.7 to −4.4	−3.6 to −4.1	−2.9 to −3.6
Polyphosphate		nd	−20.1	nd
Others		7.3		

† Shoulders covering this range. nd = not detected.

centrations being detected by molybdate colourimetry (Table 4). For example, orthophosphate in the NaOH–EDTA extracts of the calcareous grassland soil constituted between 1 and 4% extracted P when determined by molybdate colourimetry, but between 20 and 34% when the extracts were analysed by solution ^{31}P NMR

Table 4. Concentrations (μg P g^{-1} soil) of P functional classes in NaOH–EDTA extracts of a blanket peat, acid grassland soil and calcareous grassland soil determined by solution ^{31}P NMR spectroscopy. Values in parentheses indicate the percentage of the total signal area (i.e. percentage of the total extracted P). Rounding may result in an apparent total signal area of 100 ± 1%.

Soil type and sampling date	Depth of sample	Inorganic P			Organic P			Monoester to diester ratio
		Orthophosphate	Pyrophosphate	Polyphosphate	Orthophosphate monoesters	Orthophosphate diesters	Phosphonates	
Blanket peat								
January	0–5 cm	88 (22)	22 (5)	nd	180 (45)	76(19)	36 (9)	2.38
May†	0–5 cm	82 (19)	19 (4)	nd	200 (46)	70 (16)	37 (9)	2.84
September	litter	82 (23)	11 (3)	nd	180 (51)	79 (22)	trace	2.27
	0–5 cm	72 (18)	14 (4)	nd	209 (53)	71 (18)	29 (7)	2.94
	5–10 cm	78 (21)	10 (3)	nd	209 (56)	42 (11)	34 (9)	4.97
Acid grassland soil								
January	0–5 cm		42 (5)	nd	629 (82)‡	44 (6)	50 (7)	–
May	0–5 cm	117 (17)	10 (1)	35 (5)	331 (47)	114 (17)	90 (13)	2.84
September	litter	139 (22)	69 (11)	nd	254 (40)	168 (26)	11 (2)	1.51
	0–5 cm	217 (26)	18 (2)	nd	363 (43)	101 (12)	136 (16)	3.58
	5–10 cm		7 (1)	nd	570 (75)‡	120 (16)	64 (8)	–
Calcareous grassland soil								
January	0–5 cm	95 (20)	25 (5)	nd	335 (69)	29 (6)	trace	11.52
May	0–5 cm	101 (24)	16 (4)	nd	285 (67)	18 (4)	2 (< 1)	15.47
September	0–5 cm	201 (34)	19 (3)	nd	351 (60)	16 (3)	trace	21.58
	5–10 cm	173 (31)	19 (3)	nd	343 (63)	14 (3)		24.41

† An additional signal at 7.3 ppm represented 6% of the total signal area. ‡ Inorganic orthophosphate and orthophosphate monoesters were an inseparable single signal at approximately 6.0 ppm. nd, not detected.

spectroscopy. Signals from inorganic orthophosphate were upfield of literature reports (e.g., Turner et al. (2003) (in press)), but the assignment was confirmed by spiking extracts with K_2HPO_4. Interestingly, the chemical shift of these spikes varied depending on the concentration. For example in the blanket peat extracts, the chemical shift of the added orthophosphate spike ranged from 5.3 and 6.1 ppm at high and medium P concentrations (16 and 125 scans to obtain acceptable signal) to 6.6 ppm at low concentrations (15,000 scans to obtain acceptable signal). Thus, the chemical shift of the spike only coincided with the sample signal at concentrations similar to those present in the original sample.

Signals between –1.0 and 2.0 ppm were assigned to orthophosphate diesters, which constituted a greater proportion of the total signal area in the blanket peat (11–19%) and the acid grassland soil (6–17%) compared to the calcareous grassland soil (3–6%) (Table 4). Within the diester region, a clear signal close to 0 ppm in extracts of all soils was assigned to DNA, whilst a shoulder between 0.4 and 2.0 ppm was assigned to phospholipids. The phospholipids include phosphatidyl ethanolamine (1.8 ppm), phosphatidyl serine (1.6 ppm), and phosphatidyl choline (0.7 ppm), although the latter compound degrades rapidly in alkaline solutions, so is unlikely to contribute greatly to the measured concentrations of orthophosphate diesters reported here (Turner et al. 2003 (in press)). Signals in this region have also been assigned to teichoic acids, which occur in bacterial cell walls (e.g., Condron et al. (1990)). However, teichoic acids are unlikely to be present in these soils, because cell walls are assembled from P-free teichuronic acid units in P-limited environments (Stewart and Tiessen 1987).

Phosphonates were consistently present in the two acid soils, but only trace amounts were detected in the calcareous grassland soil (Figures 1 and 2, Table 4). Two phosphonates were identified (Table 3): signals at 19.5 ppm were assigned to an unspecified phosphonolipid, whilst signals at 20.8 ppm were assigned to 2-aminoethylphosphonic acid (Newman and Tate 1980).

Signals in the –4.0 ppm region were assigned to pyrophosphate, which occurred in extracts of all soils at relatively similar proportions (1–5% extracted P). A signal at –20.1 ppm in the acid grassland soil May sample accounted for 5% of the total extracted P. This was assigned to inorganic polyphosphate, due to the absence of corresponding signals in the –9 ppm region that would indicate organic polyphosphates (Turner et al. 2003 (in press)). A small signal at 7.3 ppm was detected in the May extract of the blanket peat, which could indicate an aromatic orthophosphate diester (Bedrock et al. 1994; Turner et al. 2003 (in press)).

Phosphorus composition of litter and variations with soil depth

Similar amounts of P were extracted per g of material from the litter and soil layers, but the P composition was different. The extract of the blanket peat litter contained similar proportions of orthophosphate monoesters, orthophosphate diesters and pyrophosphate to the 0–5 cm soil layer, but only traces of phosphonates were present compared to the substantial proportions in the 0–5 cm layer (Figure 1). In addition, a signal at 4.6 ppm in the 0–5 cm sample was not present in the litter

sample. In acid grassland litter, the proportions of inorganic orthophosphate and orthophosphate monoesters were similar to the 0–5 cm soil layer, but there were greater proportions of orthophosphate diesters and pyrophosphate (Figure 2). Again, only small amounts of phosphonates were present. A distinct shoulder in the region assigned to phospholipids was present in both litter samples.

In the 5–10 cm samples of all three soils the proportions of P compounds were similar to those in the 0–5 cm samples, suggesting their stability in these soils. The 5–10 cm acid grassland soil extract gave a poor quality spectra, possibly due to the high concentrations of humic material and paramagnetics (Figure 2). However, a strong phosphonate signal and a shoulder in the labile diester region were evident.

Seasonal variation in soil phosphorus fractions

The variability in total P and extractable P between sampling dates, almost certainly due to inherent spatial variability, caused difficulty in detecting temporal changes in the concentrations of P fractions with any degree of certainty. This was partially overcome by using the ratio of orthophosphate monoesters to orthophosphate diesters to indicate relative changes in organic P forms. Thus, the P composition of the 0–5 cm soil layers appeared to vary little during the three sampling occasions, but a seasonal trend in the orthophosphate monoester-to-diester ratios was apparent for all three soils (Table 4). The trend was greatest in the calcareous grassland soil, with the ratio increasing from 11.5 to 21.6 between January to September. For the two acidic soils, the changes were less clear, but the ratio increased between January to September from 2.4 to 2.9 in the blanket peat and from 2.8 to 3.6 in the acid grassland soil. Furthermore, the ratios increased with depth in all three soils, for example from 2.27 in the litter layer to 4.97 in 5–10 cm layer of the blanket peat.

Discussion

Soil phosphorus composition

In Upper Teesdale soils, a large proportion of the total P extracted by NaOH–EDTA was in organic forms. This is probably due to the long history of atmospheric N deposition onto Widdybank Fell, because Scottish peat soils (Bedrock et al. 1994) and *Sphagnum-Carex* peat and associated litter layers in Finland (Kaila 1956) subject to lower rates of N deposition contained greater proportions of inorganic orthophosphate. For example, alkaline extracts of virgin Scottish blanket peat contained 60% inorganic orthophosphate determined by solution ^{31}P NMR spectroscopy (Bedrock et al. 1994). Furthermore, calcareous soils such as that in the current study rarely contain more than 50% organic P (Harrison 1987).

Plant and microbial communities in Upper Teesdale on all three soil types are strongly P-limited, as indicated by high rates of bryophyte and soil phosphatase activity (Turner et al. 2001, 2002a). This suggests that the soil organic P is rela-

tively recalcitrant and that soil properties exert a stronger control on its dynamics than biological processes. The stable nature of orthophosphate monoesters such as inositol phosphates are well known (Turner et al. 2002b), but orthophosphate diesters are considered more labile due to their rapid hydrolysis in soils and under cultivation (Bowman and Cole 1978; Condron et al. 1990). In the acidic soils studied here, orthophosphate diesters appeared to be relatively stable, which may be due to the low solubility of nucleic acids at low pH (Greaves and Wilson 1969), or their association with humic compounds (Bedrock et al. 1994; Makarov et al. 1997). Despite this, changes in the orthophosphate monoester-to-diester ratios suggested that orthophosphate diesters were preferentially degraded during the summer months, notably in the calcareous grassland soil. Organic P accumulation in soils during the cold winter months followed by degradation during the warm summer months has been attributed to reduced biological activity during the winter (e.g., Dormaar (1972)). Our results suggest that, at least in the calcareous grassland soil, this may involve preferential hydrolysis of orthophosphate diesters. The stable nature of organic P in the soils of Upper Teesdale suggests that compounds must first released from complexation with the soil before they can be hydrolysed by phosphatase enzymes, implying that seasonal degradation of orthophosphate diesters is controlled by variations in their solubility (Magid and Nielsen 1992). This may be linked to wetting and drying cycles during the spring and early summer, which solubilise substantial amounts of organic P by a combination of microbial cell lysis and physical disruption of organic matter (Turner and Haygarth 2001). Orthophosphate diesters could, therefore, provide an important source of P for biological uptake during the growing season in this strongly P-limited environment.

Phosphonates and pyrophosphate were present in substantial quantities and may represent important, but poorly understood, biological sources of P in the soils of Upper Teesdale. The presence of large amounts of phosphonates in the acidic soils, where they originated in the soil rather than the litter layers, appears to confirm their stability in moist, acidic conditions (Tate and Newman 1982). In contrast, the traces of phosphonates detected in the calcareous soil suggest that they are synthesised, but rapidly degraded, in alkaline environments. The origins of soil phosphonates are unclear; protozoa probably constitute the main source, although bacteria, amoeba, fungi and snails can all produce 2-aminothyl phosphonic acid (Hilderbrand 1983).

Pyrophosphate was present in all soil extracts and in the litter layers of the two acidic soils, notably in the acid grassland litter. This compound is synthesised in soils by microorganisms (Pepper et al. 1976), but may also originate from the degradation of polyphosphates. Pyrophosphate is rapidly hydrolysed by phosphatase enzymes, but can persist in soil for many months following sorption (Blanchar and Hossner 1969). Inorganic polyphosphate was detected in only one sample in the current study (acid grassland soil 0–5 cm sampled in May 2000). This is unsurprising because it probably functions as P storage compounds in microorganisms during times of P surplus (Pepper et al. 1976), a situation likely to occur only rarely in Upper Teesdale soils. The absence of polyphosphate in the other samples was unlikely to be due to hydrolysis during extraction or analysis, because long-chain

polyphoshates degrade only slowly in NaOH–EDTA (Turner et al. 2003 (in press)) and substantial amounts have been detected in other studies using the same procedures (Cade-Menun and Preston 1996; Dai et al. 1996).

Differences in orthophosphate determined by molybdate colourimetry and solution ^{31}P NMR spectroscopy

Inorganic orthophosphate determined by solution ^{31}P NMR spectroscopy in NaOH–EDTA extracts was consistently and considerably greater when than when determined by molybdate colourimetry. The same phenomenon has been found to a lesser extent in other studies using NaOH extraction (Newman and Tate 1980; Bedrock et al. 1994; Guggenberger et al. 1996). Explanations could include the underestimation of organic P by solution ^{31}P NMR spectroscopy, or hydrolysis of organic P during analytical procedures (Bedrock et al. 1994). However, reports of substantial concentrations of orthophosphate in dialysed NaOH extracts (Guggenberger et al. 1996) and in humic acids precipitated from alkaline soil extracts (e.g., Bedrock et al. (1994) and Makarov et al. (1997)), suggest that the most likely explanation is the presence of orthophosphate in complexed forms in NaOH–EDTA extracts. These could include the simple occlusion of orthophosphate within high molecular weight humic complexes, or bound to humic compounds through cation bridges (Gerke 1992; Bedrock et al. 1997). The presence of such compounds is not precluded by the use of EDTA in the extraction solution and they could even exist as EDTA–metal–phosphate complexes (Elgavish and Granot 1979). The complexation of orthophosphate in solution would prevent its detection by molybdate colourimetry, but not by solution ^{31}P NMR spectroscopy. The exception would be inorganic orthophosphate bound through paramagnetic Fe, although metal bridges would predominantly involve Al in acid soils (Bedrock et al. 1997). The presence of large concentrations of complexed orthophosphate in these NaOH–EDTA extracts indicates that conventional colourimetric procedures can substantially overestimate the extracted organic P. This further suggests that much of the alkali-unextractable soil P may be inorganic orthophosphate strongly bound to organic matter through metal bridges (Anderson 1980).

Implications for plant acquisition of phosphorus from organic compounds

The large concentrations of relatively stable organic P compounds in Upper Teesdale soils suggest that organisms able to access such compounds could have a competitive advantage in P-limited environments. When coupled with other effects of atmospheric N deposition, this may contribute to observed shifts in species composition (Van der Eerden et al. 1991; Bobbink et al. 1998). Considerable loss of bryophyte diversity and lichen abundance on Widdybank Fell during the last 25 years has been attributed to climatic fluctuations resulting from the construction of a water storage reservoir (Huntley et al. 1998), but it is possible that enhanced P limitation may have been a factor.

Organisms have various strategies for acquiring P from recalcitrant organic compounds, including the inducement of rhizosphere pH changes, secretion of chelating organic acid anions (e.g., citrate, malate) and synthesis of phosphatase enzymes. These processes have been extensively investigated in terms of the solubilisation of inorganic P (Jones 1998; Hinsinger 2001), yet have been neglected in terms of the acquisition of P from organic compounds, despite the dominance of organic P in low P soils where these processes are most likely to occur.

A further mechanism is the association of plant roots with mycorrhizal fungi, which are known to secrete extracellular phytase when grown in P-limited conditions (Antibus et al. 1992). This enzyme is involved in the dephosphorylation of inositol phosphates, which dominate the orthophosphate monoester pool in most soils (Turner et al. 2002b). Thus, organisms capable of using inositol phosphates may have a competitive advantage in P-limited environments. However, studies on the utilisation of inositol phosphates as a P source are contradictory and more focussed research is required on the biological availability of these enigmatic compounds (Turner et al. 2002b). In this respect, it is interesting to note that the ability of plants to access different soil N pools has recently been shown to control the community composition of N-limited tundra systems (McKane et al. 2002). Such resource partitioning may also operate for P compounds in P-limited systems such as Upper Teesdale, and may partly explain the complex ecology of this environment.

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