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The phosphorus composition of temperate pasture soils determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy

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Abstract

Information on the composition and dynamics of soil phosphorus (P) remains limited, but is integral to understanding soil biogeochemical cycles. We used solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy to characterise NaOH–EDTA extractable P in 29 permanent pasture soils from England and Wales (total carbon 29–80 g kg^{-1} soil, clay 219–681 g kg^{-1} soil, pH 4.4–6.8). Total P ranged between 376 and 1981 mg P kg^{-1} soil, of which between 45 and 88% was extracted with NaOH–EDTA. The extracts were dominated by orthophosphate monoesters (29–60% extracted P) and inorganic orthophosphate (21–55% extracted P), with smaller concentrations of orthophosphate diesters (2–10% extracted P), pyrophosphate (1–7% extracted P), phosphonates (0–3% extracted P), and traces of polyphosphates. Orthophosphate diesters were subclassified into phospholipids (1–7% extracted P) and DNA (1–6% extracted P). Signals slightly downfield of inorganic orthophosphate were tentatively assigned to aromatic orthophosphate diesters similar in structure to *R*-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate. Such signals are rarely detected in soil extracts, but were present in relatively large concentrations in the samples analysed here (2–5% extracted P). Relationships between functional P groups and soil properties suggested that the various functional groups are involved in the soil P cycle to different extents. In particular, concentrations of orthophosphate monoesters appeared to be controlled by the potential for chemical stabilisation in soil, whereas DNA and pyrophosphate were strongly correlated with the microbial biomass, suggesting an active involvement in biological nutrient turnover.

1. Introduction

Information on the chemical composition of soil phosphorus (P) remains limited, but is fundamental to understanding plant nutrition, soil biogeochemical cycles, and the transfer of soil P to watercourses (Haygarth and Jarvis, 1999; Frossard et al., 2000). In particular, the chemical nature and dynamics of soil organic P remain enigmatic, despite constituting up to 90% of the total P in some soils (Dai et al., 1996; Cade-Menun et al., 2000; Turner et al., 2003a), and providing a source of P for plant uptake (Gahoonia and Nielsen, 1992; Chen et al., 2002). Indeed, a large proportion of the organic P in most soils remains unidentified (Harrison, 1987; Magid et al., 1996).

Phosphorus originating from plant, animal, and microbial sources occurs in a range of complex compounds, which exhibit widely different behaviour in the soil environment (Anderson, 1967). This leads to the

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rapid degradation and disappearance of some compounds, but the stabilisation and persistence of others (Stewart and Tiessen, 1987). For example, mononucleotides and other monophosphate esters are degraded within hours of release (Bowman and Cole, 1978), whereas inositol phosphates react strongly in most soils and accumulate to form the major class of organic P (Celi et al., 1999; Turner et al., 2002). Clearly, a comprehensive understanding of P dynamics in natural and managed ecosystems requires detailed structural information on soil P.

Limitations on research into the composition of soil P have been primarily analytical. For example, speciation of soil P can involve difficulties with the extraction, separation and detection of these often recalcitrant compounds. Further, conventional chemical fractionation procedures provide no structural information, and the classification of such fractions in terms of plant availability is known to be in error, since plants can readily access soil P pools that are resistant to chemical extraction (Gahoonia and Nielsen, 1992; Chen et al., 2002). However, soil P composition can be conveniently determined using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy. This technique obviates the requirement for the complex separation procedures involved in chromatography, and has significantly advanced our knowledge of soil P composition during the last two decades (Preston, 1996; Condron et al., 1997). Initial studies revealed the presence of phosphonates in soils (Newman and Tate, 1980; Tate and Newman, 1982) and there is now a wealth of information on soil P composition in a wide range of environments (e.g., Ogner, 1983; Condron et al., 1985; Adams and Byrne, 1989; Bedrock et al., 1994; Makarov et al., 1997; Mahieu et al., 2000; Amelung et al., 2001). The procedure is further improved by extraction with a combination of NaOH and EDTA (Bowman and Moir, 1993), which recovers more soil P than NaOH alone (Cade-Menun and Preston, 1996). Indeed, up to 90% total P has been recovered from high organic matter soils using this technique (Dai et al., 1996; Cade-Menun et al., 2000; Turner et al., 2003a).

Recently we reported detailed information on solution ^{31}P NMR chemical shift assignments of P compounds in NaOH–EDTA soil extracts, which refined previous compound identification (Turner et al., 2003b). In particular, signals in the orthophosphate diester region were classified into phospholipids (0 to 2 ppm) and DNA (−1 to 0 ppm). Here we report the application of this technique to the investigation of the P composition of temperate pasture soils. By analysing a large number of soils under uniform land-use (permanent lowland pasture), but with a range of physical and chemical properties, we aimed to derive empirical information on the processes controlling functional classes of soil P.

2. Methods

2.1. Soil sampling and preparation

Twenty-nine lowland permanent pasture soils were sampled during October 1998 from sites around England and Wales, selected from the National Soil Inventory database (National Soil Resources Institute, Cranfield University, UK) to give a range of physical and chemical properties (Table 1). Mean annual temperature across the sites varied between 8.5 and 11 °C, whilst mean annual rainfall varied between 600 and 850 mm. In terms of organic matter development, we consider that climate is relatively comparable across the selected sites.

Intact soil blocks (15×20 cm) were taken to 10 cm from each site, sieved (4 mm) to remove large roots, stones and macrofauna and left to equilibrate for 1 week at 10–15 °C. Soils were then air-dried for 7 days at 30 °C and re-sieved (2 mm) before extraction.

2.2. Soil phosphorus extraction and analysis by molybdate colorimetry

Phosphorus was extracted by shaking 5 g of soil with 100 ml of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at 20 °C (Cade-Menun and Preston, 1996). The extracts were centrifuged at 10,000×g for 30 min, frozen at −30 °C, then lyophilized over several days. Inorganic orthophosphate was determined in diluted extracts (1:100) by molybdate colorimetry and flow injection analysis with P detection at 690 nm (Tecator, 1983). Extracts must be diluted at least 25-fold to prevent interference from EDTA during colorimetric analysis. The short reaction time in the flow injection procedure minimises interference from humic acid precipitation and ensures that acid-induced hydrolysis of organic and condensed P compounds is negligible. Total P was determined by a similar procedure following acid-persulphate digestion of the samples (Rowland and Haygarth, 1997). Organic P was calculated as the difference between total P and inorganic orthophosphate. This fraction also includes inorganic polyphosphates (Shand et al., 2000), but we use the term organic P for clarity. All results are means of three replicate analyses with standard errors (not shown) less than ±5% of the mean value.

2.3. Solution ^{31}P NMR spectroscopy

Freeze dried NaOH–EDTA extracts (approximately 200 mg) were re-dissolved in 1 ml of 1 M NaOH and 0.1 ml D_2O (for signal lock) and transferred to 5-mm NMR tubes. The addition of NaOH ensures consistent chemical shifts and optimum spectral resolution at a solution pH >12. Solution ^{31}P NMR spectra were obtained

Table 1
Physical and chemical properties of the twenty nine permanent pasture soils from England and Wales (soils are ranked in order of their total C content)

	Soil series	Location	USDA class	Textural class[a]	pH (water)	Clay	Total C	Total N	Oxalate extraction Al	Oxalate extraction Fe
						(g kg^{-1} soil)				
1.	Brockhurst	Honiton, Devon	Haplaquepts	Clay	5.0	379	28.9	3.74	1.09	4.67
2.	Dunkeswick	Harrogate, Yorkshire	Haplaquepts	Clay loam	4.7	346	29.6	3.16	1.19	6.19
3.	Wick	Northallerton, Yorkshire	Dystrochrepts	Sandy clay loam	5.5	219	30.6	3.25	0.85	4.48
4.	Denbigh	Kendal, Cumbria	Dystrochrepts	Clay loam	5.5	273	30.8	3.40	2.44	3.44
5.	Nupend	Kirkham, Lancashire	Hapludalfs	Clay loam	5.0	240	32.8	3.57	1.13	4.43
6.	Brickfield	Corbridge, Northumberland	Haplaquepts	Sandy clay	4.8	338	36.9	3.63	1.02	3.66
7.	Dunkeswick	Derby, Derbyshire	Haplaquepts	Clay	6.1	483	38.9	2.85	1.29	4.67
8.	Brickfield	Haydon Bridge, Northumberland	Haplaquepts	Sandy clay	4.8	430	39.8	3.40	1.07	4.29
9.	Whimple	Leamington Spa, Warwickshire	Hapludalfs	Clay loam	5.5	328	40.1	4.05	0.80	3.22
10.	Salop	Whitchurch, Shropshire	Haplaquepts	Sandy clay	5.1	318	42.3	4.45	1.05	5.15
11.	Whimple	Lower Langford, Somerset	Hapludalfs	Clay	5.1	338	43.7	4.73	1.00	4.69
12.	Newport	Wem, Shropshire	Udipsamments	Sandy clay loam	4.9	250	44.0	4.58	0.82	3.14
13.	Denbigh	Atherstone, Warwickshire	Dystrochrepts	Clay	6.2	401	44.9	4.43	2.06	6.15
14.	Clifton	Chorley, Lancashire	Haplaquepts	Clay	4.4	457	45.4	4.08	1.46	5.53
15.	Brickfield	Llangefni, Anglesey	Haplaquepts	Clay loam	4.8	335	46.0	4.83	1.53	5.33
16.	Wick	Haltwhistle, Northumberland	Dystrochrepts	Sandy clay loam	5.9	261	47.2	4.61	1.95	6.30
17.	Brickfield	Dwyran, Anglesey	Haplaquepts	Clay	5.0	362	47.2	5.04	1.84	6.60
18.	Oxpasture	Cheddar, Somerset	Hapludalfs	Silty clay	4.8	424	47.5	4.93	2.48	8.94
19.	Whimple	Stafford, Staffordshire	Hapludalfs	Clay loam	5.1	299	48.0	4.83	0.80	2.92
20.	Hallsworth	Holsworthy, Devon	Haplaquepts	Clay	4.7	484	48.4	5.37	1.60	9.96
21.	Brickfield	Hexham, Northumberland	Haplaquepts	Sandy clay	5.0	359	49.6	4.66	3.24	9.01
22.	Moor Gate	Camelford, Cornwall	Dystrochrepts	Clay loam	5.6	313	56.0	5.92	4.95	6.34
23.	Denbigh	Camelford, Cornwall	Dystrochrepts	Clay	4.5	445	58.7	6.35	1.44	7.65
24.	Nordrach	Buxton, Derbyshire	Paleudalfs	Clay	6.8	541	60.2	5.49	3.30	9.60
25.	Newchurch	Burnham-on-Sea, Somerset	Fluvaquents	Clay	5.9	547	64.4	6.52	1.35	5.93
26.	Denchworth	Leppington, Yorkshire	Haplaquepts	Clay	5.8	579	66.3	6.99	1.09	7.09
27.	Worcester	Chew Stoke, Somerset	Hapludalfs	Clay	6.0	567	67.7	7.57	0.91	2.69
28.	Whimple	Radbourne, Derbyshire	Hapludalfs	Clay	5.2	629	68.8	7.33	1.51	4.35
29.	Fladbury	Glastonbury, Somerset	Fluvaquents	Clay	5.0	681	80.4	8.70	2.44	12.88

[a] Based on topsoil texture.

using a Bruker AMX 600 spectrometer operating at 243 MHz. We used a 30° pulse width, a total acquisition time of 1.5 s (pulse delay 0.808 s, acquisition time 0.672 s) and broadband proton decoupling. The delay time used here allows sufficient spin-lattice relaxation between scans for P compounds in NaOH–EDTA (Cade-Menun et al., 2002). Temperature was regulated at 24 °C, although it is now recommended that solution ^{31}P NMR spectra of soil extracts be acquired at a standardised temperature of 20 °C (Cade-Menun et al., 2002; Turner et al., 2003b). The number of scans collected (4000–40,000) depended on the P concentration of the freeze-dried extract.

Chemical shifts were determined relative to 85% H_3PO_4 after convolution with a Lorentzian width of 10 Hz. We used the deconvolution process of the Bruker WinNMR program to determine chemical shift and area of individual peaks. Compounds were identified by adding model compounds as spikes to soil extracts in the NMR tube (Turner et al., 2003b). The precise chemical shifts may vary slightly among soils, almost certainly due to differences in ionic strength, pH, or paramagnetic ion concentrations, although the relative positions will remain consistent.

2.4. Determination of soil properties

Total carbon (C) and nitrogen (N) were determined simultaneously using a Carlo-Erba model NA2000 analyser (Carlo-Erba, Milan, Italy). Total soil P was determined by NaOH fusion (Smith and Bain, 1982). Textural information was obtained by wet sieving followed by analysis using a Micromeritics Sedigraph 5100 with a Micromeritics Mastertech 51 automatic sampler (Norcross, GA, USA). Soil pH was determined in a 1:2.5 soil/deionised water ratio. Microbial C, N and P

concentrations were determined by fumigation-extraction procedures and were reported previously (Turner et al., 2001). Oxalate-extractable aluminium (Al) and iron (Fe) were determined by extraction with ammonium oxalate/oxalic acid (pH 3.0) for 2 h, with analysis by inductively-coupled plasma atomic-emission spectrometry (ICP–AES) (Schoumans, 2000).

2.5. Statistical analysis

Concentrations are expressed on the basis of oven-dry soil (105 °C). A correlation matrix (r values) was calculated to investigate relationships between soil properties and P compounds, which were then investigated visually by plotting on x–y scatter graphs using standard procedures in Microsoft Excel 2002. Regression models were calculated using Sigma Plot 2000.

3. Results

3.1. Soil properties

Total C concentrations ranged between 28.9 and 80.4 g C kg^{-1} soil, total N concentrations between 2.85 and 8.70 g N kg^{-1} soil, clay contents between 219 and 681 g kg^{-1}, and pH values between 4.4 and 6.8 (Table 1). Concentrations of microbial C (412–3412 mg C kg^{-1} soil), N (57–346 mg N kg^{-1} soil) and P (31–239 mg P kg^{-1} soil) were relatively large in these soils (Turner et al., 2001). Oxalate-extractable Al concentrations ranged between 0.80 and 3.30 g Al kg^{-1} soil, and oxalate-extractable Fe ranged between 2.69 and 12.88 g Fe kg^{-1} soil.

3.2. Whole soil and NaOH–EDTA extractable phosphorus

Total soil P ranged between 376 and 1981 mg P kg^{-1} soil (Table 2). Recovery in NaOH–EDTA ranged between 45 and 88%, although relatively consistent proportions of the total P were extracted from most soils (mean recovery 73%; Fig. 1). Inorganic orthophosphate concentrations in the NaOH–EDTA extracts determined by molybdate colorimetry ranged between 51 and 546 mg P kg^{-1} soil, equivalent to between 12 and 54% extracted P (Table 2).

3.3. Soil phosphorus composition determined by solution ^{31}P NMR spectroscopy

3.3.1. Inorganic phosphorus

Inorganic P compounds identified in the NaOH–EDTA extracts included orthophosphate, pyrophosphate and polyphosphate (Table 3, Fig. 2). Inorganic orthophosphate (6.24 ppm) ranged between 74 and 561 mg P kg^{-1} soil, equivalent to between 21 and 55% extracted P (Table 4). These values were consistently greater than those determined by molybdate colorimetry (Fig. 3). The regression model for the relationship between inorganic orthophosphate determined by solution ^{31}P NMR spectroscopy and that determined by molybdate colorimetry suggested an average difference of 52 mg P kg^{-1}soil.

Pyrophosphate (−4.05 ppm) was present in extracts of all soils. Concentrations ranged between 8 and 95 mg P kg^{-1} soil, equivalent to between 1 and 7% extracted P (Table 4). Inorganic polyphosphates were detected in only 6 soils at concentrations up to 10 mg P kg^{-1} soil (2% extracted P).

Table 2
Total soil P determined by NaOH fusion and NaOH–EDTA extractable P fractions determined by molybdate colorimetry in twenty-nine permanent pasture soils from England and Wales

Soil series			NaOH–EDTA extractable P		
		Total soil P	Total P[a]	Inorganic P[b]	Organic P[b]
		(mg P kg^{-1} soil)			
1.	Brockhurst	1020	731 (72)	246 (34)	485 (66)
2.	Dunkeswick	519	411 (79)	96 (23)	314 (77)
3.	Wick	834	617 (74)	298 (48)	320 (52)
4.	Denbigh	1021	805 (79)	258 (32)	547 (68)
5.	Nupend	923	674 (73)	278 (41)	396 (59)
6.	Brickfield	585	471 (81)	71 (15)	400 (85)
7.	Dunkeswick	821	522 (64)	265 (51)	257 (49)
8.	Brickfield	376	330 (88)	51 (15)	279 (85)
9.	Whimple	568	348 (61)	72 (21)	276 (79)
10.	Salop	821	633 (77)	106 (17)	528 (83)
11.	Whimple	988	713 (72)	241 (34)	473 (66)
12.	Newport	962	743 (77)	210 (28)	533 (72)
13.	Denbigh	1321	887 (67)	370 (42)	516 (58)
14.	Clifton	571	432 (76)	52 (12)	380 (88)
15.	Brickfield	1106	908 (82)	301 (33)	607 (67)
16.	Wick	1312	1005 (77)	546 (54)	459 (46)
17.	Brickfield	854	672 (79)	113 (17)	558 (83)
18.	Oxpasture	887	639 (72)	131 (20)	509 (80)
19.	Whimple	833	640 (77)	167 (26)	473 (74)
20.	Hallsworth	997	729 (73)	213 (29)	516 (71)
21.	Brickfield	626	472 (75)	113 (24)	359 (76)
22.	Moor Gate	1004	676 (67)	174 (26)	502 (74)
23.	Denbigh	1542	1248 (81)	223 (18)	1025 (82)
24.	Nordrach	1074	781 (73)	221 (28)	560 (72)
25.	Newchurch	784	350 (45)	74 (21)	276 (79)
26.	Denchworth	900	623 (69)	128 (21)	494 (79)
27.	Worcester	989	611 (62)	98 (16)	513 (84)
28.	Whimple	1007	698 (69)	91 (13)	607 (87)
29.	Fladbury	1981	1538 (78)	455 (30)	1083 (70)

[a] Values in parentheses are the proportion (%) of the total soil P.

[b] Values in parentheses are the proportion (%) of the NaOH–EDTA extractable P.

3.3.2. Organic phosphorus

Organic P compounds identified in the NaOH–EDTA extracts included orthophosphate monoesters, orthophosphate diesters and phosphonates (Fig. 2, Table 3). Orthophosphate monoesters were the major P fraction in all soils. Concentrations ranged between 154 and 751 mg P kg^{-1} soil (Table 4), equivalent to between 29 and 60% total extracted P, and between 71 and 91% extracted organic P (sum of orthophosphate monoesters, orthophosphate diesters and phosphonates). Several clear signals were identified within the orthophosphate monoester region. Distinct signals at 5.90, 4.95, 4.57 and 4.45 ppm in all extracts indicated large concentrations of *myo*-inositol hexakisphosphate (Table 3). A signal at 4.10 ppm in all extracts was assigned to choline phosphate, whilst a signal at 3.27 ppm represented glucose-1-phosphate. Clear signals at 4.85 and 5.12 ppm in most extracts almost certainly represented β-glycerophosphate and phosphatidic acid, respectively. These compounds appear during the chemical degradation of phosphatidyl choline in NaOH–EDTA, so may be artifacts of the analytical procedure (Turner et al., 2003b).

Concentrations of total orthophosphate diesters ranged between 11 and 109 mg P kg^{-1} soil (Table 4), equivalent to between 2 and 10% total extracted P, and between 3 and 15% extracted organic P. Two groups of orthophosphate diesters were identified (Turner et al., 2003b). A signal in all extracts at −0.26 ppm was assigned to DNA. Concentrations ranged between 5 and 71 mg P kg^{-1} soil, equivalent to between 1 and 6% total extracted P, and between 2 and 8% extracted organic P (Table 4). Signals between 0 and 1.8 ppm were assigned to phospholipids, including phosphatidyl ethanolamine (1.75 ppm), phosphatidyl serine (1.57 ppm), and phos-

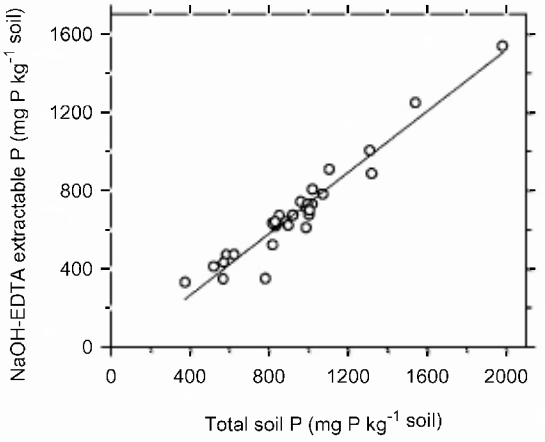

Fig. 1. Relationship between total soil P and NaOH–EDTA extractable P in permanent pasture soils from England and Wales. The regression line describes the model: [NaOH–EDTA extractable total P] = 0.783 ± 0.040 [Total soil P] − 48.8 ± 40.8; $P < 0.0001$, $F = 373$, $R^2 = 0.93$.

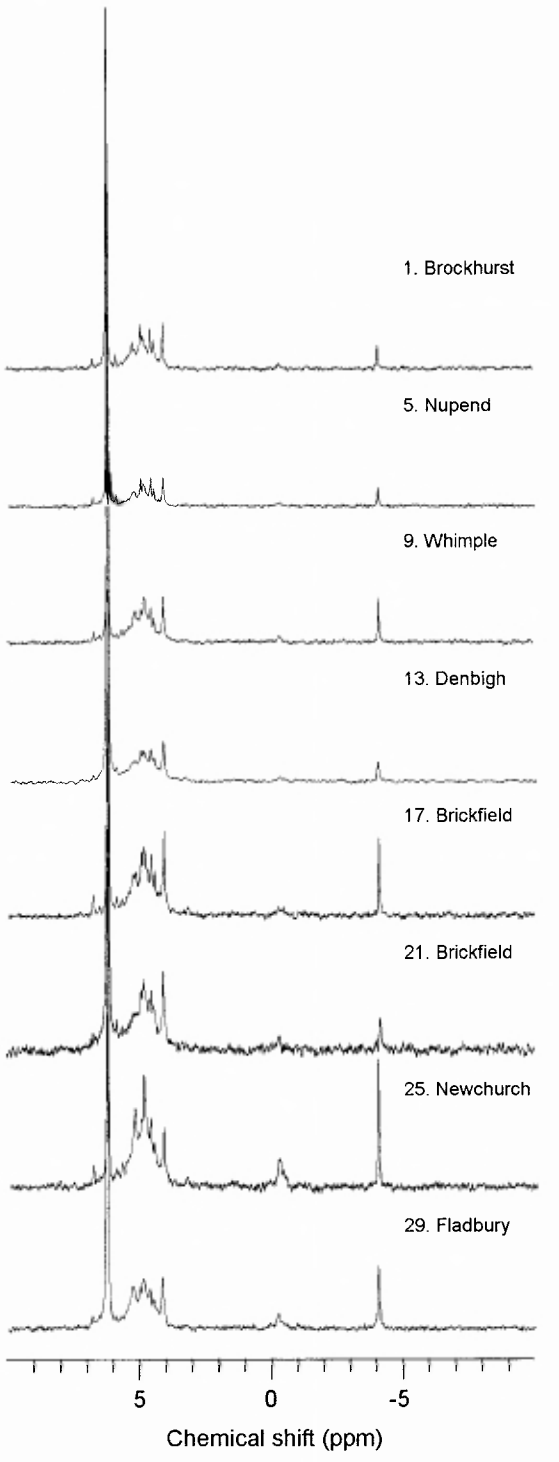

Fig. 2. Selected solution ^{31}P NMR spectra of NaOH–EDTA extracts of permanent pasture soils from England and Wales. The soils are numbered in order of increasing total C concentrations (Table 1).

Table 3
Solution ^{31}P NMR chemical shift assignments detected in NaOH–EDTA extracts of permanent pasture soils from England and Wales[a]

Chemical shift		Assignment
Mean	Range	
20.72	20.56 to 21.78	2-Aminoethyl phosphonic acid
19.15	18.99 to 19.27	Phosphonolipid[b]
7.43	7.19 to 7.58	Unknown, but possibly aromatic diesters similar in structure to *R*-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate
6.78	6.72 to 6.88	
6.55	6.48 to 6.63	
6.24	6.18 to 6.34	Inorganic orthophosphate
5.90	5.84 to 6.00	Inositol hexakisphosphate[c]
5.68	5.61 to 5.77	Unidentified
5.39	5.30 to 5.47	Glucose-6-phosphate
5.24	5.18 to 5.29	Unidentified
5.12	5.04 to 5.16	Phosphatidic acid
4.95	4.90 to 5.00	Inositol hexakisphosphate[c]
4.85	4.80 to 4.89	β-Glycerophosphate
4.72	4.67 to 4.78	Mononucleotides/ethanolamine phosphate
4.57	4.53 to 4.65	Inositol hexakisphosphate[c]
4.45	4.38 to 4.49	Inositol hexakisphosphate[c]
4.26	4.20 to 4.35	Mononucleotide
4.10	4.06 to 4.14	Choline phosphate
3.64	3.52 to 3.82	Unidentified
3.27	3.13 to 3.43	Glucose-1-phosphate
1.75		Phosphatidyl ethanolamine
1.57		Phosphatidyl serine
0.78		Phosphatidyl choline
−0.26	−0.15 to −0.36	DNA
−4.05	−3.92 to −4.15	Pyrophosphate
−3.63, −9.61		Adenosine diphosphate
−20.18		Inorganic polyphosphate

[a] Assignments are based on the addition of model P compounds to soil NaOH–EDTA extracts (Turner et al., 2003b).
[b] Identified from literature assignments (Newman and Tate, 1980).
[c] *myo*-Inositol hexakisphosphate gives four signals in the ratio 1:2:2:1, corresponding to the positions of the P groups on the inositol ring (Turner et al., 2003b).

phatidyl choline (0.78 ppm). Concentrations of phospholipids ranged between 5 and 48 mg P kg^{-1} soil, equivalent to between 1 and 7% total extracted P, and between 2 and 11% extracted organic P (Table 4). However, these concentrations are likely to be underestimates, because phosphatidyl choline degrades rapidly in alkaline solution (Makarov et al., 2002; Turner et al., 2003b). Signals from RNA appear in this region (0.54 ppm), but degrade rapidly to orthophosphate monoesters (signals between 4.28 and 4.75 ppm) within a few hours, so are unlikely to contribute significantly to the measured concentrations of phospholipids.

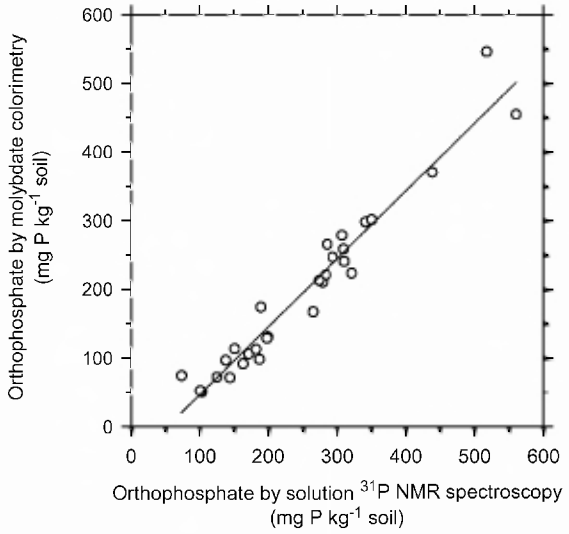

Fig. 3. Relationship between concentrations of inorganic orthophosphate (mg P kg^{-1} soil) determined by solution ^{31}P NMR spectroscopy and molybdate colorimetry in NaOH–EDTA extracts of permanent pasture soils from England and Wales. The regression line describes the model: [Inorganic orthophosphate by molybdate colorimetry] = 0.989±0047 [Inorganic orthophosphate by NMR] − 52.03±12.94; $P<0.0001$, $F=445$, $R^2=0.94$.

Signals between 6.5 and 7.5 ppm slightly upfield of inorganic orthophosphate were tentatively assigned to aromatic orthophosphate diesters, because the aromatic diester *R*-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate appears at 7.4 ppm in alkaline soil extracts (Turner et al., 2003b). A signal at 6.78 ppm was present in extracts of all soils, whilst signals at 6.55 and 7.43 ppm were present only in extracts of sixteen and four soils, respectively. Concentrations of these compounds ranged between 8 and 51 mg P kg^{-1} soil, equivalent to between 2 and 5% total extracted P, and between 3 and 11% extracted organic P (Table 4).

Phosphonates were not detected in three soils, whilst several others contained only trace amounts. Concentrations up to 16 mg P kg^{-1} soil were detected, equivalent to 3% total extracted P and 5% extracted organic P (Table 4). The main phosphonate signals at 20.72 and 19.15 ppm were assigned to 2-aminoethylphosphonic acid and an unidentified phosphonolipid, respectively (Newman and Tate, 1980). Most soil extracts contained only a single signal at 20.72 ppm, but extracts of five soils, mainly with low inorganic P concentrations, contained both phosphonate signals.

Weak signals at 10, 12, and 15 ppm in some soils indicated the presence of trace amounts of phosphonic acid esters similar in structure to β-naphthyl-phosphonate (Turner et al., 2003b). Additional weak signals in two soils at −3.63 and −9.61 ppm indicated the

Table 4
Concentrations of functional P classes in NaOH–EDTA extracts of permanent pasture soils from England and Wales determined by solution ^{31}P NMR spectroscopy[a]

Soil no.	Inorganic P			Organic P						Monoester-to-diester ratio
	Orthophosphate	Pyrophosphate	Polyphosphates	Orthophosphate monoesters	Orthophosphate diesters			Phosphonates	Unknown[b]	
					Total diesters	DNA	Phospholipids			
	(mg P kg^{-1} soil)									
1.	294 (40)	19 (3)	n.d.	374 (51)	14 (2)	7 (1)	7 (1)	5 (<1)	25 (3)	25.9
2.	138 (33)	14 (3)	2 (<1)	205 (50)	23 (6)	9 (2)	14 (3)	12 (3)	18 (4)	9.0
3.	342 (55)	9 (1)	n.d.	209 (34)	23 (4)	8 (1)	15 (2)	10 (2)	26 (4)	9.1
4.	310 (39)	14 (2)	2 (<1)	390 (48)	31 (4)	12 (1)	20 (2)	16 (2)	41 (5)	12.4
5.	308 (46)	16 (2)	n.d.	272 (40)	51 (8)	13 (2)	37 (6)	3 (<1)	24 (4)	5.4
6.	144 (30)	13 (3)	n.d.	284 (60)	13 (3)	6 (1)	8 (2)	3 (<1)	14 (3)	21.2
7.	286 (55)	9 (2)	10 (2)	154 (29)	33 (6)	9 (2)	24 (5)	7 (1)	24 (5)	4.7
8.	103 (31)	8 (2)	n.d.	190 (58)	13 (4)	6 (2)	7 (2)	6 (2)	10 (3)	14.4
9.	125 (36)	14 (4)	n.d.	176 (50)	11 (3)	5 (1)	6 (2)	3 (<1)	19 (5)	15.8
10.	171 (27)	27 (4)	n.d.	381 (60)	28 (4)	13 (2)	15 (2)	3 (<1)	24 (4)	13.8
11.	311 (44)	19 (3)	n.d.	326 (46)	27 (4)	11 (2)	16 (2)	n.d.	29 (4)	11.9
12.	279 (38)	25 (3)	n.d.	366 (49)	38 (5)	17 (2)	22 (3)	7 (1)	28 (4)	9.5
13.	439 (50)	23 (3)	n.d.	363 (41)	41 (5)	13 (2)	27 (3)	6 (<1)	14 (2)	8.9
14.	101 (23)	16 (4)	n.d.	259 (60)	29 (7)	13 (3)	15 (4)	14 (3)	14 (3)	9.0
15.	351 (39)	12 (1)	n.d.	435 (48)	60 (7)	24 (3)	36 (4)	14 (2)	36 (4)	7.3
16.	518 (52)	17 (2)	3 (<1)	389 (39)	38 (4)	9 (1)	29 (3)	11 (1)	29 (3)	10.2
17.	151 (23)	36 (5)	n.d.	397 (59)	65 (10)	21 (3)	44 (7)	2 (<1)	21 (3)	6.1
18.	199 (31)	23 (4)	n.d.	354 (55)	42 (7)	20 (3)	23 (4)	7 (1)	14 (2)	8.4
19.	265 (41)	21 (3)	n.d.	306 (48)	25 (4)	10 (2)	15 (2)	5 (<1)	17 (3)	12.2
20.	274 (38)	36 (5)	3 (<1)	349 (48)	44 (6)	27 (4)	17 (2)	n.d.	22 (3)	7.9
21.	182 (39)	15 (3)	n.d.	249 (53)	15 (3)	9 (2)	5 (1)	3 (<1)	8 (2)	16.9
22.	189 (28)	21 (3)	10 (1)	368 (54)	49 (7)	20 (3)	29 (4)	13 (2)	27 (4)	7.5
23.	322 (26)	32 (3)	n.d.	751 (60)	82 (7)	33 (3)	48 (4)	12 (<1)	51 (4)	9.2
24.	284 (36)	20 (3)	n.d.	412 (53)	44 (6)	22 (3)	21 (3)	3 (<1)	19 (2)	9.4
25.	74 (21)	24 (7)	n.d.	204 (58)	33 (9)	20 (6)	13 (4)	n.d.	15 (4)	6.1
26.	198 (32)	46 (7)	n.d.	301 (48)	59 (9)	28 (5)	30 (5)	4 (<1)	16 (3)	5.1
27.	187 (31)	27 (4)	n.d.	334 (55)	35 (6)	14 (2)	20 (3)	5 (<1)	23 (4)	9.6
28.	163 (23)	47 (7)	n.d.	412 (59)	47 (7)	27 (4)	20 (3)	13 (2)	17 (2)	8.8
29.	561 (36)	95 (6)	n.d.	733 (48)	109 (7)	71 (5)	38 (3)	9 (<1)	31 (2)	6.7

[a] Values in parentheses are the proportion (%) of the total extracted P calculated from total signal area. Rounding may give total peak areas of 100±2%. n.d., not detected.

[b] Tentatively assigned to aromatic diesters similar in structure to *R*-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (Turner et al., 2003b).

presence of ADP in trace amounts. Organic polyphosphates are rarely detected in soils, but these two soils contained large microbial biomass concentrations, suggesting that ADP may have been present in live microbial cells at the time of extraction. Signals from ADP and phosphonic acid esters were too weak to quantify.

3.4. Relationships between soil properties and phosphorus functional classes

Total soil C and N were strongly positively correlated with clay, oxalate Fe and microbial C, N and P (Table 5). Total soil P was positively correlated with total C, oxalate Fe, and the microbial biomass. Total organic P determined by solution ^{31}P NMR spectroscopy was strongly positively correlated with total C, oxalate Fe, and the microbial biomass (Table 6). Similarly, and as expected from their dominance in NaOH-extractable organic P, orthophosphate monoesters were strongly positively with the same soil properties (Table 6, Fig. 4).

Concentrations of DNA and pyrophosphate were strongly positively correlated with total C, total soil organic P, oxalate Fe, clay content, and the microbial biomass (Table 6, Fig. 4). As a proportion of the extractable (organic) P they were strongly correlated

Table 5
Correlation coefficients for the relationships between soil properties of the twenty-nine permanent pasture soils from England and Wales

	Total C	Total soil P	Clay	Soil pH	Oxalate Al	Oxalate Fe
Total soil P	0.51**					
Clay	0.79***	n.s.				
Soil pH	n.s.	n.s.	n.s.			
Oxalate Al	n.s.	n.s.	n.s.	n.s.		
Oxalate Fe	0.48**	0.50**	0.48**	n.s.	0.54**	
Microbial C	0.74***	0.40*	0.63***	n.s.	n.s.	n.s.
Microbial N	0.73***	0.55**	0.54**	n.s.	n.s.	n.s.
Microbial P	0.65***	0.42*	0.54**	n.s.	n.s.	n.s.

Table 6
Correlation coefficients for relationships between soil properties and functional P groups determined by solution ^{31}P NMR spectroscopy in NaOH–EDTA extracts of permanent pasture soils from England and Wales[a]

	Inorganic phosphates		Organic P				
	Orthophosphate	Pyrophosphate	Total organic P[b]	Orthophosphate monoesters	DNA	Phospholipids	Unknown[c]
Total C	n.s (−0.43*)	0.74*** (0.68***)	0.55**	0.51**	0.75*** (0.73***)	n.s.	n.s. (−0.51**)
Total P	0.82***	0.67***	0.88***	0.86***	0.75***	0.68***	0.62***
Clay	n.s. (−0.41*)	0.65*** (0.67***)	n.s.	n.s.	0.63*** (0.72***)	n.s.	n.s. (−0.43*)
pH	n.s.	n.s.	n.s.	n.s. (−0.38*)	n.s.	n.s.	n.s.
Oxalate-Al	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Oxalate-Fe	n.s.	0.58***	0.51**	0.49*	0.70*** (0.59***)	n.s.	n.s. (−0.46*)
Microbial C	n.s. (−0.50**)	0.86*** (0.82***)	0.51**	0.47**	0.77*** (0.72***)	n.s.	n.s. (−0.49**).
Microbial N	n.s. (−0.46*)	0.80*** (0.69***)	0.65***	0.62***	0.75*** (0.60***)	0.46*	n.s.
Microbial P	n.s.	0.83*** (0.71***)	0.53**	0.49**	0.71*** (0.57**)	n.s.	n.s. (−0.43*)

Significance at the *5, **1 and ***0.1% levels; n.s., not significant.

[a] Values in parentheses are correlation coefficients for the proportion of the total (organic) extracted P. Data for polyphosphates and phosphonates are not presented, because their concentrations constituted <1% total P in most soils.

[b] Determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy.

[c] Tentatively assigned to aromatic diesters similar in structure to *R*-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (Turner et al., 2003b).

with clay, total C, oxalate Fe and the microbial biomass (Table 6). Phospholipids and aromatic diesters were positively correlated with total soil P and total soil organic P (Table 6). The concentrations of phosphonates and polyphosphates were not correlated with any soil property, but phosphonates were proportionally greatest where the microbial biomass was smallest.

4. Discussion

Pasture soils typically contain large concentrations of organic P relative to cultivated soils (Harrison, 1987), although the high concentrations in the soils studied here probably also reflect the warm, moist climate, coupled with the relatively large clay contents (Anderson, 1980). The soil organic P composition is similar to that of other temperate grassland soils from around the world, despite a greater recovery of total soil P than previous studies that have used NaOH alone (e.g., Newman and Tate, 1980; Tate and Newman, 1982; Hawkes et al., 1984; Condron et al., 1985; Guggenberger et al., 1996). This is surprising, because recalcitrant soil P would be expected to consist of orthophosphate monoesters in the form of inositol phosphates (Turner et al., 2002). Where high P recovery was achieved by sequential extraction, the proportions of orthophosphate monoesters were similar to those reported here (Condron et al., 1990). The concentrations of orthophosphate monoesters are, however, likely to be overestimated in studies involving alkaline extraction, due to the degradation of some orthophosphate diesters, specifically RNA and phosphatidyl choline, during extraction and analysis (Makarov et al., 2002; Turner et al., 2003b). This appears unavoidable considering the requirement for alkaline extraction and

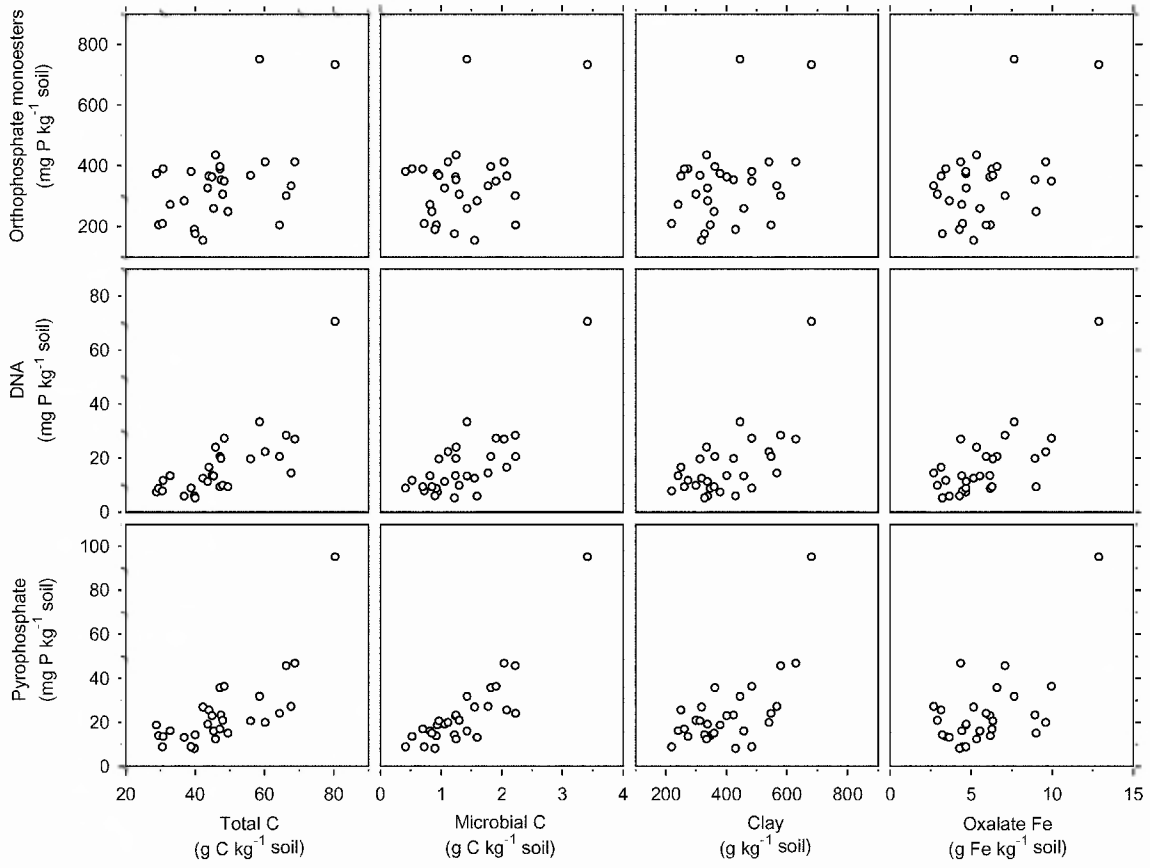

Fig. 4. Relationships between soil properties (total C, microbial C, clay, oxalate Fe) and the concentrations of orthophosphate monoesters, DNA and pyrophosphate in NaOH–EDTA extracts of permanent pasture soils from England and Wales.

lengthy machine times. Indeed, degradation of orthophosphate diesters may have been substantial in the current study, because most soils contained relatively high microbial biomass concentrations. Solid-state ^{31}P NMR spectroscopy offers a promising alternative for the analysis of soil P composition, because compounds can be investigated without the need for extraction and potential degradation. However, the technique currently lacks the sensitivity required for application to most soils (Condron et al., 1997).

Orthophosphate monoesters and diesters exhibited different relationships with soil properties, suggesting that the processes controlling their abundance in soil also differ. Orthophosphate diesters were strongly correlated with the microbial biomass, which is unsurprising as they are the major P class in most microorganisms (Webley and Jones, 1971; Magid et al., 1996). Indeed, much of the measured orthophosphate diesters may have been extracted directly from live microbial cells: microbial P concentrations in these soils ranged between 31 and 239 mg P kg^{-1} soil (Turner et al., 2001) compared with concentrations of phospholipids and DNA between 11 and 109 mg P kg^{-1} soil. However, orthophosphate diesters can become stabilised in soils, primarily through adsorption onto clays (Greaves and Wilson, 1969), or by association with humic compounds (Makarov et al., 1997; Mahieu et al., 2000). This results in a preferential accumulation of orthophosphate diesters in the clay fraction (Amelung et al., 2001), although the greatest concentrations of microbial biomass are also associated with clay particles (Van Gestel et al., 1996). It therefore seems likely that a combination of direct release from microbial cells and stabilisation by association with clays and organic matter contribute to the observed correlations between DNA and the microbial biomass. However, it is clear that orthophosphate diesters are intimately linked with biological nutrient turnover in the soil, which supports suggestions that they represent a labile pool of soil organic P (Dick and Tabatabai, 1978; Harrison, 1982; Condron et al., 1990; Frossard et al., 2000).

Similarly, concentrations of pyrophosphate were closely linked to those of orthophosphate diesters and showed similar relationships to the microbial biomass. Pyrophosphate and long-chain polyphosphates originate in the soil from microbial activity (Ghonsikar and

Miller, 1973; Pepper et al., 1976). However, unlike long-chain polyphosphates, which are rapidly degraded, pyrophosphate can be stabilised by adsorption in the soil and remain for many months (Blanchar and Hossner, 1969). Thus, pyrophosphate concentrations probably reflect both microbial turnover and the potential for stabilisation in the soil.

In comparison to orthophosphate diesters and pyrophosphate, concentrations of orthophosphate monoesters were less strongly correlated with total C, clay, and the microbial biomass. Indeed, the statistical significance of these correlations is probably misleading, due to the strong influence of two outlying values (Fig. 4). This suggests that processes controlling the stabilisation of the orthophosphate monoesters are different to those controlling other functional P groups, almost certainly because a large fraction of the orthophosphate monoesters in the soils studied here were higher inositol phosphates. These compounds constitute only a small fraction of organic P inputs to the soil from plants and microbes, but their high charge density means that they are react strongly with clays, metals, and organic matter (Celi et al., 1999; Turner et al., 2002). This protects them from biological degradation and prevents their involvement in short-term organic matter turnover. Therefore, the potential for stabilisation was primarily responsible for the accumulation of orthophosphate monoesters in these soils.

The apparent differences in the processes controlling concentrations of orthophosphate monoesters compared to DNA and pyrophosphate suggests that only a small proportion of soil organic P is actively involved in the short-term soil P cycle (Stewart and Tiessen, 1987). This is confirmed by results from recent studies that have directly measured basal organic P mineralisation rates in soil (0.2–1.7 mg P kg^{-1} soil day^{-1}) (Lopez-Hernandez et al., 1998; Oehl et al., 2001). In turn, this offers a partial explanation for the apparent disparities between the cycling of soil C, N and organic P (McGill and Cole, 1981), because the accumulation of the major part of the soil organic P is controlled by processes separate to those controlling biological P turnover. It is important to note, however, that several of the correlations discussed above were strongly influenced by one or two outlying values. For example, orthophosphate monoesters were significantly correlated with total C and clay concentrations, yet the uncertainty in these relationships is clearly shown in Fig. 4. This highlights the need for caution in interpreting such correlations where analytical error is uncertain, and suggests that more manipulative experiments are required to confirm our hypotheses.

Signals between 6.5 to 7.5 ppm in the spectra of most soils were tentatively assigned to aromatic orthophosphate diesters, because *R*-(−)-1,1′-binaphthyl-2-2′-diyl hydrogen phosphate gives a signal at 7.4 ppm in NaOH–EDTA (Turner et al., 2003b). Three signals in this region were detected in the various soil extracts, but little information exists on the possible structure or origin of these compounds. Similar signals have been reported at 6.9 ppm in NaOH–NaF extracts of Russian grassland soils (Amelung et al., 2001) and at 7.2 ppm in a cropped Scottish mineral soil (Bedrock et al., 1994), but this is the first report of such consistent concentrations across a range of soils. However, our assignment remains uncertain, because binaphthyl phosphate, in which the P group occurs in a seven-member ring with considerable strain, is rather dissimilar to other aromatic orthophosphate diesters. Indeed, the chemical shifts of aromatic diesters with more open structures appear in the negative region of the spectrum (e.g. bis-*para*-nitrophenyl phosphate at −10.6 ppm, bis-methylumbelliferyl phosphate at −7.2 ppm; Turner et al., 2003b). An alternative assignment for the most upfield signals is the C-2 phosphate of inositol hexakisphosphate stereoisomers other than *myo* (i.e., D-*chiro*, *scyllo*, *neo*), although there is no literature information on the solution ^{31}P NMR chemical shifts of these compounds to support this hypothesis. Unlike DNA and pyrophosphate, the signals assigned to aromatic diesters were not significantly correlated with the microbial biomass, suggesting that they are relatively uninvolved in biological organic P turnover in the soil. More information is clearly needed on these organic P compounds, including confirmation of our assignment to aromatic orthophosphate diesters.

Other organic compounds present in these soils appeared in only trace quantities, although this does not preclude them from involvement in soil P turnover. The presence of phosphonates has been ascribed to wet, cold, or acidic conditions, which retards the microorganisms capable of synthesising phosphonatase enzymes (Tate and Newman, 1982). However, we found no evidence for greater concentrations of phosphonates in more acid soils. Little is known about the origins or transformations of phosphonates in soils, although bacteria, amoeba, fungi and snails can all produce 2-aminoethyl phosphonic acid (Hilderbrand, 1983).

The difference between inorganic orthophosphate determined by solution ^{31}P NMR spectroscopy and by molybdate colorimetry has been previously reported for high organic matter upland soils (Bedrock et al., 1994; Turner et al., 2003a). Problems associated with distinguishing between inorganic and organic forms of P in soil extracts were also highlighted by Magid et al. (1996). The most likely explanation is the presence of inorganic orthophosphate in complexed forms in NaOH–EDTA extracts, which could include inorganic orthophosphate occluded within high molecular weight humic complexes, or bound to humic complexes through cation bridges (Gerke, 1992; Bedrock et al., 1994). The presence of such complexes is not precluded

by the use of EDTA in the extractant and they could even exist as EDTA–metal–phosphate complexes (Elgavish and Granot, 1979). The degradation of organic P compounds during the analytical procedure seems an unlikely explanation, because alkali-labile orthophosphate diesters degrade to orthophosphate monoesters rather than inorganic orthophosphate (Anderson, 1967; Turner et al., 2003b), whilst the acid conditions of the molybdate reaction would be expected to overestimate inorganic orthophosphate by hydrolysing acid-labile organic P and polyphosphates. Inorganic polyphosphates degrade to short chain lengths in NaOH (Hupfer and Gächter, 1995), but are relatively stable in NaOH–EDTA, suggesting that their absence in the soils studied here is not due to their degradation during analysis (Turner et al., 2003b). The implication for the measurement of soil organic P is that conventional extraction procedures involving P detection by molybdate colorimetry may overestimate organic P, because some inorganic orthophosphate occurs in complexes that preclude reaction with molybdate.

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