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Phosphatase activity in temperate pasture soils: Potential regulation of labile organic phosphorus turnover by phosphodiesterase activity

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Abstract

Phosphatase enzymes regulate organic phosphorus (P) turnover in soil, but a clear understanding remains elusive. To investigate this, phosphomonoesterase and phosphodiesterase activities were determined by using *para*-nitrophenol (*p*NP) analogue substrates in a range of temperate pasture soils from England and Wales. Substrate-induced phosphatase activity ranged between 2.62 and 12.19 μmol *p*NP g^{-1} soil h^{-1} for phosphomonoesterase and between 0.25 and 2.24 μmol *p*NP g^{-1} soil h^{-1} for phosphodiesterase. Activities were correlated strongly with soil pH and labile organic P extracted in sodium bicarbonate, although the relationships differed markedly for the two enzymes. Acidic soils contained high phosphomonoesterase activity, low phosphodiesterase activity, and high concentrations of labile organic P, whereas the reverse was true in more neutral soils. As most of the organic P inputs to soil are phosphate diesters, it therefore seems likely that phosphodiesterase activity regulates labile organic P turnover in pasture soils. The low phosphodiesterase activity in acidic soils may be linked to the dominance of fungi or an effect of sorption on the enzyme. These results suggest that greater emphasis should be placed on understanding the role of phosphodiesterase activity in the cycling of soil organic P.

Keywords: Pasture; Soil; Phosphatase; Phosphomonoesterase; Phosphodiesterase; Organic phosphorus

1. Introduction

Organic phosphorus (P) is abundant in soils and can contribute to the P nutrition of plants and microbes following hydrolysis and the release of free phosphate (Condron et al., 2005). This process is catalysed by phosphatase enzymes, which are actively secreted into the soil by many plants and microbes in response to a demand for P, or passively released from decaying cells (Quiquampoix and Mousain, 2005).

Of the phosphatases present in soil, phosphomonoesterases are the most studied. This group of enzymes act on a range of low molecular weight P compounds with monoester bonds, including mononucleotides, sugar phosphates, and polyphosphates (Reid and Wilson, 1971). They cannot initiate the cleavage of phosphate from phytic acid (*myo*-inositol

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hexakisphosphate), although they can catalyse the hydrolysis of lower-order inositol phosphates (Cosgrove, 1980).

Phosphodiesterases are far less studied in both soils and soil organisms. This seems a significant oversight, because phosphodiesterase is involved in the degradation of phospholipids and nucleic acids, which constitute the majority of the fresh organic P inputs to soil (Cosgrove, 1967). Phosphomonoesterase and phosphodiesterase are both necessary to release free phosphate from a phosphate diester. Initial hydrolysis by phosphodiesterase releases a phosphate monoester, which must then by hydrolysed by phosphomonoesterase to release free phosphate for biological uptake (Fig. 1).

Understanding and modelling soil organic P turnover and its contribution to plant nutrition requires information on factors regulating soil phosphatase activity. However, a clear understanding of such factors remains elusive, despite numerous attempts to relate phosphatase activities to P pools in soil (e.g., Harrison, 1983; Spier and Cowling, 1991; Margesin and Schinner, 1994; Olander and Vitousek, 2000).

We investigate this for permanent lowland pasture soils from England and Wales. We adopted an empirical approach by analysing a large number of soils under similar vegetation and climate, but containing a range of chemical and physical properties. Our aim was to derive novel information on the link between soil phosphatase activity and the turnover of labile organic P.

2. Methods

2.1. Soil sampling

Twenty-nine lowland permanent pasture soils from England and Wales were selected from the National Soil Inventory database (National Soil Resources Institute, Cranfield University, UK) to give a wide range of physical and chemical properties (Table 1). Mean annual temperature across the sites ranged between 8.5 and 11 °C, and mean annual rainfall ranged between 600 and 850 mm. A representative bulk soil sample was taken to 10 cm depth from each site, coarsely sieved (4 mm) while still field-moist, and allowed to equilibrate for one week at approximately 15 °C. Subsamples were air-dried (30 °C) for 7 days and re-sieved (2 mm) prior to analysis.

2.2. Phosphatase assays

Phosphomonoesterase activity was assayed using *para*-nitrophenyl phosphate as an analogue phosphate monoester substrate (Tabatabai, 1994). Fresh soil (1.00 g) was weighed into screw cap glass test tubes and incubated for 1 h in a water bath at 37 °C with 4 ml of Tris–maleate buffer and 1 ml of 25 mM substrate dissolved in buffer (5 mM final concentration). The buffer contained 0.5 M Tris hydroxymethyl (aminomethane) and 0.5 M maleic acid and was adjusted with dilute HCl to pH 6.5, which is the optimum pH for phosphomonoesterase activity in most soils (Eivazi and Tabatabai, 1977). The reaction was terminated by

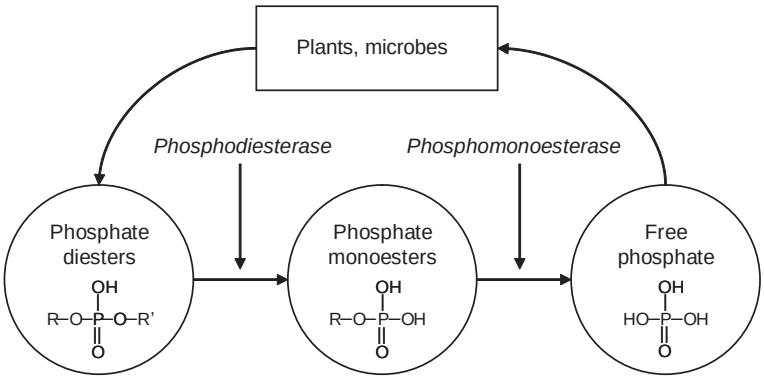

Fig. 1. A simplified conceptual model of the turnover of organic phosphorus inputs from plants and microbes in soil. Organic phosphorus inputs to soil from plants and microbes are mainly phosphate diesters, which must be hydrolysed by phosphodiesterase and phosphomonoesterase prior to the release of free phosphate for biological uptake. R and R′ represent organic moieties.

Table 1
Properties of twenty-nine pasture soils from England and Wales

Soil no.	Location	pH	Clay (g kg^{-1} soil)	Total C (g kg^{-1} soil)	Total N (g kg^{-1} soil)	Microbial C (g kg^{-1} soil)	Total organic P (mg kg^{-1} soil)	$NaHCO_3$ extraction	
								Inorganic P (mg kg^{-1} soil)	Organic P (mg kg^{-1} soil)
1	Honiton, Devon	5.0	379	28.9	3.74	0.94	418	24	29
2	Harrogate, Yorkshire	4.7	346	29.6	3.16	0.92	258	21	33
3	Northallerton, Yorkshire	5.5	219	30.6	3.25	0.72	267	35	23
4	Kendal, Cumbria	5.5	273	30.8	3.40	0.52	479	28	17
5	Kirkham, Lancashire	5.0	240	32.8	3.57	0.82	350	34	28
6	Corbridge, Northumberland	4.8	338	36.9	3.63	1.59	314	21	31
7	Derby, Derbyshire	6.1	483	38.9	2.85	0.41	217	17	11
8	Haydon Bridge, Northumberland	4.8	430	39.8	3.40	0.90	219	13	19
9	Leamington Spa, Warwickshire	5.5	328	40.1	4.05	1.22	208	11	14
10	Whitchurch, Shropshire	5.1	318	42.3	4.45	1.55	435	17	28
11	Lower Langford, Somerset	5.1	338	43.7	4.73	1.06	383	26	23
12	Wem, Shropshire	4.9	250	44.0	4.58	2.08	439	48	41
13	Atherstone, Warwickshire	6.2	401	44.9	4.43	1.24	424	26	17
14	Chorley, Lancashire	4.4	457	45.4	4.08	1.43	316	19	46
15	Llangefni, Anglesey	4.8	335	46.0	4.83	1.25	545	45	36
16	Haltwhistle, Northumberland	5.9	261	47.2	4.61	0.70	467	47	19
17	Dwyran, Anglesey	5.0	362	47.2	5.04	1.82	484	14	22
18	Cheddar, Somerset	4.8	424	47.5	4.93	1.24	418	15	24
19	Stafford, Staffordshire	5.1	299	48.0	4.83	1.30	354	29	32
20	Holsworthy, Devon	4.7	484	48.4	5.37	1.91	416	22	32
21	Hexham, Northumberland	5.0	359	49.6	4.66	0.85	275	15	21
22	Camelford, Cornwall	5.6	313	56.0	5.92	0.96	457	11	17
23	Camelford, Cornwall	4.5	445	58.7	6.35	1.43	895	24	46
24	Buxton, Derbyshire	6.8	541	60.2	5.49	1.12	478	16	21
25	Burnham-on-Sea, Somerset	5.9	547	64.4	6.52	2.23	252	9	13
26	Leppington, Yorkshire	5.8	579	66.3	6.99	2.22	380	14	18
27	Chew Stoke, Somerset	6.0	567	67.7	7.57	1.78	397	14	19
28	Radbourne, Derbyshire	5.2	629	68.8	7.33	2.04	488	12	20
29	Glastonbury, Somerset	5.0	681	80.4	8.70	3.41	882	24	44

The soils are ranked in order of total carbon concentration.

adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. The mixture was centrifuged for 10 min at 1500×g, and then 1 ml of the supernatant was diluted with deionised water and the absorbance of the released *para*-nitrophenol (*p*NP) read at 410 nm.

Phosphodiesterase was assayed by a similar procedure (Tabatabai, 1994). The buffer was 50 mM Tris adjusted with dilute H_2SO_4 to pH 8, which is the optimum pH for phosphodiesterase in most soils (Browman and Tabatabai, 1978). The substrate was 5 mM bis-*para*-nitrophenyl phosphate (1 mM final concentration) and the reaction was terminated by adding 4 ml of 0.1 M Tris–NaOH, pH 12 (instead of 0.5 *M* NaOH, which hydrolyses bis-*para*-nitrophenyl phosphate). After centrifugation, samples were diluted with 0.1 M Tris–NaOH (pH 10) and the absorbance read at 410 nm.

For both enzymes, assays were performed in triplicate and corrected for a blank (substrate added immediately after the addition of $CaCl_2$ and NaOH)

and for adsorption of released *p*NP (Vuorinen, 1993). Alkaline phosphatase activity (pH 11.0 optimum) was not determined as it was likely to make a small contribution to the total activity in these acidic soils. Substrate concentrations should be at least five times the K_m value of the enzyme to ensure zero-order kinetics for the reaction. The concentrations used here were probably sufficient for this, although it is now recommended that concentrations of around 15 mM should be used for both enzymes assayed here to ensure substrate saturation (Tabatabai, 1994).

2.3. Labile phosphorus determination

Labile P was defined by extraction in 0.5 M $NaHCO_3$ (Olsen et al., 1954). This procedure is used widely to estimate labile pools of inorganic and organic P in sequential extraction schemes (e.g., Hedley et al., 1982). Air-dried (7 days at 30 °C) soils were extracted for 30 min with 0.5 M $NaHCO_3$ (adjusted to pH 8.5 with NaOH) in a 1:20 soil to solution ratio. Each extract was filtered through a Whatman No. 42 filter paper (Whatman Ltd., Maidestone, UK) and analysed for phosphate by molybdate colorimetry (Murphy and Riley, 1962) following acidification with dilute H_2SO_4 to remove carbonate. Total P in the extracts was determined by the same method following persulphate digestion (Rowland and Haygarth, 1997). Labile organic P was calculated as the difference between total P and phosphate. Phosphate is termed inorganic P here for simplicity. However, inorganic pyrophosphate was also present in these soils (Turner et al., 2003), some of which may have been recovered in the bicarbonate extracts.

Air-drying caused considerable increases in both inorganic and organic P fractions (Turner and Haygarth, 2003). However, changes were consistent across all soils and did not influence the nature or significance of the relationships with soil properties or phosphatase activity. Therefore, only data for air-dried soils are presented.

2.4. Determination of soil properties

Total carbon and nitrogen were determined simultaneously using a Carlo-Erba model NA2000 analyser (Carlo-Erba, Milan, Italy). Soil organic P was determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy (Turner et al., 2003). Total inorganic P was estimated by extraction in 0.5 *M* H_2SO_4 for 1 h (Saunders and Williams, 1955). Textural information was obtained by wet sieving followed by analysis using a Micromeritics Sedigraph 5100 and Mastertech 51 automatic sampler (Micrometrics, Norcross GA, USA). Soil pH was determined in a 1 2.5 soil to deionised water ratio. Microbial nutrients were determined by fumigation–extraction procedures and were reported previously (Turner et al., 2001). Oxalate-extractable aluminium and iron were determined by extraction with ammonium oxalate/oxalic acid (pH 3.0) for 2 h with detection by inductively-coupled plasma optical-emission spectrometry (ICP–OES) (Schoumans, 2000).

2.5. Data analysis

Phosphatase activity is expressed as μmol *p*NP released g^{-1} soil h^{-1} and also as activity per g microbial carbon; the latter expression is useful for examining the factors controlling enzyme synthesis (Johnson et al., 1998). All values of phosphatase activity and soil properties are expressed on the basis of oven-dried soil (105 °C for 24 h). The similarity in bulk density of the soils meant that there was no requirement to express the results on a soil volume basis. A correlation matrix (*r* values) was calculated to investigate the relationships between variables, which were then investigated visually by plotting on an *x*–*y* scatter. Regression models were calculated using Sigma Plot 6.0.

3. Results

3.1. Soil properties

Some soil properties are presented in Table 1. Labile inorganic P ranged between 9 and 48 mg P kg^{-1} soil, while labile organic P ranged between 11 and 46 mg P kg^{-1} soil. Total carbon concentrations ranged between 28.9 and 80.4 g kg^{-1} soil, total nitrogen concentrations between 2.85 and 8.70 g kg^{-1} soil, clay contents between 219 and 681 g kg^{-1} soil, and pH values between 4.4 and 6.8. Total organic P concentrations ranged between 208 and 895 mg kg^{-1} soil. Microbial carbon (0.41–3.41 g kg^{-1} soil) and P

(31–239 mg kg^{-1} soil) concentrations were relatively high in these soils. Concentrations of oxalate-extractable aluminum ranged between 0.80 and 3.30 g kg^{-1} soil and oxalate-extractable iron ranged between 2.92 and 12.58 g kg^{-1} soil.

3.2. Soil phosphatase activity

Phosphomonoesterase activity ranged between 2.62 and 12.19 (mean 7.67) μmol *p*NP g^{-1} soil h^{-1} (Table 2). Phosphodiesterase activity was lower, ranging between 0.25 and 2.24 (mean 0.94) μmol *p*NP g^{-1} soil h^{-1} (Table 2). The ratio of phosphomonoesterase to phosphodiesterase ranged widely between 2.5 and 30.8 (mean 11.3). Phosphomonoesterase and phosphodiesterase activities were not correlated significantly.

When normalised for microbial carbon, phosphomonoesterase ranged between 2.96 and 10.05 (mean 6.22) μmol *p*NP g^{-1} soil h^{-1} and phosphodiesterase between 0.26 and 2.12 (mean 0.76) μmol *p*NP g^{-1} soil h^{-1}. The two expressions of phosphodiesterase activity were correlated positively (r=0.37; P<0.05), but those of phosphomonoesterase were not. Adsorption of the hydrolysis product, *p*NP, varied between 8% and 26%. Without correction, this would represent a considerable error in the determination of phosphatase activity.

Table 2
Phosphomonoesterase and phosphodiesterase activities in twenty-nine pasture soils from England and Wales

Soil no.	Phosphomonoesterase (μmol *p*NP g^{-1} soil h^{-1})		Phosphodiesterase (μmol *p*NP g^{-1} soil h^{-1})		Phoshomonoesterase to phosphodiesterase ratio
	Total activity	Activity per g microbial carbon	Total activity	Activity per g microbial carbon	
1	7.10±0.15	7.55±0.16	0.42±0.01	0.45±0.01	17
2	7.59±0.07	8.23±0.08	0.25±0.02	0.27±0.02	31
3	7.07±0.40	9.77±0.55	0.99±0.06	1.37±0.09	7
4	4.41±0.23	8.46±0.44	0.40±0.02	0.76±0.04	11
5	5.56±0.27	6.80±0.33	0.45±0.07	0.55±0.08	12
6	9.97±0.40	6.25±0.25	0.44±0.04	0.28±0.02	23
7	2.62±0.40	6.36±0.98	0.73±0.04	1.78±0.09	4
8	9.05±0.32	10.05±0.35	0.74±0.02	0.82±0.03	12
9	6.36±0.06	5.21±0.05	0.97±0.02	0.80±0.02	7
10	9.71±0.13	6.26±0.08	0.80±0.08	0.52±0.05	12
11	7.12±0.23	6.73±0.21	0.69±0.04	0.66±0.04	10
12	8.47±0.52	4.07±0.25	0.54±0.03	0.26±0.02	16
13	8.04±0.85	6.50±0.69	1.40±0.22	1.13±0.18	6
14	10.48±0.08	7.33±0.05	0.39±0.03	0.27±0.02	27
15	9.67±0.36	7.75±0.29	0.66±0.01	0.53±0.01	15
16	4.91±0.13	7.03±0.19	1.48±0.05	2.12±0.08	3
17	9.89±0.41	5.43±0.23	0.66±0.05	0.36±0.03	15
18	6.84±0.07	5.51±0.06	0.63±0.01	0.51±0.01	11
19	6.84±0.22	5.26±0.17	0.83±0.04	0.64±0.03	8
20	8.31±0.12	4.36±0.06	0.93±0.11	0.49±0.07	9
21	6.72±0.33	7.90±0.38	0.58±0.03	0.68±0.03	12
22	7.15±0.45	7.41±0.47	0.59±0.06	0.62±0.06	12
23	9.98±0.19	6.99±0.14	0.46±0.05	0.33±0.03	21
24	5.20±0.42	4.66±0.38	2.07±0.02	1.86±0.02	3
25	8.54±0.35	3.84±0.16	1.80±0.07	0.81±0.03	5
26	7.23±0.15	3.25±0.07	2.24±0.20	1.01±0.11	3
27	5.25±0.12	2.96±0.07	1.92±0.10	1.08±0.06	3
28	10.00±0.37	4.91±0.18	1.32±0.15	0.65±0.07	8
29	12.19±0.25	3.57±0.07	1.82±<0.01	0.53±<0.01	7
Mean	7.67±2.13	6.22±1.85	0.94±0.57	0.76±0.48	11

Data are expressed as total activity and activity per g microbial carbon. Values are means±standard deviation of three replicate assays.

3.3. Relationships between phosphatase activities and labile phosphorus fractions

Phosphatase activities were not correlated with labile inorganic P ($P>0.05$), but were correlated strongly with labile organic P (Table 3). The relationship between phosphomonoesterase activity and labile organic P was positive and linear, whereas that for phosphodiesterase activity was best described by an inverse first-order model (Fig. 2). The relationship for phosphodiesterase was only statistically significant when a single outlying point was excluded (Fig. 2). The outlying soil contained the greatest clay and organic carbon concentrations of all the soils analysed, but there was no obvious reason why it did not fit the model, especially as it fitted well when normalised for microbial carbon ($P<0.001$; Fig. 2). When phosphomonoesterase activity was normalised for microbial carbon, it was not correlated significantly with labile P fractions ($P>0.05$). The phosphomonoesterase-to-phosphodiesterase ratio was correlated positively with labile organic P ($P<0.001$; Table 3).

3.4. Relationships with soil properties

Both phosphomonoesterase and phosphodiesterase activities were linearly correlated with soil pH, although the nature of the relationships differed markedly (Fig. 3). Phosphomonoesterase activity was correlated negatively ($P<0.001$), whereas phosphodiesterase activity was correlated positively ($P<0.001$). There was no significant correlation between soil pH and phosphomonoesterase activity normalised for microbial carbon (Table 3), but the correlation with phosphodiesterase was stronger when activity was normalised. The ratio of phosphomonoesterase-to-phosphodiesterase was strongly correlated negatively with soil pH ($P<0.001$; Table 3).

Phosphomonoesterase activity was correlated positively with total nitrogen and total organic P ($P<0.05$; Table 3). In contrast, phosphodiesterase was positively correlated with clay concentration, total carbon, and total nitrogen ($P<0.001$), but was not correlated with total organic P ($P>0.05$). Both phosphomonoesterase and phosphodiesterase activities were correlated positively with microbial carbon and microbial P. When normalised for microbial carbon, phosphomonoesterase was correlated negatively with clay content, total carbon, total nitrogen, and microbial carbon and P, while phosphodiesterase was correlated positively with soil pH (Table 3).

Labile organic P was correlated negatively with soil pH ($P<0.01$; Table 3), although the relationship was best described by an inverse second-order model ($P<0.0001$; Fig. 3). In particular, it was noticeable that labile organic P concentrations increased markedly in soils with pH<5.3 (Fig. 3). Labile organic P

Table 3
Correlation coefficients (r values) for relationships between phosphatase activities, labile phosphorus fractions, and soil properties for twenty-nine pasture soils from England and Wales

	pH	Clay	Total carbon	Total nitrogen	Total organic phosphorus	Total inorganic phosphorus	Microbial carbon	Microbial phosphorus
Labile phosphorus fractions								
Inorganic phosphorus	−0.14	**−0.55****	−0.32	−0.26	0.22	**0.58*****	−0.16	−0.02
Organic phosphorus	**−0.71*****	0.00	0.06	0.13	**0.54****	0.31	**0.37***	**0.49****
Phosphatase activities								
Phosphomonoesterase	**−0.60*****	0.27	0.32	**0.37***	**0.62*****	−0.27	**0.67*****	**0.66*****
Phosphodiesterase	**0.69*****	**0.64*****	**0.73*****	**0.67*****	−0.33	025	**0.49****	**0.42***
Phosphomonoesterase per g microbial carbon	−0.28	**−0.58*****	**−0.69*****	**−0.70*****	−0.01	0.05	**−0.74*****	**−0.64*****
Phosphodiesterase per g microbial carbon	**0.83*****	0.08	0.09	−0.05	**−0.59*****	**0.44***	−0.35	−0.36
Phosphomonoesterase to phosphodiesterase ratio	**−0.76*****	−0.30	**−0.43***	−0.37	**0.63*****	**−0.39***	−0.10	−0.07

Statistically significant relationships are indicated in bold lettering. Significance levels are denoted by *, ** and ***, representing the 5%, 1% and 0.1% levels, respectively.

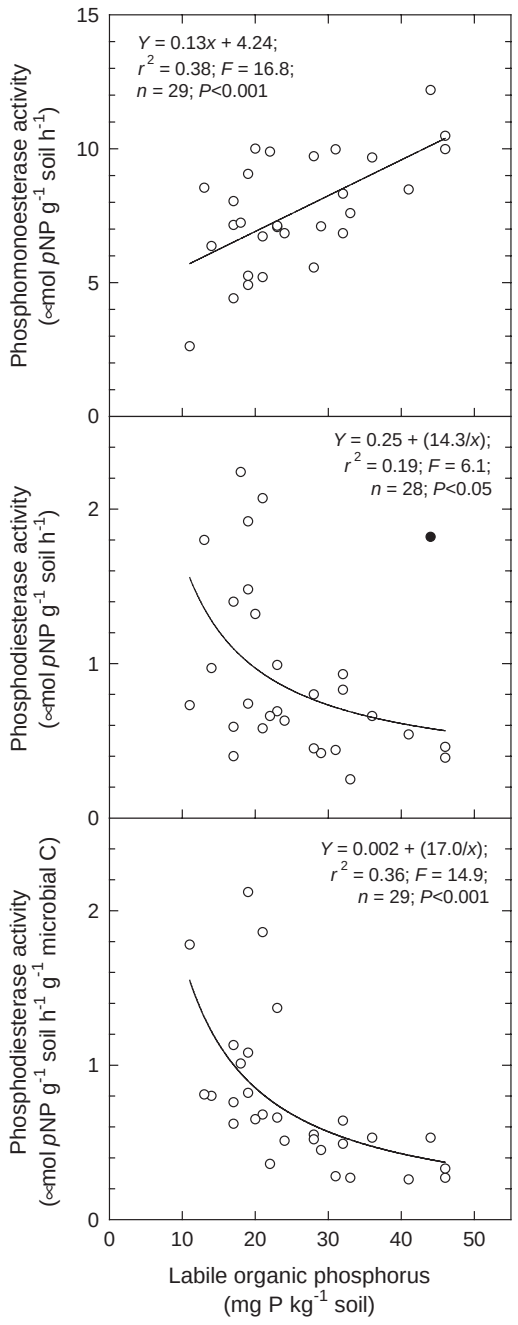

Fig. 2. Relationships between labile organic phosphorus and phosphatase activities of 29 permanent pasture soils from England and Wales: phosphomonoesterase activity (top), phosphodiesterase activity (centre) and phosphatase activity expressed normalised for microbial carbon (bottom). The filled circle on the centre graph represents an outlying value that was not included in the regression model.

was also positively correlated with total organic P, microbial carbon and microbial P (Table 3). Labile inorganic P was positively correlated with total inorganic P ($P<0.01$) and negatively correlated with clay content ($P<0.01$; Table 3).

4. Discussion

This study provides the first regional data on phosphatase activities in permanent pasture soils of England and Wales. Values are similar to those reported in the literature for grassland soils using the same procedure. For example, acid phosphomonoesterase activity in pasture soils of New Zealand ranged between 8.3 and 16.7 μmol *p*NP g^{-1} soil h^{-1} (Sarathchandra and Perrott, 1981). Much greater activities were detected in high organic matter grassland soils from the uplands of northern England (Johnson et al., 1998; Turner et al., 2002a), although these were influenced by long-term pollutant nitrogen deposition from the atmosphere.

For phosphodiesterase, relatively high activities between 3.1 and 25.2 μmol *p*NP g^{-1} soil h^{-1} were reported from a New Zealand pasture soil during 3 years of repeated measurements (Ross et al., 1995), although much lower activities between 0.06 and 0.12 μmol *p*NP g^{-1} soil h^{-1} were detected during a seasonal cycle in another New Zealand grassland soil (Chen et al., 2003). Corresponding phosphomonoesterase activities in the latter study ranged between 2.0 and 4.3 μmol *p*NP g^{-1} soil h^{-1}.

In studies of individual organisms, the expression of phosphatase activity increases in response to the degree of P limitation (Raghothama, 1999; Torriani-Gorini, 1994). In the soils studied here, no link was found between phosphatase activity and labile inorganic P, although activities were strongly correlated with labile organic P. Relationships were different for the two phosphatases studied and were determined mainly by soil pH. Low phosphodiesterase activities in acidic soils coincided with high concentrations of labile organic P, whereas the reverse was true in more neutral soils. Most organic P inputs to soil are phosphate diesters such as nucleic acids (Cosgrove, 1967), which must be hydrolysed by both phosphodiesterase and phosphodiesterase to release phosphate (Fig. 1). Our results therefore suggest strongly that

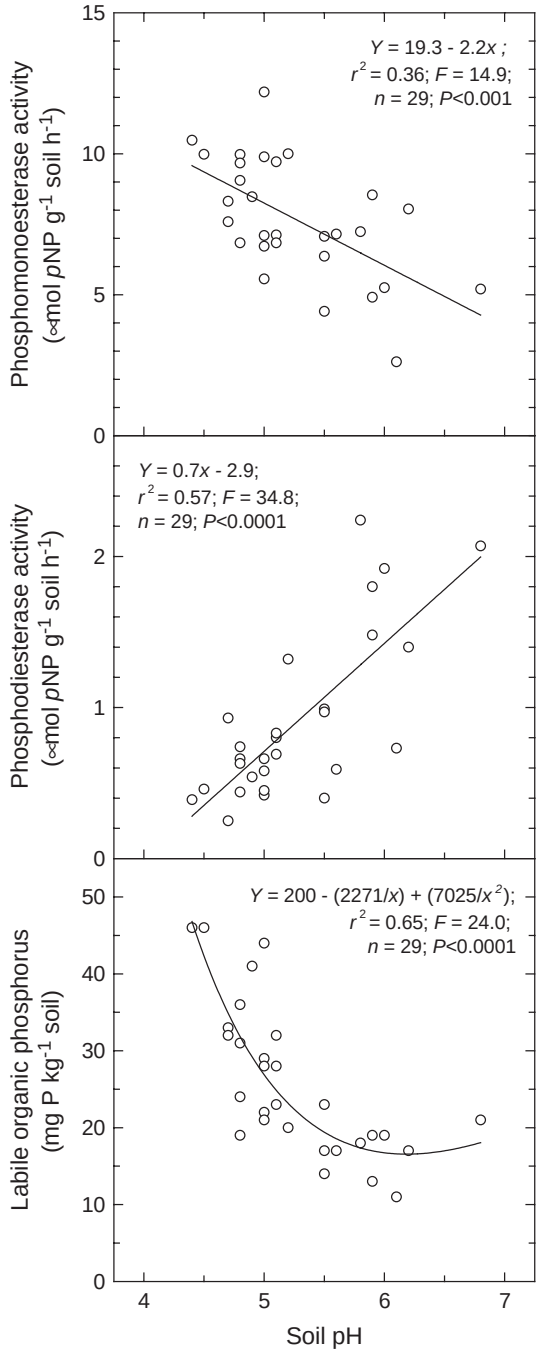

Fig. 3. Relationships between soil pH and phosphatase activities of twenty-nine pasture soils from England and Wales: phosphomonoesterase activity (top), phosphodiesterase activity (centre) and labile organic phosphorus (bottom).

phosphodiesterase activity is the rate-limiting step that regulates labile organic P turnover in pasture soils. This hypothesis is supported by evidence that RNA was hydrolysed much more slowly than simple phosphate monoesters (glycerophosphate and 3′ mononucleotides of RNA) in a temperate sandy loam under grass in central USA (pH 6.5, total C 0.9%) (Bowman and Cole, 1978). Further, large proportions of the water-extractable organic P in some Australian pasture soils occurred as phosphate diesters, with labile monoesters present in negligible amounts (Turner et al., 2002b).

The differences in the influence of soil pH on the two phosphatases may be due in part to the dominant microbes present in acidic and neutral soils (Paul and Clark, 1996). The greater rates of phosphomonoesterase activity in more acidic soils reported here and in previous studies (Juma and Tabatabai, 1978; Harrison, 1983) suggest that fungi are the main source of this enzyme, because these organisms dominate the microbial biomass in acidic soils. Plants are also an important source of acid phosphomonoesterase (Quiquampoix and Mousain, 2005). In contrast, the greater phosphodiesterase activity in neutral soils suggests synthesis mainly by bacteria or actinomycetes, which are both more abundant in neutral soils. There is evidence that nucleic acid degradation in soil is mediated primarily by Gram negative bacteria similar to *Pseudomonas* (Greaves and Wilson, 1970), while actinomycetes appear to be the main group of organisms responsible for degrading phospholipids (Ko and Hora, 1970). Some plants also secrete phosphodiesterase under conditions of severe P-deficiency (Abel et al., 2000), but this probably makes a negligible contribution to the total phosphodiesterase activity in most soils.

Most studies of fungal phosphatase have focused on phosphomonoesterase (e.g., Antibus et al., 1992), but there is evidence that some fungi also synthesise phosphodiesterase and use nucleic acids in their environment (Leake and Miles, 1996; Myers and Leake, 1996). This means that dominance by fungi does not explain completely the low phosphodiesterase activity detected in acidic soils, which raises the intriguing possibility that phosphodiesterase is synthesised in acidic soils, but is inactivated rapidly, perhaps by sorption to soil constituents. Phosphomonoesterase is known to be strongly sorbed in soil,

although it retains some residual activity in the immobilised state (Skujins, 1976). The immobilised enzymes may even accumulate to the extent that they are the dominant source of activity (Kiss et al., 1975). There is no comparable data for phosphodiesterase, but this warrants further study given the apparent importance of this enzyme in the turnover of soil organic P.

5. Conclusion

Analysis of a range of permanent lowland pasture soils revealed strong correlations between labile organic P and phosphatase activities, although relationships were different for the two enzymes studied. The highest rates of phosphomonoesterase occurred in acidic soils and coincided with large concentrations of labile organic P, whereas the highest rates of phosphodiesterase activity occurred in more neutral soils and coincided with the lowest concentrations of labile organic P. Given that most organic P inputs to soil are phosphate diesters, this suggests strongly that phosphodiesterase activity regulates labile organic P turnover in pasture soils. The effect of soil pH on the two phosphatases may be linked to the dominant groups of organisms in acidic and neutral soils, or to the rapid inactivation of phosphodiesterase in acidic soils. The results demonstrate clearly that greater emphasis should be placed on understanding the role of phosphodiesterase in the turnover of soil P.

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