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Seasonal phosphatase activities of mosses from Upper Teesdale, northern England

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Summary

Changes in tissue nutrient concentrations and surface phosphatase activities of eight moss species were measured over one year in terrestrial and semi-aquatic environments on Widdybank Fell, Upper Teesdale National Nature Reserve, northern England. Rates of phosphatase activity in apical regions of moss shoots differed markedly between species, but were generally greatest in the winter and least in the summer in most species. Mean values for phosphomonoesterase activity (μmol *para*-nitrophenol g^{-1} d.wt h^{-1}) ranged from 18.2 for *Polytrichum commune* to 85.8 for *Palustriella commutata* var. *falcata*. Mean phosphodiesterase activity ranged from 3.1 for *Polytrichum commune* to 86.2 for *Hylocomium splendens*. In contrast, tissue nitrogen and phosphorus concentrations remained relatively constant throughout the year. Phosphatase activities were negatively correlated with tissue phosphorus concentration for several species, although few relationships were detected between ambient nutrient concentrations and phosphatase activity, tissue nitrogen, or tissue phosphorus concentration. These results demonstrate that phosphatase activities can provide a sensitive indicator of nutrient stress in terrestrial and semi-aquatic mosses, notably in the ectohydric *Hylocomium splendens*. However, further studies at sites with a wide range of nutrient levels are required to determine whether the technique can be used to indicate ambient nutrient status.

KEYWORDS: bryophytes, *Hylocomium splendens*, moss, nitrogen, phosphodiesterase, phosphomonoesterase, phosphorus, uplands.

Introduction

Mosses growing at terrestrial and aquatic sites display 'surface' phosphatase activity, which varies in response to the concentrations of nitrogen (N) and phosphorus (P) in the moss tissue (Press & Lee, 1983; Christmas & Whitton, 1998; Turner *et al*., 2001). This in turn reflects the relative availability of these nutrients in the ambient environment. For example, mosses growing in the uplands of northern England, including *Hylocomium splendens*, *Polytrichum commune* and *Sphagnum* spp. show greater activity than the same species growing in subarctic Sweden, almost certainly linked to a greater degree of P limitation in the English uplands (Press & Lee, 1983; Turner *et al*., 2001). The situation in northern England is at least partly due to pollutant N deposition from the atmosphere (Hicks *et al*., 2000), because this has been reported to enhance biological P limitation in natural and semi-natural ecosystems (Aber *et al*., 1989). Moss phosphatase activity may therefore be a sensitive indicator of ambient nutrient status, including the impact of pollutant deposition of reactive N compounds from the atmosphere (Turner *et al*., 2001).

Mosses show both phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activity (Christmas & Whitton, 1998; Turner *et al*., 2001). The expression of these enzymes indicates that mosses possess the ability to use various organic phosphate esters present in the ambient environment. Activity is quantified in the laboratory by measuring the hydrolysis of synthetic substrates in liquid medium. It is usually assumed that most, if not all, hydrolysis takes place in the cell wall, although the possibility of intracellular hydrolysis cannot be ruled out. It is probable that mosses also recycle cellular organic P at times of P stress, a common process in vascular plants (Plaxton & Carswell, 1999) that also occurs for N in *Hylocomium splendens* (Eckstein & Karlsson, 1999). It is probable that

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both mechanisms operate together, with mosses opportunistically using ambient organic compounds when available, while efficiently recycling internal nutrients during times of P stress. However, if internal organic phosphates are hydrolysed by phosphatases prior to translocation around the moss, their activity is unlikely to be detected in assays for 'surface' (cell wall) activity.

It seems likely that nutrient availability at sites in upland areas is often highly variable. In the case of Widdybank Fell in Upper Teesdale, northern England, concentrations of P in soil solution and stream water vary seasonally by at least three orders of magnitude, with the highest concentrations occurring in occasional pulses during spring and early summer (Livingstone & Whitton, 1984; Whitton *et al.*, 1998; Turner *et al.*, 2003a). Phosphorus in these pulses is present largely in organic forms, confirming the potential importance of phosphatase as a means to access P in this environment. In contrast, combined N concentrations (nitrate, ammonium, organic N) are relatively plentiful, and soluble N: P ratios suggest that P availability limits productivity for much of the year, at least for non-vascular phototrophs. However, some species may be N-limited during the spring and early summer (Turner *et al.*, 2003a), as was also reported for the Dee catchment in north-east Scotland (Edwards *et al.*, 2000). It therefore seems possible that the phosphatase activities of mosses may vary seasonally in response to the ambient nutrient supply and the rate at which accumulated nutrients are used for growth.

The aim of the study reported here was to determine possible changes in phosphatase activities of terrestrial and semi-aquatic mosses at Widdybank Fell during an annual cycle, and to investigate whether any such changes could be related to ambient or tissue nutrient concentrations. If such changes were evident, it was hoped to establish which species may be used as indicators of seasonal changes and the best time of year for using these species to assess long-term nutrient availability at a site.

Materials and Methods

Sampling sites and species

The study was conducted in the Upper Teesdale National Nature Reserve, northern England, an upland area containing relict late-glacial plant assemblages that are sufficiently rare to be of international importance (Clapham, 1978). There are three distinct soil types and related plant communities:

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

The grasslands are grazed by sheep. Small streams (sikes) drain the area into Cow Green Reservoir, fed by acidic drainage from blanket peat bogs and alkaline drainage from calcareous springs.

Samples were collected from Widdybank Fell (UK Ordnance Survey Grid Ref. NY 820 300; 54° 40′ N; 2° 15′ W, maximum altitude 519 m a.s.l.; 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. The area is subject to pollutant N deposition from the atmosphere, with annual rates of deposition onto Great Dun Fell, close to Widdybank Fell, between 20 and 40 kg ha^{-1} depending on altitude (Hicks *et al.*, 2000). There have been floristic changes on the Fell in recent decades, notably reductions in bryophyte diversity and lichen abundance (Huntley *et al.*, 1998). Changes in microclimate induced by the creation of an adjacent reservoir may have influenced some of these changes, but almost certainly do not explain them all. Some terrestrial and aquatic species are strongly limited by P availability (Jeffrey & Piggott, 1973; Livingstone & Whitton, 1984; Turner *et al.*, 2001), but it is difficult to assess the extent to which atmospheric pollution has enhanced this.

Mosses and associated soil/water were sampled monthly during a seasonal cycle between November 1999 and October 2000 from a range of environments on Widdybank Fell, including terrestrial and aquatic locations with both acidic and calcareous environments (Table 1). Terrestrial species were sampled from the three soil types, while semi-aquatic mosses were sampled from areas adjacent to two streams (Slapestone Sike and Red Sike), which combine drainage from blanket peat and grassland with subterranean drainage from limestone (Livingstone & Whitton, 1984). Seven taxa were sampled, though for simplicity the two varieties of *Palustriella commutata* are termed 'species'. One terrestrial species was sampled from both an acidic and a calcareous environment, while five species were sampled twice-weekly during the spring period between 19 March and 31 May 2000 to investigate short-term variations in phosphatase activity. The nomenclature is based on Blockeel & Long

Table 1. *Moss species and the environments on Widdybank Fell from which they were sampled.*

Species	Environment
Dicranum scoparium	Calcareous grassland
Hylocomium splendens	Calcareous grassland
Plagiothecium nemorale	Acid grassland
Polytrichum commune	Acid grassland
Palustriella commutata var. *commutata*	Calcareous spring draining blanket peat and calcareous grassland into Slapestone Sike
Palustriella commutata var. *falcata*	Stream bank of Red Sike, draining blanket peat and calcareous grassland
Racomitrium lanuginosum	Blanket peat
Racomitrium lanuginosum	Calcareous grassland
Sphagnum cuspidatum	Acidic spring draining blanket peat

(1988), which differs from that widely used in the literature for *Palustriella commutata* var. *falcata* (= *Cratoneuron commutatum* var. *falcatum*) and *P. commutata* var. *commutata* (= *C. commutatum* var. *commutatum*).

Whole plants were sampled from healthy clumps dominated by the particular species and returned immediately to the laboratory, where they were stored in the dark at 4°C until analysis within 24 h of collection. The mosses were washed in assay medium and 2-cm tips were removed for assays, except for *Sphagnum cuspidatum*, of which the capitulum (terminal group of stem branches) was used. The use of tips means that the region of active growth is included, which ensures minimum contamination by epiphytes and soil particles. It also seems likely that the tips best reflect recent changes in the environment (see Discussion).

Meteorological data

Daily mean temperature and rainfall data, plus the concentrations of nitrate, ammonium and phosphate in rainfall, were obtained from the Environmental Change Network automatic weather station situated at Moor House. This site is approximately 10 km to the southwest of Widdybank Fell, and at higher altitude (847 m a.s.l.). Meteorological records at Moor House for the study period are shown in Fig. 1. Temperatures were highest between June and September, and lowest between December and February. Most rainfall fell in this cold winter period. Nitrate and ammonium concentrations in rainfall were each usually less than 500 μg N l^{-1}, whereas inorganic P concentrations were <10 μg P l^{-1} for most of the year (Turner *et al.*, 2003a).

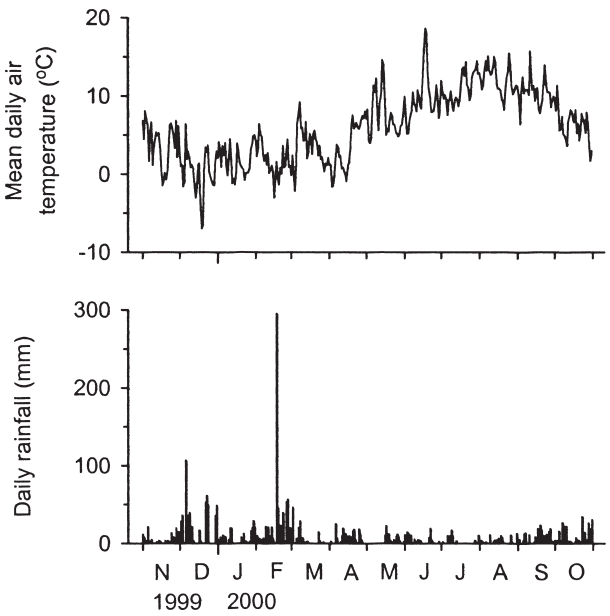

Figure 1. Mean daily temperature and rainfall between 1 November 1999 and 31 October 2000 at Moor House, Upper Teesdale, northern England.

Determination of tissue nutrients

Tissue N and P concentrations were determined by digestion in sulphuric acid and hydrogen peroxide, with selenium catalyst (Novozamsky *et al.*, 1983). Ammonium and phosphate in the digests were determined colourimetrically using a Skalar segmented flow analyzer (Skalar UK Ltd, York, UK); ammonium was determined by reaction with sodium salicylate and sodium nitroprusside, while phosphate was determined by reaction with ammonium molybdate. Due to the small amounts of material sampled relative to that required for tissue analysis (due to the National Nature Reserve status of the sampling site), only single replicates of bulked tissue were analysed.

Determination of moss phosphatase activities

Rates of phosphatase activity were determined by standardized methodology (Turner *et al.*, 2001), using *para*-nitrophenol phosphate (*p*NPP) and bis- *para*-nitrophenol phosphate (bis-*p*NPP) as analogue substrates for phosphomonoesterase (PMEase) and phosphodiesterase (PDEase), respectively. Moss tips were placed in glass vials with 2.9 ml dimethylglutaric acid buffer (pH 5.0) made in assay medium. Two-cm apical tips were used for all mosses except *Sphagnum cuspidatum*, for which 1-cm capitula were used. Assays were initiated by adding 0.1 ml substrate (500 μM final concentration) and the vials were incubated for 15 minutes in a shaking water bath (*ca* 100 strokes min^{-1}) at 20°C and low level laboratory light (15–20 μmol photon m^{-2} s^{-1}). Four replicate assays plus blanks were analysed for each species on a particular day.

To terminate the assay and develop the yellow colour, 2.5 ml assay mixture was transferred to 0.25 ml terminator solution in a test tube. The terminator consisted of 1.1 M NaOH (100 mM final), 27.5 mM EDTA (2.5 mM final) and 0.55 M K_2HPO_4 (50 mM final). This was designed to inactivate any free enzyme and raise the pH to >11, but not cause hydrolysis of bis-*p*NPP, which occurs slowly at pH > 12. Absorbance was measured at 405 nm; values > 0.8 were diluted with deionized water. The activity was determined using calibration curves constructed from *para*-nitrophenol (*p*NP) standards (0–25 μM) made in assay medium. The values of the blank and control vials were subtracted from the final measured value. Only small amounts of chemical hydrolysis were detected from the substrates. Following analysis, the mosses were dried at 105°C for 24 h and then weighed to 0.0001 g on a microbalance. Enzyme activity is expressed as μmol *p*NP released g^{-1} d.wt h^{-1}. The assay medium was a modification of the No. 10 medium of Chu (1942), an important change from the original medium being the inclusion of EDTA as chelator and the absence of N and P. The final assay medium was obtained by dilution from stock solutions and contained: 243.7 μM $CaCl_2$, 188.6 μM $NaHCO_3$, 101.4 μM $MgSO_4$, 57.38 μM KCl, 4.5 μM $FeCl_3$, 4.2 μM Na_2.EDTA, 11.56 μM H_3BO_3, 2.28 μM $MnCl_2$, 0.078 μM $CuSO_4$, 0.035 μM $CoSO_4$, 0.028 μM

Na_2MoO_4, 0.019 μM $ZnSO_4$ and 0.135 μM $NiSO_4$. Mosses were always analysed within 24 h of collection in the field-moist state, because drying caused variable and in some cases substantial changes in phosphatase activity (Turner *et al.*, 2001).

Soil and water sampling and analysis

Soil and water sampling and analysis were described previously (Turner *et al.*, 2003a). Briefly, soil cores to 5 cm depth were taken from each of the three soil types (blanket peat, acid organic grassland soil, calcareous grassland soil) and water-soluble nutrients extracted by shaking 5 g of moist soil with 50 ml of deionised water for 1 h. Extracts were centrifuged ($3000 \times g$ for 30 minutes) and filtered through 0.45-μm membranes (cellulose acetate; Sartorius Ltd, Epsom, UK).

Soil water extracts and filtered (0.45 μm) stream water samples were analysed for N and P fractions. Nitrate and ammonium were determined using a Skalar segmented flow analyzer. Total P was determined by molybdate reaction following acid-persulphate digestion (Rowland & Haygarth, 1997). Inorganic orthophosphate was determined by reaction with molybdate (Murphy & Riley, 1962), with organic P estimated as the difference between total and inorganic P. Total P and inorganic orthophosphate were determined using 5 and 10-cm path lengths, respectively, giving detection limits of approximately 1 μg P l^{-1}.

Data analysis

Correlation coefficients (r values) for relationships between tissue nutrients, phosphatase activities and ambient nutrients were calculated using standard procedures in Microsoft Excel 5.0. Regression models were calculated using Sigma Plot 6.0. Coefficients of variation (%) were calculated to assess the relative seasonal variability in tissue nutrients and phosphatase activities (SD/mean*100). Values were calculated using month and residual variance component estimates of all species (Kendall & Stuart, 1963), with 95% confidence intervals calculated using Fieller's theorem (Zerbe, 1978).

RESULTS

Ambient nutrient concentrations

Summary information on soluble nutrient concentrations in soil solution and stream water in the various environments is shown in Table 2, and has been previously reported (Turner *et al.*, 2003a). The wide range of P concentrations apparent in Table 2 conceals the strong seasonal variability in organic P concentrations in this environment, despite relatively constant concentrations of N fractions (Fig. 2). Reactive (inorganic) P concentrations were close to the detection limit of the analytical procedure throughout the season (Table 2).

Moss phosphatase activity

There were marked differences in phosphatase activities amongst species (Table 3). Mean seasonal PMEase activity ranged from 18.2 μmol *p*NP g^{-1} d.wt h^{-1} for *Polytrichum commune* to 85.8 μmol *p*NP g^{-1} d.wt h^{-1} for *Palustriella commutata* var. *falcata*, while mean PDEase activity ranged from 3.1 μmol *p*NP g^{-1} d.wt h^{-1} for *Polytrichum commune* to 86.2 μmol *p*NP g^{-1} d.wt h^{-1} for *Hylocomium splendens*. PMEase and PDEase activities were strongly correlated in all species (r values between 0.63 and 0.95; $P < 0.05$), except *Plagiothecium nemorale* ($r = 0.54$; $P > 0.05$). The mean PDEase: PMEase ratio was least in *Polytrichum commune* (0.19) and greatest in *Hylocomium splendens* (1.12).

Table 2. *Summary of soluble nutrient concentrations in soil and streams of the various environments on Widdybank Fell from which mosses were sampled (Turner et al., 2003a). Samples were collected between November 1999 and October 2000.*

Environment		Nitrogen			Phosphorus	
		NO_3-N	NH_4-N	Organic N[a]	PO_4-P	Organic P
Stream/spring water (µg l^{-1})						
Acid spring	Median	<10	45	447	<1.0	14.2
	Range	<10	<10–260	15–1340	<1.0–5.1	0–85.9
Calcareous spring	Median	185	<10	48	1.1	2.4
	Range	100–300	<10–40	20–125	<1.0–5.9	0.3–211
Red Sike	Median	165	<10	200	<1.0	4.4
	Range	100–290	<10–130	25–460	<1.0–7.0	0.1–271
Soil solution (µg g^{-1} soil)						
Acid grassland soil	Median	0.49	3.22	8.70	0.21	0.98
	Range	<0.1–5.75	<0.1–9.32	0.66–20.1	<0.01–1.62	0.21–3.29
Blanket peat	Median	<0.1	1.45	15.5	0.05	0.41
	Range	<0.1–0.43	<0.1–9.33	3.15–31.6	<0.01–0.81	0.05–2.88
Calcareous grassland soil	Median	1.16	2.66	7.33	0.09	0.37
	Range	0.39–4.25	0.21–9.49	2.13–17.6	0.01–0.32	0.10–33.6

[a] Values for stream/spring water are for total organic N determined in unfiltered samples.

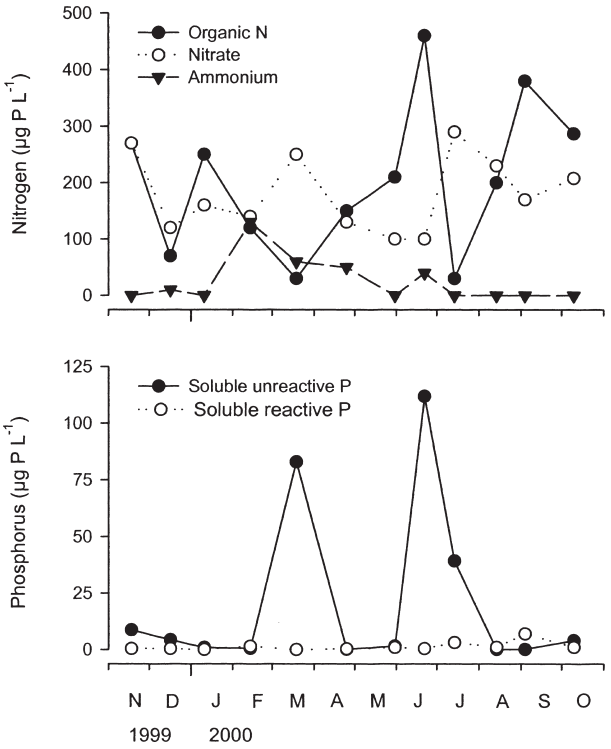

Figure 2. Concentrations of N (nitrate, ammonium and total organic N) and P (reactive and unreactive P) in stream water from Red Sike between November 1999 and October 2000, demonstrating the temporal variability of organic P in this environment. Note the different *Y*-axis scales.

Strong seasonal changes were apparent in phosphatase activities (Fig. 3). There was a general trend for maximum rates in winter and minimum rates in summer, this being most clearly demonstrated in *Hylocomium splendens* and *Sphagnum cuspidatum*. The PDEase: PMEase ratio remained relatively constant during the seasonal cycle, except for *Polytrichum commune* and *Palustriella commutata* var. *commutata*. However, the results for these species may reflect the fact that their PDEase activity was often close to the detection limit. Coefficients of variation for seasonal phosphatase activity were 37.1 ± 2.9% for PMEase (95% confidence interval 31.6– 43.0) and 47.7 ± 3.9 for PDEase (95% confidence interval 40.3–55.9), reflecting the relatively large seasonal variations for individual mosses.

Tissue nutrients

Mean seasonal tissue N concentration ranged from 7.6 mg N g^{-1} d.wt in *Racomitrium lanuginosum* growing on blanket peat to 20.2 mg N g^{-1} d.wt in *Sphagnum cuspidatum* (Table 3). Mean tissue P concentration ranged from 1.00 mg P g^{-1} d.wt in *Racomitrium lanuginosum* growing on blanket peat to 1.81 mg P g^{-1} d.wt in *Plagiothecium nemorale* (Table 3). For individual mosses the concentrations of tissue N and P were not correlated at statistically significantly levels ($P > 0.05$), the exception being *Palustriella commutata* var. *falcata* ($r = 0.59$, $P < 0.05$).

Concentrations of both N and P were slightly greater in the warmer months (May to August) and least in winter (November to February), although the differences were relatively small compared to the seasonal changes in phosphatase activities (Fig. 3). There were also slight seasonal differences in the tissue N: P ratio. Coefficients of variation for seasonal tissue nutrients were significantly lower than those of phosphatase activities, being 14.7 ± 3.9% for tissue N (95% confidence interval 12.7–16.7) and 18.9 ± 1.3% for tissue P (95% confidence interval 16.3–21.6). This reflected the relatively stable concentrations of tissue nutrient concentrations.

Short-term changes in phosphatase activity

The phosphatase activities of five species were determined intensively between March and June 2000. Activities in four species were reasonably consistent during this period, although sharp increases were noted on two occasions in *Sphagnum cuspidatum* (Fig. 4), which appeared to correspond to similar, but much smaller, changes in *Hylocomium splendens*, *Palustriella commutata* var. *commutata* and *Racomitrium lanuginosum*. The relative changes in PMEase and PDEase activities suggested that PDEase activity responded more slowly, lagging behind the more rapid changes in PMEase (Fig. 4).

Relationships between phosphatase activities, tissue nutrients, and environmental variables

The phosphatase activity of most mosses was negatively correlated with tissue P concentrations, with significant values ($P < 0.05$) for five species for PMEase and two species for PDEase (Table 4, Fig. 5). Only the PMEase activity of *Racomitrium lanuginosum* (growing on blanket peat) was significantly correlated (positively) with tissue N concentration. In almost all species there were positive correlations between phosphatase activities and tissue N: P ratio, with significant values in three species (Fig. 5).

For all mosses, negative correlations existed between rates of phosphatase activity and mean monthly air temperature, although these were only statistically significant in four (Table 4). The relationships were particularly strong for PMEase and PDEase activities of *Hylocomium splendens* (Fig. 6), but were also statistically significant for the PMEase activities of *Sphagnum cuspidatum*, *Palustriella commutata* var. *commutata* and *Racomitrium lanuginosum* growing on blanket peat (Table 4).

There were few significant correlations between moss phosphatase activities and ambient nutrients (r values not shown). PDEase activity of *Racomitrium lanuginosum* in calcareous grassland was significantly correlated with soluble inorganic P ($r = -0.76$, $P < 0.01$), and ambient organic P was significantly correlated with the PMEase and PDEase activities of *Sphagnum cuspidatum* ($r = -0.72$ and -0.73,

Table 3. *Phosphatase activities and tissue nutrient concentrations of 2-cm moss tips (1-cm capitula for Sphagnum cuspidatum) sampled between November 1999 and October 2000 from Widdybank Fell. Means are of 12 monthly samples (± standard deviation).*

Species		PMEase	PDEase	PDEase: PMEase ratio	Tissue N	Tissue P	N: P ratio
		μmol *p*NP g^{-1} d.wt h^{-1}			mg g^{-1} d.wt		
Dicranum scoparium	Mean	33.7 (8.75)	19.4 (6.20)	0.58 (0.13)	8.4 (1.63)	1.35 (0.20)	6.4 (1.55)
	Range	20.5–48.8	12.9–34.5	0.44–0.83	6.2–11.3	0.91–2.84	3.7–9.2
Hylocomium splendens	Mean	78.8 (23.3)	86.2 (26.5)	1.12 (0.23)	14.3 (1.76)	1.35 (0.23)	10.9 (2.46)
	Range	34.5–119.0	52.0–131.9	0.86–1.63	11.8–18.0	0.91–1.74	8.0–17.4
Palustriella commutata var. *commutata*	Mean	39.6 (20.4)	10.5 (6.86)	0.26 (0.17)	15.3 (2.22)	1.50 (0.31)	10.6 (2.45)
	Range	5.3–70.8	0.7–19.4	0.04–0.68	10.3–18.5	0.87–1.88	7.2–15.8
Palustriella commutata var. *falcata*	Mean	85.8 (29.6)	43.9 (16.9)	0.51 (0.10)	10.5 (2.87)	1.08 (0.29)	10.1 (2.62)
	Range	17.1–124.5	7.1–67.8	0.40–0.76	7.4–17.8	0.64–1.73	5.9–14.3
Plagiothecium nemorale	Mean	78.2 (28.1)	50.6 (21.3)	0.69 (0.29)	13.4 (0.76)	1.81 (0.30)	7.6 (1.30)
	Range	35.1–143.4	22.7–97.8	0.31–1.35	12.6–15.4	1.41–2.32	5.6–9.3
Polytrichum commune	Mean	18.2 (8.80)	3.1 (1.45)	0.19 (0.10)	15.7 (1.92)	1.57 (0.20)	10.2 (1.67)
	Range	4.5–38.0	0.6–5.84	0.03–0.41	13.6–19.6	1.38–2.10	8.2–13.4
Racomitrium lanuginosum (blanket peat)	Mean	39.5 (8.23)	18.2 (5.14)	0.47 (0.11)	7.6 (0.62)	1.00 (0.21)	8.0 (2.07)
	Range	29.2–57.2	12.0–27.9	0.35–0.69	6.4–8.3	0.71–1.33	5.4–11.8
Racomitrium lanuginosum (calcareous grassland)	Mean	44.6 (11.0)	30.2 (4.73)	0.70 (0.14)	8.0 (0.98)	1.06 (0.17)	7.8 (1.24)
	Range	29.0–63.1	22.3–38.9	0.50–0.96	6.9–10.1	0.74–1.23	5.9–9.5
Sphagnum cuspidatum	Mean	77.3 (28.2)	51.1 (20.6)	0.62 (0.20)	20.2 (2.48)	1.61 (0.36)	12.9 (2.44)
	Range	9.6 – 119.7	0.2–76.8	0.02–0.82	15.9–25.4	1.18–2.55	7.5–17.2

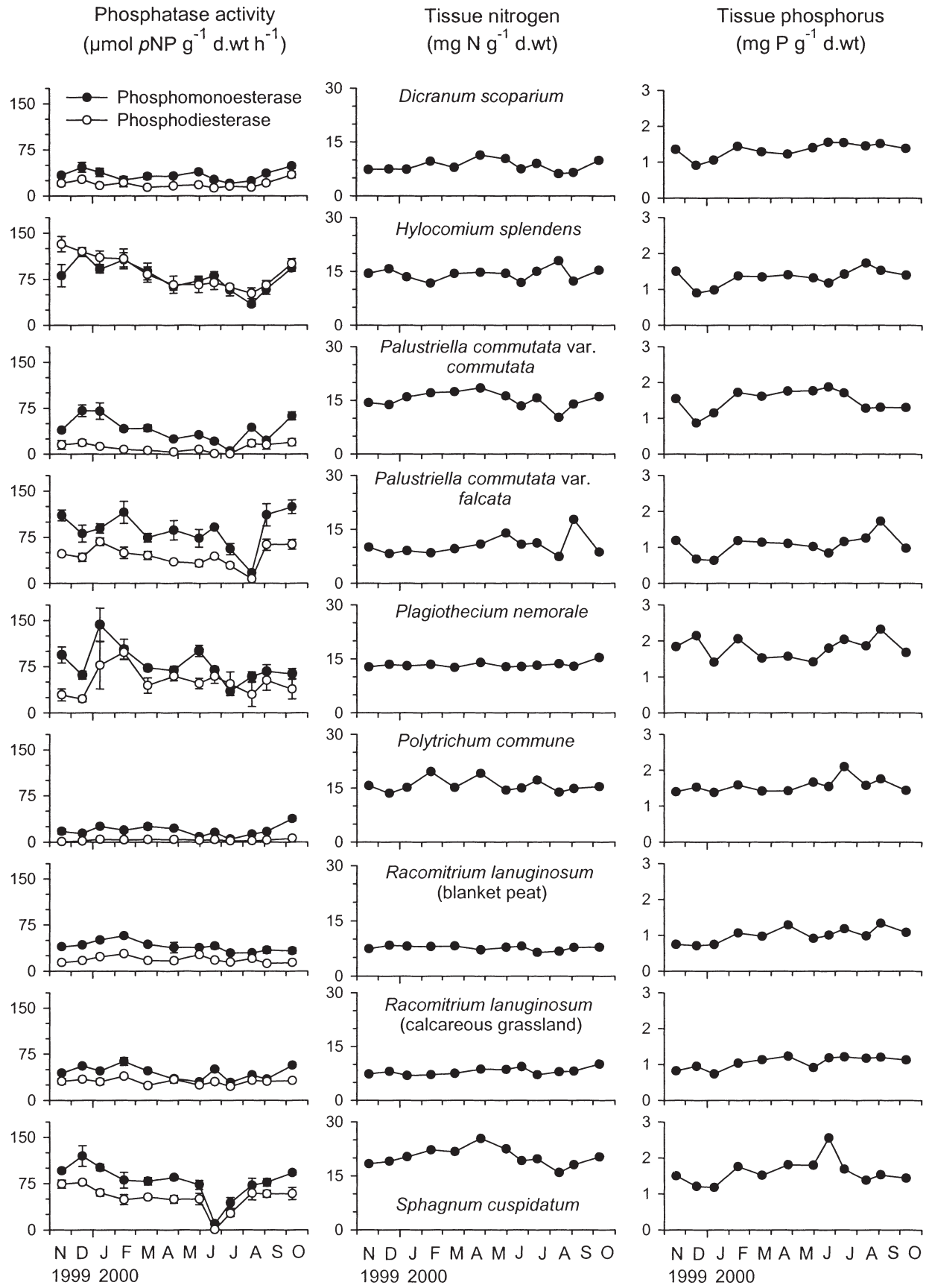

Figure 3. Changes in phosphatase activities (µmol *para*-nitrophenol g^{-1} d.wt h^{-1}) and tissue nutrients (mg g^{-1} d.wt) of 2-cm tips (1-cm capitula for *Sphagnum cuspidatum*) of nine species sampled between November 1999 and October 2000 from Widdybank Fell. Phosphatase values are mean ± SE of four replicate assays, while tissue nutrient concentrations are single analyses of bulked tissue samples.

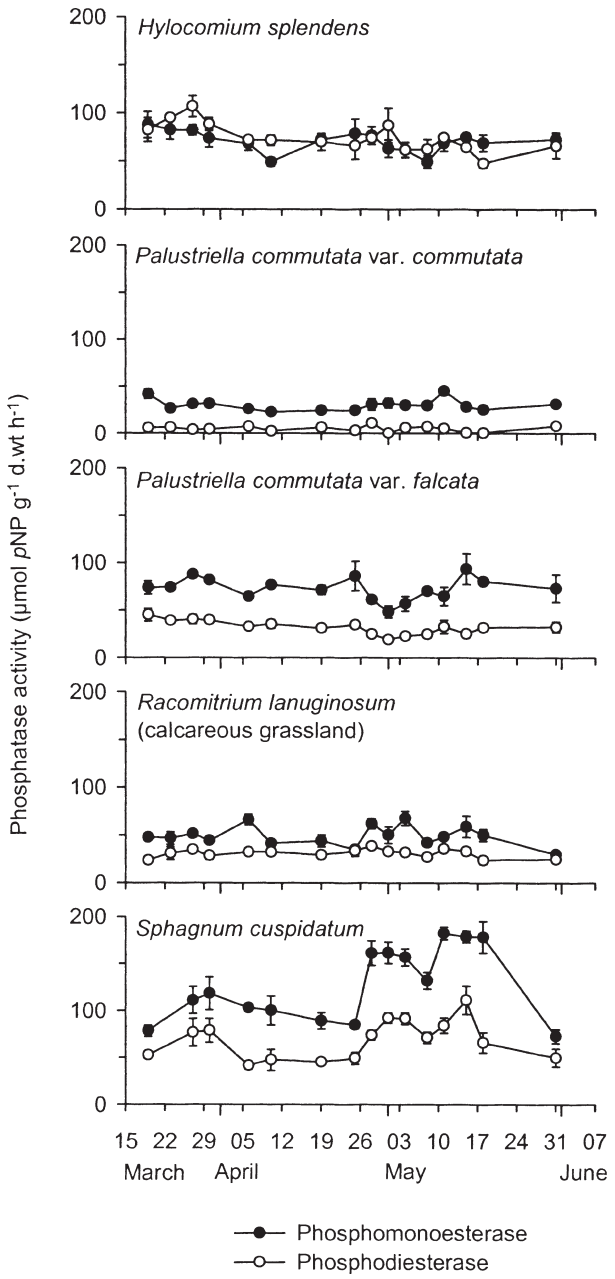

Figure 4. PMEase and PDEase activities (µmol *para*-nitrophenol g^{-1} d.wt h^{-1}) of five species sampled intensively during the spring period (19 March to 31 May 2000) from different environments on Widdybank Fell. Values are mean ± SE of four replicate assays.

respectively, $P < 0.01$). Of the ambient N fractions, PMEase activity of *Dicranum scoparium* was negatively correlated with ammonium ($P < 0.01$), while the PDEase activity of *Hylocomium splendens* was positively correlated with nitrate ($P < 0.05$). Tissue P concentrations in *Palustriella commutata* var. *falcata* were positively correlated with soluble inorganic P concentrations in stream water ($r = 0.77$, $P < 0.01$) and those of *Sphagnum cuspidatum* were positively correlated with soluble organic P in the acidic spring water ($r = 0.63$, $P < 0.05$). Phosphatase activities of mosses during the intensive sampling period were not significantly correlated with ambient nutrient concentrations, except for *Sphagnum cuspidatum*, which was positively correlated with soluble organic P ($r = 0.78$, $P < 0.01$).

Discussion

Phosphatase activities in the shoot apices of the species studied all showed seasonal changes, with the lowest rates of activity during the summer. Seasonal differences were greatest in *Hylocomium splendens* and smallest in *Racomitrium lanuginosum*. Low rates of phosphatase activity coincided with the highest concentrations of P and the lowest N: P ratio in the shoot apices. However, few relationships were evident with ambient P concentrations. This was not unexpected, because P concentrations in rainfall, soil solutions and drainage waters were near or below the limit of detection for much of the year, with P only becoming available at higher levels in occasional pulses during spring and early summer (Turner *et al.*, 2003a). Further, it seems likely that aquatic mosses reflect P concentrations in the surrounding water quite closely, whereas the dependence of terrestrial mosses on soil nutrients probably varies greatly amongst species (Bates, 2000). For example, species such as *Plagiothecium nemorale* growing on a mat of poorly decomposed grass litter might be less sensitive to changes in soil-solution nutrients than species having good contact with the soil, such as *Polytrichum commune*. Indeed, the low rates of surface phosphatase activity *P. commune*, coupled with its primitive vascular system and rudimentary cuticle (Proctor, 2000), suggest that this species utilises organic P from the substratum to a greater extent than other mosses. This is likely to occur through phosphatase activity of rhizoids, although there is no data to indicate whether this might involve 'surface' phosphatase or the secretion of enzymes as occurs in vascular plant roots.

In Upper Teesdale, pulses of organic P occur during the spring and early summer, yet rates of phosphatase activities were lowest during this time. This suggests that phosphatase activities are probably induced in response to the degree of internal nutrient stress rather than as a direct response to the presence of organic phosphate in the ambient environment. Presumably, rates of phosphatase activities in mosses growing in Upper Teesdale remain high enough to make effective use of unpredictable organic P pulses whenever they occur. A rather similar phenomenon was reported for *Sphagnum rubellum* and *Aulacomnium turgidum* in the Alaskan tundra, whereby these species displayed high affinity uptake mechanisms for inorganic phosphate, despite this compound being relatively scarce in the environment (Kielland & Chapin, 1994). This was hypothesized to be an adaptation to a highly variable phosphate supply, which occurred in infrequent pulses during the year. Mosses therefore appear to display considerable physiological buffering with respect to phosphatase activities and tissue nutrient concentrations as an adaptation to a highly variable supply of nutrients in their environment.

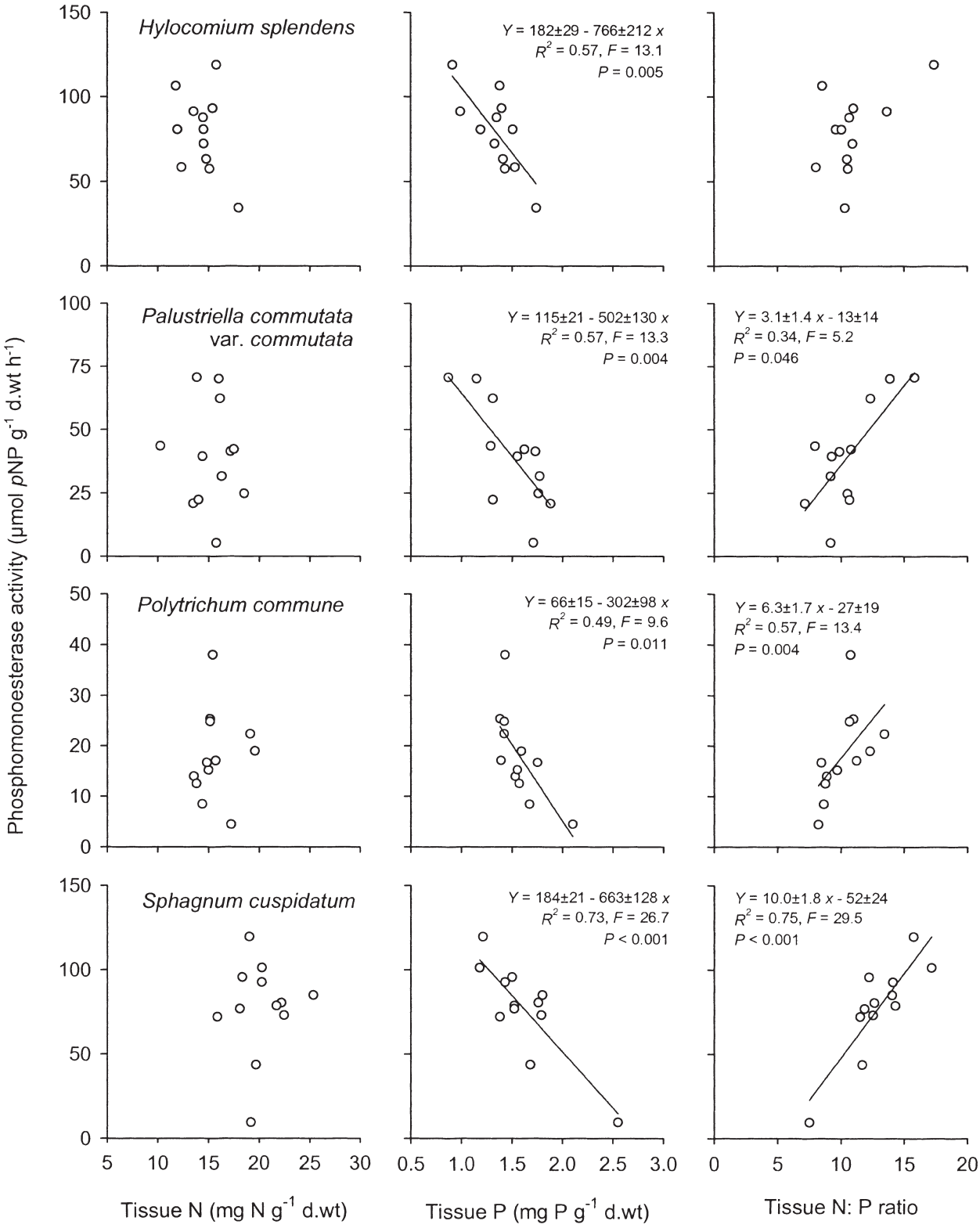

Figure 5. Relationships between PMEase activity (µmol *para*-nitrophenol g^{-1} d.wt h^{-1}) and tissue N, tissue P and tissue N: P ratio of four species sampled from contrasting environments between November 1999 and October 2000 from Widdybank Fell. Significant relationships ($P < 0.05$) are indicated by regression lines.

Table 4. *Correlation coefficients (r values) for relationships between rates of PMEase and PDEase activity and tissue N and P concentrations in 2-cm tips (1-cm capitula for Sphagnum cuspidatum) sampled between November 1999 and October 2000 from Widdybank Fell. Bold type indicates statistically significant correlations at the 5% (*), 1% (**), or 0.1% (***) levels.*

Species and enzyme	Tissue N	Tissue P	N: P ratio	Air temp.
Dicranum scoparium				
PMEase	0.12	**–0.59***	0.48	–0.47
PDEase	0.21	–0.27	0.34	–0.32
Hylocomium splendens				
PMEase	–0.34	**–0.75****	0.51	**–0.81****
PDEase	–0.15	–0.47	0.39	**–0.77****
Palustriella commutata var. *commutata*				
PMEase	–0.06	**–0.76****	**0.76****	**–0.63***
PDEase	–0.45	**–0.83*****	0.51	–0.15
Palustriella commutata var. *falcata*				
PMEase	0.23	0.03	0.15	–0.43
PDEase	0.22	–0.11	0.36	–0.43
Plagiothecium nemorale				
PMEase	–0.26	–0.49	0.42	–0.52
PDEase	–0.09	–0.11	0.10	–0.24
Polytrichum commune				
PMEase	0.10	**–0.70***	**0.58***	–0.42
PDEase	0.20	–0.34	0.40	–0.14
Racomitrium lanuginosum (blanket peat)				
PMEase	**0.65***	–0.37[a]	0.50	**–0.76****
PDEase	0.24	–0.29	0.24	–0.29
Racomitrium lanuginosum (calcareous grassland soil)				
PMEase	0.09	–0.22	0.23	–0.57
PDEase	0.09	–0.07	0.08	–0.41
Sphagnum cuspidatum				
PMEase	0.09	**–0.85*****	**0.86*****	**–0.69***
PDEase	–0.13	**–0.87*****	**0.71****	–0.49

[a] $r = -0.60$, $P = 0.053$, when a single outlying value is removed.

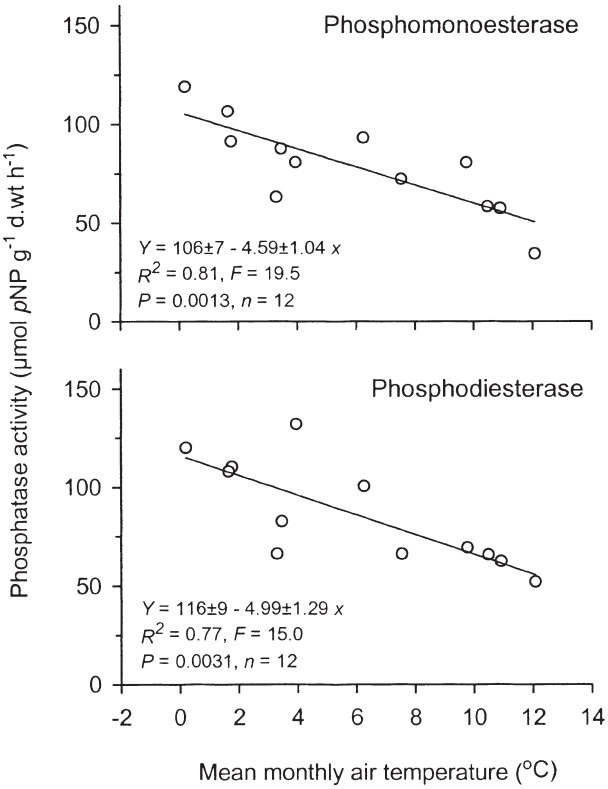

Figure 6. Relationships between mean monthly air temperature (°C) and phosphatase activities (µmol *para*-nitrophenol g^{-1} d.wt h^{-1}) of *Hylocomium splendens* growing on calcareous grassland in Upper Teesdale, northern England, between November 1999 and October 2000.

Based on the assumption that phosphatase activity corresponds to tissue P concentrations, it is likely that reduced rates of phosphatase activities in the summer were probably linked to translocation of P from older parts of the plant to the apical tips during periods of maximum growth. It will therefore be easier to assess the relationship between mosses and their environment when more is known about P storage and recycling within the whole plant. Vascular plants commonly recycle internal nutrients to maintain constant cytoplasmic concentrations of phosphate (Plaxton & Carswell, 1999; Raghothama, 1999), and a recent literature review concluded that internal recycling probably represents a widespread and important mechanism in moss nutrition (Bates, 2000). However, there is relatively little information about nutrient translocation in species other than those having obvious conducting pathways (e.g. *Polytrichum* spp.). Internal recycling of cellular N was detected in a ^{15}N tracer study of *Hylocomium splendens* in northern Sweden (Eckstein & Karlsson, 1999), while *Rhytidiadelphus squarrosus* can grow for a period in the absence of external nutrients, presumably through internal recycling (Wells & Brown, 1996). Specific evidence for P translocation is limited, although P concentrations in younger segments of *Hylocomium splendens* growing in Swedish boreal forest were greater than those in the older segments (Tamm, 1953), and some evidence exists for P movement from older to younger parts of *Sphagnum recurvum* (Rydin & Clymo, 1989).

The seasonal pattern of changes in moss phosphatase activities found during the current study correspond well with changes in the phosphatase activity of the cyanobacterium *Rivularia* reported for the same and other local streams in Upper Teesdale in various years (Livingstone & Whitton, 1984, Whitton *et al.*, 1998). However, it seems likely that changes in the seasonal pattern sometimes occur because of the effects of periodic burning in late autumn and winter for maintenance of heather (*Calluna vulgaris*) moorland. Wind action at the time of burning might spread inorganic nutrients to adjacent areas. Not only would P be derived from plant ash, but also from the burnt soil (Cade-Menun *et al.*, 2000). Much of the soil P at Widdybank Fell, as in many upland regions, occurs in recalcitrant organic forms that are mostly, if not completely, unavailable to plants for much of the time (Turner *et al.*, 2003b), but these are converted to readily available inorganic forms during moorland burning. Mosses are notable early colonizers of burnt ground (Bates, 2000) and it seems possible that young plants might accumulate sufficient P to permit future internal recycling with minimal external inputs.

Moss phosphatase as an indicator of environmental nutrient status

It is suggested that repeat measurements of phosphatase activity in a combination of terrestrial and aquatic mosses would provide a simple and relatively cheap means of indirectly monitoring long-term changes in nutrient status, such as those associated with atmospheric N deposition. Ideally this would be conducted on at least two occasions per year, once in winter and once in summer, although it is likely that more frequent (i.e., monthly) monitoring would be required. Although PMEase and PDEase activities were strongly correlated, little extra effort is required to measure both activities. If it is possible only to sample once a year, then winter measurements are probably preferable because of the higher activity, though care should be taken to avoid an area likely to have been locally influenced by nutrient input from moorland burning. *Hylocomium splendens* showed marked seasonal changes in phosphatase activities, but nevertheless this species is especially useful for monitoring calcareous sites due to the close relationship between its phosphatase activity and tissue nutrients, and its ability to grow in environments with a wide range of nutrient levels (Shaw & Goffinet, 2000). Dried material of some algae shows marked phosphatase activity after many years, so it is also possible that reliable information about past nutrient conditions could be obtained from herbarium mosses.

The lack of relationships between phosphatase activity and ambient nutrient concentrations probably reflects the fact that it requires some days for phosphatase activity to respond to the internal P content of the shoot. This means that phosphatase activity is unsatisfactory here as an indicator of short-term environmental changes. However, phosphatase activities in the aquatic mosses *Rhynchostegium riparioides* and *Fontinalis antipyretica* were closely related to the N: P ratio in ambient river water when sampled from sites with a wide range of nutrient levels (Christmas & Whitton, 1998). A similar broad survey is required for selected terrestrial moss species growing in a range of environments, in order to determine the suitability of the phosphatase activity as an indicator of ambient nutrient status.

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