

**The Role of Acid Phosphatases in the
Phosphorus Nutrition of Arctic Tundra Plants**

by

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(ABSTRACT)

The acid phosphomonoesterase activity associated with two major rooting strategies in arctic tundra plants was examined: that of Eriophorum vaginatum, a dominant plant in tussock tundra ecosystems, with its predominantly non-mycorrhizal root system; and that of ectomycorrhizal roots.

Eriophorum has phosphatase activity which is evenly distributed along its root surface, has a pH optimum at soil pH (3.5-4.0), and continues at substantial rates at 1°C. Inorganic phosphorus inhibits activity only 7 to 19%. In addition, Eriophorum has phosphatase activity associated with all the "below-ground" components of its tussock growth form: dead roots, leaf sheaths, and soil. Plants with higher tissue phosphorus growing in soils with higher available phosphate in general had higher live and dead root, leaf sheath, and soil phosphatase activity in both natural and manipulated sites of higher plant productivity. Yearly and seasonal variation sometimes exceeded differences among treatments, suggesting that enzyme activity would not provide a reliable measure of plant or soil phosphorus levels. Experiments with radiolabeled inositol hexaphosphate showed that Eriophorum is able to hydrolyze and absorb inorganic phosphate from an organic phosphate source. A comparison of enzyme hydrolysis rates with inorganic phosphate assimilation rates indicates that organic phosphate hydrolysis may occur as rapidly as inorganic phosphate absorption. Inorganic phosphate released by root

surface phosphatase activity could satisfy approximately 65% of the annual phosphate demand of Eriophorum.

Phosphatases of two ectomycorrhizal fungi (Cenococcum geophilum and Entoloma sericeum) responded similarly to growth in axenic culture at 2 or 50 micromolar KH_2PO_4 or sodium inositol hexaphosphate: surface V_{max} estimates were significantly greater for 2 micromolar- than for 50 micromolar-grown isolates. The presence of constitutive extracellular soluble phosphatase activity resulted in the appearance of inorganic phosphate in media initially supplied only with organic phosphate. The surface acid phosphatase activity of field-collected ectomycorrhizal roots of arctic Salix and Betula, however, did not respond in a consistent way to differences in soil characteristics. Activity differed more among "color types" or fungal types than among sites of different soil characteristics.

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Prologue

Phosphorus is essential to the growth of plants. It is an integral component of adenosine triphosphate, phospholipids, nucleic acids, sugar phosphates, phosphoproteins, and other compounds. Although it typically constitutes less than one percent of plant dry weight, phosphorus plays an essential role in structure, metabolic regulation, the encoding of genetic information, and energy transfer.

As a nearly universally deficient nutrient (Halstead and Mc Kercher 1975), phosphorus is viewed as one of the most important nutrients in the biogeochemical sedimentary cycles (Smith 1974). Its major abiotic reservoirs are rock and natural phosphate deposits. With no gaseous phase to its cycle, phosphorus movement occurs primarily within ecosystems rather than globally. The movement of phosphorus from rock deposits through soil, plant, animal, ocean and back to sedimented rock deposits, one complete cycle, may take millions of years, much longer than the cycles of carbon, hydrogen, oxygen, and nitrogen (Williams 1978).

In arctic tundra ecosystems, the total amount of phosphorus is not unusually small, but input from weathering of rock material or atmospheric dust is quite low. The major external source of phosphorus is inorganic ions in precipitation (Gersper *et al.* 1980).

Dead soil organic matter serves as the major reservoir for nutrients in arctic tundra ecosystems (Chapin et al. 1980). The slow rate of decomposition in these cold soils has been hypothesized to be the main limitation to phosphorus availability (Chapin and Van Cleve 1978). Mineralization and nutrient turnover rates of 40-80 years have been reported for tussock tundra (Chapin et al. submitted). Indeed, more than 95 percent of the total organic matter in tundra ecosystems is found below ground (Gersper et al. 1980). Approximately 65 percent of all the phosphorus in tundra soils is organic phosphorus, and in soil solution the organic phosphorus concentration may be as much as 21 times higher than the inorganic phosphate concentration (Gersper et al. 1980). In most ecosystems, the largest fraction of total soil phosphate is organic with much occurring as inositol phosphate (Dalal 1977).

The mineralization of soil organic phosphorus is carried out by phosphatases, a class of enzymes which hydrolyze phosphoester bonds. These can be either monoester hydrolases (EC 3.1.3), including phytases, nucleotidases, sugar phosphatases, and glycerophosphatase, or diester hydrolases (EC 3.1.4), including nucleases and phospholipases. Soil phosphatases are produced by soil microorganisms, mycorrhizal fungi, and plant roots. They can exist free in the soil (usually adsorbed to soil particles) or can be associated with living or dead bacteria, fungi, and plant roots.

Smith (1974) proposed that the assimilation of minerals by green plants constitutes one end of the mineral cycle and the release of materials by decomposers the other end. The discovery of phosphatases on plant root and mycorrhizal fungal surfaces suggested a short-cut in that cycle: perhaps plants can be responsible for decomposition (via hydrolysis of organic phosphate compounds) and assimilation, both "ends" of the mineral cycle.

In the 1920's it was reported that red clover and oats could use the organic phosphate compound phytin as a source of phosphate (Whiting and Heck 1926, Heck

and Whiting 1927). Rogers et al. in 1940 observed that corn and tomato could take up phytin and lecithin directly and that these plants hydrolyzed several other organic phosphate compounds before absorbing the phosphate. Radishes were also reported to use phytin and lecithin as phosphorus sources (Szember 1960), and phytin was hydrolyzed around the plant roots in the absence of microbes. "Substantial phytase activity" was then reported for gram, pea, wheat, and barley (Saxena 1964), and in agricultural soils that had been cropped organic phosphate concentrations were lower than in soils that had been incubated with microbes (Sekhon and Black 1969). It was evident that crop plants were making use of organic phosphate, but not much was known about plants' mechanisms for obtaining phosphate from this source.

Cytochemical and histochemical localization experiments provided evidence for cell wall-bound phosphatases, the enzymes that might account for the observations reported above, in potato tubers (Sugawara et al. 1981), sycamore (Crasnier et al. 1980), and pea (Bowen and Bryant 1978). More specifically relevant to the apparent utilization of soil organic phosphate was the localization of cell wall phosphatases on root surfaces. Hall (1969) and Hall and Davie (1971) demonstrated that phosphatases were present on the root surface of corn and other higher plants. Felipe et al. (1979), in an investigation of the role of phosphatases in corn nutrition, localized the enzyme in the gelatinous walls between the root cap and the epidermis and in the newly-forming primary walls at mitosis. Woolhouse (1969), working with ecotypes of Agrostis tenuis, also demonstrated the presence of root surface phosphatases. Ridge and Rovira (1971) and McLachlan (1980) have shown that wheat has root surface phosphatases. Coupe and d'Auzac (1979) demonstrated with four different species that phosphatase activity measured using excised roots was approximately the same as activity measured using purified cell walls.

Research on the role of mycorrhizal fungi in the enhancement of phosphate uptake for higher plants led to similar studies on mycorrhizae. Phosphatase activity was found

to be associated with orchid mycorrhizal fungi (Williamson 1973) and vesicular/arbuscular mycorrhizae (MacDonald and Lewis 1978). Work with ericoid mycorrhizae showed that they were capable of utilizing organic phosphate as a sole source of phosphorus (Pearson and Read 1975), and two mycorrhizal endophytes were shown to utilize phytate salts as phosphate sources (Mitchell and Read 1981). Research focused on ectomycorrhizal fungi corroborated these findings. Beech mycorrhizal phosphatases were shown to hydrolyze a number of organic phosphate compounds (Bartlett and Lewis 1973). Ho and Zak (1979) demonstrated that Douglas fir mycorrhizae had species-specific phosphatase isozymes. Calleja *et al.* (1980) examined three ectomycorrhizal fungus species and found surface acid phosphatases which responded to decreased levels of inorganic phosphate with increased levels of enzyme activity. They also demonstrated that measurements of activity using intact hyphae were equivalent to measurements of activity derived from incubating purified cell walls, as Coupe and d'Auzac (1979) had demonstrated for plant root segments. Antibus *et al.* (1981) described the occurrence and activity of arctic willow ectomycorrhizal phosphatases, and Williamson and Alexander (1975) found phosphatase activity restricted to the fungal mantle in beech mycorrhizal roots. Alexander and Hardy (1981) reported an inverse correlation between extractable soil inorganic phosphate and Sitka spruce mycorrhizal root phosphatase activity, but Dighton (1983) surveyed eight ectomycorrhizal isolates and concluded that their phosphatase production was independent of the concentration of inorganic or organic phosphate in which they were grown.

The object of my dissertation research was to investigate the contribution of organic phosphate to plant phosphorus nutrition. Specifically, I wanted to examine the role of acid phosphomonoesterases in the phosphorus nutrition of arctic plants. In tundra ecosystems, where the smallest proportion of the system's nutrient capital is

stored in live biomass, dead soil organic matter serves the function of the major nutrient reservoir (Chapin et al. 1980). In such a system, the contribution that roots themselves make to mineralization of organic matter might be especially important.

The plant most intensively studied, Eriophorum vaginatum, was chosen for several reasons. First, it is a dominant species in vast areas of arctic tundra, indicating that it is adapted to the set of environmental characteristics associated with arctic ecosystems, including the low concentrations of available inorganic phosphate and relatively high levels of soil organic phosphate. Second, its annually-produced root system of unbranched, hairless, and predominantly non-mycorrhizal roots makes estimates of phosphatase activity based on root surface area relatively reliable. Third, a number of studies on other aspects of E. vaginatum have been published, providing extensive background information. Fourth, on-going research projects at the Toolik Lake field site in northern Alaska provided me with opportunities to collaborate with other researchers.

The other part of my dissertation research provides insight into the phosphatase activity associated with another major rooting strategy in arctic tundra ecosystems, the development of ectomycorrhizal roots. These studies were done using field-collected Salix and Betula species (dwarf shrubs with ectomycorrhizal roots) and with isolates of ectomycorrhizal fungi for controlled laboratory experiments.

The objectives of my dissertation research were as follows:

- 1) to characterize the root surface phosphatases of Eriophorum vaginatum with respect to temperature, pH, substrate concentration, and inorganic phosphate inhibition;
- 2) to investigate the effects of fertilization on the root phosphatases of E. vaginatum;

- 3) to examine the phosphatases associated with E. vaginatum and ectomycorrhizal plants in "natural experiment" field sites where plant productivity, community composition, soil nutrient levels and other parameters differ;
- 4) to determine the response of ectomycorrhizal fungal phosphatases to different concentrations of inorganic and organic phosphorus under controlled conditions;
- 5) to test, using a ^{32}P -labelled organic phosphate compound, the hypothesis that phosphate from organic phosphate compounds is absorbed by the plant after hydrolysis by root surface phosphatases.

Each chapter of the dissertation was written in a format for submission to a peer-reviewed journal. The entire manuscript, including methods, references, etc., comprises each chapter. Information specific to the introduction of each chapter is given within the chapter, as are specific objectives or hypotheses. Figures appear at the end of the chapter for which they provide illustration. A short summary section restates the major conclusions of all the chapters, and references for the Prologue are listed following the Summary.

Chapter 1. The root surface phosphatases of *Eriophorum vaginatum*: effects of temperature, pH, substrate concentration and inorganic phosphorus

Introduction

Eriophorum vaginatum L. subsp. *spissum* (Fern.) Hult. is a dominant plant species in arctic tundra ecosystems, where phosphorus is frequently a limiting nutrient^{12,31}. Most of the phosphorus present in arctic soils is in organic forms^{1,10}. Mineralization of this organic phosphorus is thought to be controlled primarily by microbial respiration¹⁰, and the slow rate of that process in cold arctic soils keeps available inorganic phosphate levels low.

The existence on plant root surfaces of phosphatases capable of hydrolyzing organic phosphorus compounds in other species^{6,15,16,18,20} suggests that soil organic phosphorus may be more directly utilized by plants than previously believed. Work with

Spirodela³, wheat²⁵, tomato⁵, and clover¹³ has shown that surface phosphatase activity increases in phosphorus-deficient plants. This enzyme is also sensitive to repression and inhibition by inorganic phosphorus²⁹.

E. vaginatum is a tussock-forming sedge that produces new roots annually^{7,35}. Its root system is dense enough to exploit most of the tussock-soil volume¹¹; an average-sized tussock (23-cm diameter) has a total live root surface area of up to 550 cm² (pers. comm., J. Kummerow 1986). Root phosphatase activity associated with this dense root system could have a significant impact on the mineralization of soil organic phosphorus and on the phosphorus nutrition of the plant. If E. vaginatum tussocks have root surface phosphatase activity that continues to function at the low temperatures common in arctic soils, significant organic phosphorus mineralization may occur within the tussock independent of microbial activity. These root surface phosphatases may make a substantial contribution to the phosphorus nutrition of E. vaginatum.

The objective of this study was to investigate the root surface acid phosphomonoesterases of Eriophorum vaginatum. We hypothesized that E. vaginatum would have active root surface phosphatases, that these enzymes would have optimal activity at pH values near soil pH, that enzyme activity would continue at significant levels at low temperatures, and that the enzyme would be sensitive to end-product inhibition by inorganic phosphate.

Methods

Field research was conducted during the summers of 1985 and 1986 at Toolik Lake (68° 38' N, 149° 34' W, 760 m elevation) located in the northern foothills of the Brooks Range in Alaska. Samples were collected from study sites in tundra dominated by tussock-forming Eriophorum vaginatum.

General Assay Procedure

Tussocks of E. vaginatum were collected and kept dark, cool, and moist until needed (not longer than 2 days). First-year root tips 1-2 cm in length were excised into 0.5 mM CaCl₂¹⁴. Surface acid phosphomonoesterase activity was measured using p-nitrophenyl phosphate (pNPP) as substrate³⁶. Incubation of roots for varying lengths of time indicated that activity measurements were linear with respect to time for at least 150 min. Further incubations were done for 1 h. The basic procedure follows with details of various assays given below. Root tips were transferred from the CaCl₂ solution to a 2.0-ml buffered pNPP solution²² and incubated at pH 4.0 and 10-12°C. After incubation, 0.5 ml from each reaction vial was added to 0.5 ml 0.5 N NaOH and mixed. Optical density at 410 nm was measured on a Bausch & Lomb Spectrophotometer 88. Root lengths and diameters were measured using a dissecting microscope. Approximate surface areas were calculated using the formula for a cylinder without ends. Enzyme activity was expressed as μmoles p-nitrophenol produced mm⁻² root surface area h⁻¹.

Temperature and pH effects

To determine the effect of assay pH on phosphatase activity, root tips were incubated in buffered pNPP adjusted to pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, or 7.0. Temperature effects were investigated by pre-equilibrating root tips at the incubation temperature for 15 min and then adding substrate for incubation at 1, 6, 8, 11, 15, 21, 25, 41, 45, or 48°C. Segments 1-2 cm in length (up to 16 cm from the root tip) were excised from 3 roots and assayed to determine the distribution of phosphatase activity along the root. Substrate concentration for the above three experiments was 50 mM pNPP.

Determination of kinetic parameters

Final substrate concentrations of .05, .1, .25, .5, .75, 1, 5, 10, 25, 50, or 100 mM pNPP were used to determine enzyme kinetic parameters. V_{max} , the maximum velocity of enzyme-catalyzed hydrolysis, and K_m , the substrate concentration at which velocity is half maximal, were calculated from Eadie-Hofstee transformations of the activity data at the above concentrations.

Effect of inorganic phosphate

The effect of inorganic phosphate on root surface phosphatase activity was determined from three assays. In one, roots were incubated in 100 or 500 μ M KH_2PO_4 and equivalent concentrations of pNPP at pH 3, 4, 5, 6, or 7. In the second,

roots were incubated at 100 mM pNPP with or without 100 mM KH_2PO_4 at pH 4.0 or pH 5.5. The third, designed to assess differences in kinetic parameters, used buffer amended to 1, 5, 10, 25, 50, or 100 mM KH_2PO_4 . K_m and V_{max} values were determined from kinetic assays (using 1, 5, 10, 25, 50, and 100 mM pNPP) done at each inorganic phosphate concentration.

Soil pH measurement

Two subsamples of soil for soil pH measurement were taken from each of the 3 tussocks used for the pH response assays. Approximately 10 g tussock soil was placed with 40 ml 0.01 M CaCl_2 in a 125-ml Nalgene bottle and shaken for 1 h. These samples were allowed to settle, and the pH of the resulting clear solution was measured⁴.

Analysis of data

For most of the assays described, 3 E. vaginatum tussocks were collected. Root tips from each tussock were excised into separate containers. At each pH, temperature, pNPP concentration, or inorganic phosphate concentration, 3 vials of root tips were incubated, 1 vial of roots from each of the 3 tussocks. Three to six root tips per vial were used. Within any set of assays, the same number of root tips per vial was used. The assay determining distribution of enzyme activity along the root was done with 3 whole roots from a single tussock. Analysis of variance and Duncan's multiple comparison tests were used to test for differences in the data. Significance values and results of the multiple comparison tests are given in figures or described in the text.

Results and Discussion

No significant effect of distance from root tip was detected for the root surface phosphatase activity of Eriophorum vaginatum (Figure 1). The uniform distribution of phosphatase activity suggests that the total contribution of these enzymes to organic phosphorus hydrolysis within the tussock could be considerable. Tussock soil is highly organic, comprised primarily of E. vaginatum roots from previous seasons in various stages of decomposition. This decaying root material is most likely the principal source of organic phosphorus for hydrolysis and uptake by the current season's roots. Calculations based on the density of live roots have led to the estimate that all available nutrients in the tussock-soil volume fall within the zone of exploitation by current season E. vaginatum roots by the end of the growing season¹¹. Presumably the organic phosphorus in soil solution would also fall within that zone of exploitation. The activity of root surface phosphatases thus adds to the ability of E. vaginatum to maintain the efficient control of nutrient cycling within the tussock hypothesized by other researchers¹¹.

Effect of incubation media pH

Optimal phosphatase activity occurred at pH 3.5-4.0 (Figure 2). Soil pH was $3.69 \pm .23$ (mean \pm sd). The optimum measured for E. vaginatum is lower than that reported for other plants: between 5.0 and 6.0 for wheat²⁴ and clover¹³ root surface phosphatases and between 4.5 and 5.0 for Aegilops roots from soil of pH 7.4-7.6³². However, the optimum for E. vaginatum roots occurs within the range of soil solution

pH values for tussock soils measured by us and reported by others¹¹. Although peak enzyme activity occurred at pH 3.5-4.0, phosphatase activities measured between pH 3.0 and 5.0 were not significantly different. If roots encounter slightly different pH environments as they grow toward the mineral soil layer and permafrost, root surface enzymes with the ability to function over a corresponding pH range could be advantageous.

Effect of incubation temperature

The natural logarithm of phosphatase activities measured for root tips assayed at temperatures ranging from 1 to 48°C was plotted against absolute temperature in an Arrhenius plot to determine apparent energy of activation (E_{act}). The E_{act} value calculated from this regression was approximately 14.0 kcal mol⁻¹ ($R^2 = 0.93$), and the average Q_{10} value was 2.2. Activity measured at 1°C was 51% of that measured at 8°C and 25% of that measured at 11°C (Figure 3), which are common summer temperatures in tussock soil^{11,35}. Enzyme activity showed no statistically significant differences across a temperature range from 1 to 15°C. This reflects variability in the data, but it also indicates that E. vaginatum root surface phosphatase activity is relatively temperature independent at low temperatures. Sustained activity at low temperatures could make a significant contribution to organic phosphorus hydrolysis very early in the spring or late in the fall. In one tundra ecosystem, nearly half the annual phosphorus return to the soil was estimated to occur during the period of snowmelt, and it has been suggested that a strong selective pressure must exist for plants to be physiologically active at that time of the season¹⁰. Our data on E. vaginatum root surface phosphatases provide evidence for enzymatic activity at temperatures just above freezing; we were unable to incubate

roots at lower temperatures. However, tundra soil phosphatase activity has been measured at -25°C ²¹; it is probable that root surface phosphatases also can function at temperatures considerably below freezing. *E. vaginatum* root growth closely follows permafrost recession; root tips are found within 1 cm of solidly-frozen ground³⁵. Thus phosphatase activity at low temperatures, coupled with low temperature-adapted phosphate absorption⁷, could continue to be advantageous throughout the growing season.

Measurement of energy of activation and Q_{10} may provide information about temperature acclimation. A lower energy of activation for the malate dehydrogenase of beach pea from colder maritime habitats than from warmer continental sites was interpreted as a mechanism to permit the reaction to proceed more rapidly at all temperatures³³. The apparent E_{act} determined for *E. vaginatum* root surface phosphatases in this study was closer to the malate dehydrogenase E_{act} values for plants from more temperate climates³³; apparently a lower E_{act} is not a mechanism for cold acclimation in *E. vaginatum* root surface phosphatases. The Q_{10} calculated from our data (approximately 2.2) is higher than that discussed for root elongation, root growth, photosynthesis, respiration, and nutrient absorption for arctic and alpine plants⁸ but is close to the Q_{10} value of 2 expected for most enzyme-catalyzed reactions¹⁷.

Determination of kinetic parameters

Kinetic assay results indicate that saturating levels of substrate are not reached until 20-50 mM pNPP. The estimates for apparent K_m and V_{max} (based on pNPP concentrations ranging from .05 to 100 mM) were 9.23 mM and 1.61×10^{-3} $\mu\text{moles mm}^{-2} \text{ h}^{-1}$ respectively. The saturation level determined is higher than average tundra

soil solution organic phosphate concentrations by at least three orders of magnitude¹. Organic phosphate compounds may appear in soil solution in pulses, as does inorganic phosphate¹⁰; a K_m larger than substrate concentrations usually available in the soil solution may allow for faster hydrolysis of these seasonal pulses of substrate²⁸. Apparent K_m values for E. vaginatum are similar in magnitude to that determined for barley root β -glycerophosphatases¹⁹ but higher by an order of magnitude than those determined for the surface phosphatases of clover roots¹³, Spirodela²⁹, and baker's yeast³⁰.

Apparent V_{max} values were similar to activities reported for Aegilops peregrina³² but lower than the phosphatase activity of clover by 50 times¹³ and of Agrostis by 2500 times³⁶. Roots assayed for this study lacked root hairs, and differences in root surface area measurements for this species and those with root hairs, in addition to differences in assay conditions, may account for some of the differences in reported enzyme activities.

Apparent K_m and V_{max} values determined using only the six higher concentrations of pNPP (1 to 100 mM) were very close to kinetic parameters determined using data from all concentrations (.05 to 100 mM). Although concentrations of 1 to 100 mM are considerably higher than soil solution substrate concentrations, use of the lower, more "realistic" concentrations does not allow determination of apparent K_m and V_{max} .

Effect of inorganic phosphorus

Except for a slight decrease in activity (less than 20%) in the presence of 100 mM KH_2PO_4 , E. vaginatum root surface phosphatases were not inhibited by inorganic

phosphate (Figures 4 and 5). This differs from reported results for the cell wall-associated acid phosphatases of baker's yeast³⁰, tobacco cells³⁴, Spirodela²⁹, and barley roots¹⁹. Only at concentrations of 100 mM, which is far higher than Eriophorum would ever experience in soil solution, did inorganic phosphate significantly inhibit phosphatase activity; even then activity was inhibited only 7% (at pH 4.0) and 19% (at pH 5.5) (Figure 4). A similar increase in inhibition with increasing pH has been reported for the acid phosphatases of Pisum sativum seed homogenates²⁶.

The slight inhibition effect detected at 100 mM phosphate was not enough to cause significant differences among the kinetic parameters, K_m and V_{max} , determined from assays done in the presence of KH_2PO_4 ranging from 1 to 100 mM. Orthophosphate in the reaction solution had no statistically significant inhibitory effect on root surface phosphatase activity at any concentration of substrate or inorganic phosphate, and there were no statistically significant differences among kinetic parameters.

Enzyme activity was not decreased at all in the presence of 100 or 500 μM KH_2PO_4 in equivalent concentrations of pNPP over a range of pH values (Figure 5). Changes in pH or pNPP concentration had significant effects on activity, but the presence of inorganic phosphate did not. The lowest concentration of inorganic phosphate tested was higher by at least two orders of magnitude than tundra soil solution inorganic phosphate concentrations, yet no inhibitory effect was detected. Organic phosphorus concentrations in tundra soil solution are reported to be 23 times higher than inorganic phosphate concentrations¹. Because of the ratio of organic to inorganic phosphate in tundra soil solution¹ and the phosphorus depletion zone typically found around roots²³, I predict that naturally-occurring concentrations of inorganic phosphate high enough to have any inhibitory effect on root surface phosphatases do not occur. A decreased ability to respond to phosphate has been reported for another species: ecotypes of Aegilops peregrina from phosphorus-poor soils

are less able to regulate their root surface phosphatases than are ecotypes from phosphorus-rich soils³². Although that finding was part of an investigation of regulation at a different level (activation of phosphatases in response to phosphate deficiency), perhaps the same is true for regulation by end-product inhibition. Certainly the regulatory function of intracellular phosphatases so critical to maintaining metabolite pools would not be as important for external phosphatases.

Comparison of enzyme activity with inorganic phosphate assimilation

To compare root surface phosphatase activity data with inorganic phosphate absorption data for E. vaginatum, I used activity measurements made at a substrate concentration equivalent to the inorganic phosphate concentration used by others in a study of inorganic phosphate uptake (50 μM)⁹. The results of this comparison indicate that E. vaginatum roots can hydrolyze organic phosphate substrate at approximately one third the rate at which they take up inorganic phosphate (Table 1). In tundra soils, limitation due to diffusion to the root surface²⁷ should present less of a problem for organic phosphate hydrolysis than for inorganic phosphate uptake.

Chapin and Tryon⁹ compared the annual phosphorus demand of E. vaginatum with the annual phosphorus supply estimated from root uptake rates. Using their equations and substituting root surface phosphatase and soil solution organic phosphate data, I estimate that hydrolysis of organic phosphate compounds could provide approximately 65% of the annual phosphorus demand of E. vaginatum. Although I do not know what soil organic phosphorus compounds might be hydrolyzed by the enzyme measured using pNPP as substrate, other researchers have found activity toward pNPP to be associated with activity toward phenyl phosphate, adenosine diphosphate,

nucleoside triphosphates, inorganic pyrophosphate³⁴, glucose-6-phosphate, β -glycerophosphate, and inositol hexa- and triphosphates².

Conclusion

The root surface phosphatases of E. vaginatum appear to be well adapted to the arctic tundra soil environment. Optimal activity occurs at the soil pH, substantial activity occurs at temperatures near freezing, and the enzyme appears to be insensitive to inhibition by inorganic phosphate. The calculations detailed above indicate that the contribution this enzyme makes to soil organic phosphorus hydrolysis and phosphorus cycling in the E. vaginatum tussock growth form could be substantial. Whether the inorganic phosphate released by this hydrolysis is subsequently absorbed and used by the plant is the subject of further study.

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Table 1. Phosphatase activity versus phosphate absorption rates.

A comparison of ^{32}P -inorganic phosphate (Pi) uptake rates for *E. vaginatum*⁹ with root surface phosphatase activity measured with an equivalent concentration of pNPP. To calculate values that could be compared, I changed dry weight to root surface area (33.48 mm² to 1 mg dw).

Substrate concentration: 50 $\mu\text{moles L}^{-1}$.

Pi uptake rate: 5.97×10^{-5} $\mu\text{moles mm}^{-2}$
 h^{-1} (from Figure 3, Chapin and Tryon 1982).

Phosphatase activity: 2.14×10^{-5} $\mu\text{moles mm}^{-2}$
 h^{-1} (mean of values from five sites).

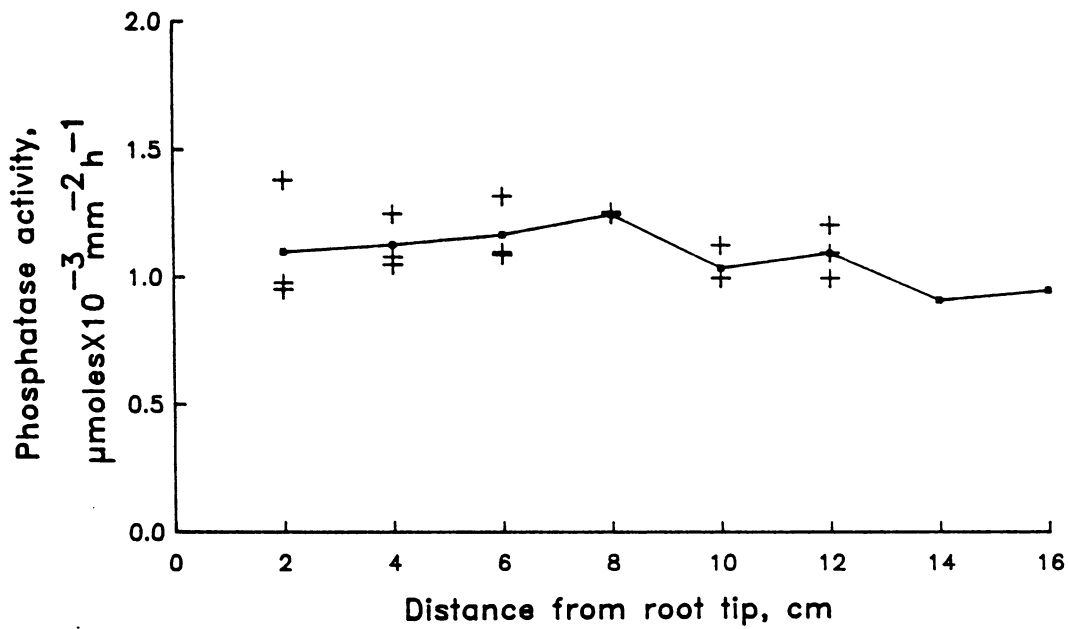


Figure 1. Distribution of phosphatase activity along root: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{mm}^{-2} \text{h}^{-1}$) on 1-2 cm root segments (mean 1.88 cm) from root tip to approximately 16 cm from root tip; no significant effect of distance from tip was detected ($p = 0.35$) (+ = individual data point; line goes through mean).

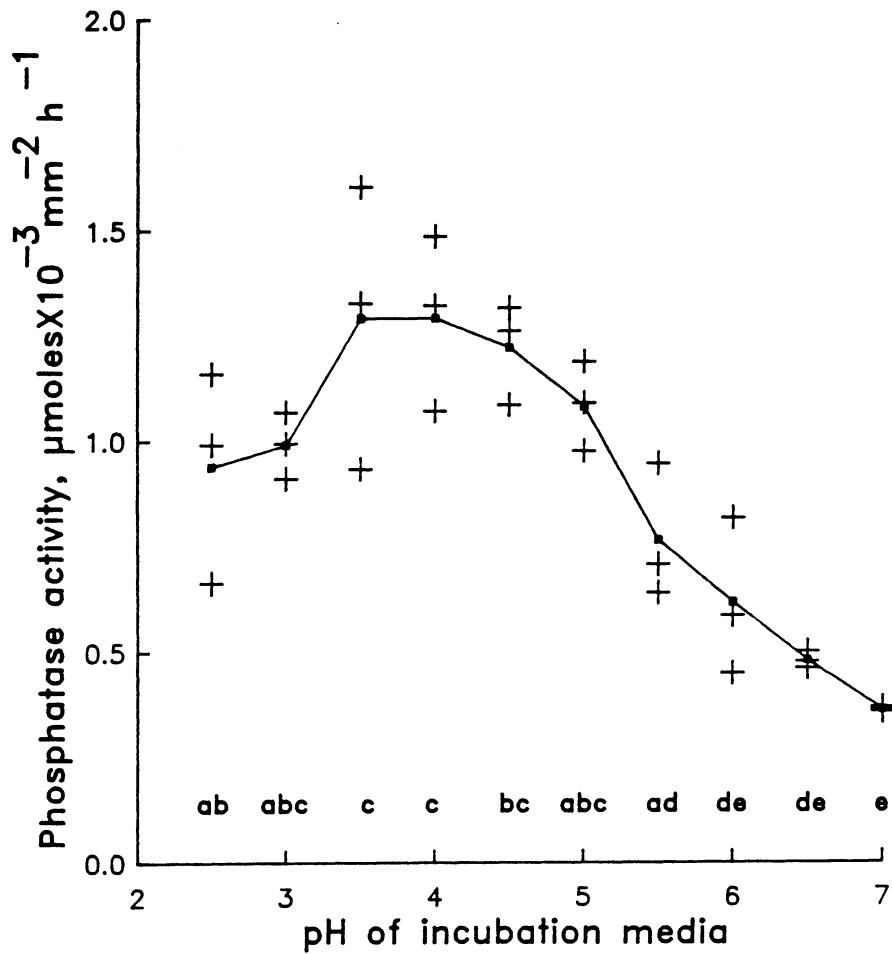


Figure 2. Effect of pH on root phosphatase activity: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{ mm}^{-2} \text{ h}^{-1}$) on root tips incubated in media differing in pH (+ = individual data point; line goes through mean; pH values marked by the same letter indicate enzyme activity not significantly different at $p = 0.05$).

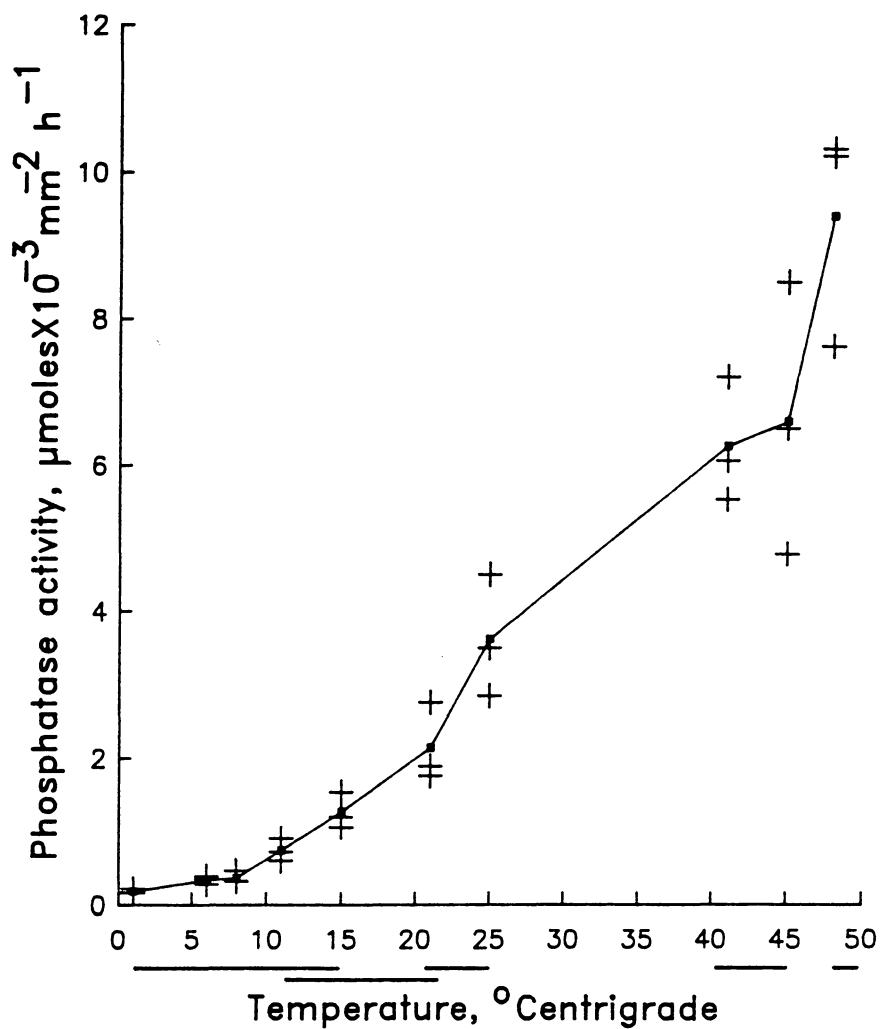


Figure 3. Effect of temperature on phosphatase activity: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{mm}^{-2} \text{h}^{-1}$) on root tips incubated at different temperatures; temperature values connected by horizontal lines under x-axis are not significantly different at $p = 0.05$ (+ = individual data point; line goes through mean).

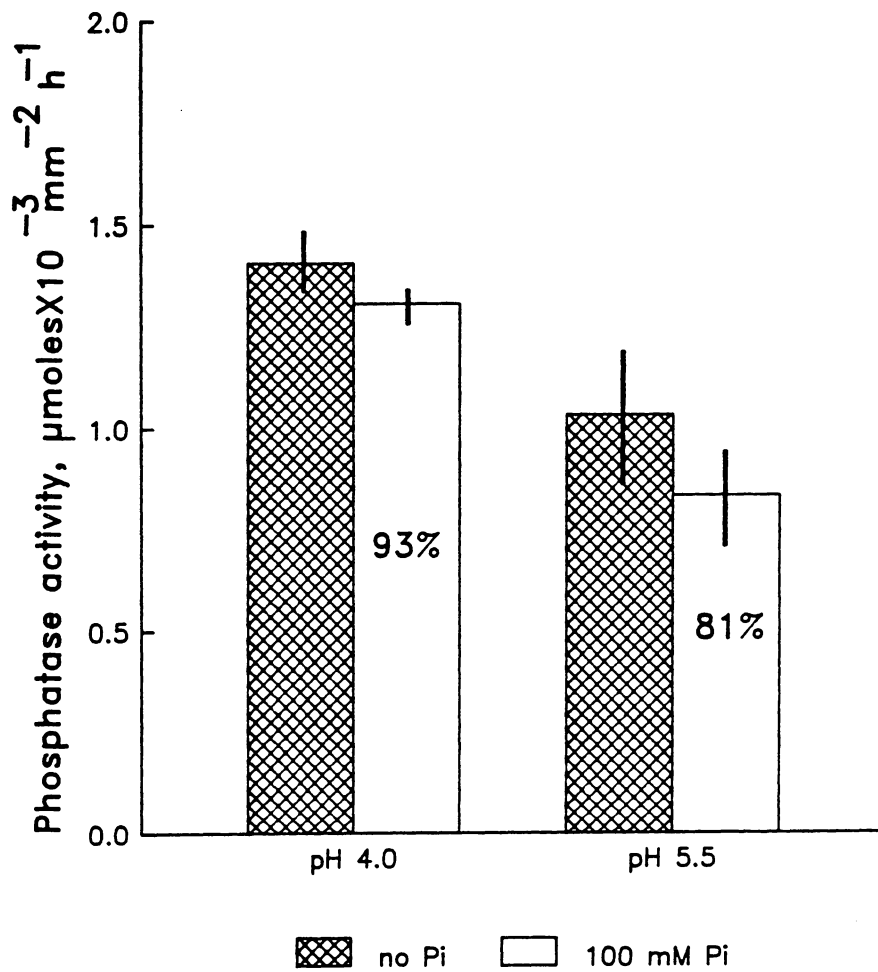


Figure 4. Inhibition of phosphatase activity by high phosphate levels: The effect of 100 mM KH_2PO_4 on root surface phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{ mm}^{-2} \text{ h}^{-1}$) for roots incubated at pH 4.0 or 5.5 in 100 mM pNPP (effect of inorganic phosphate significant at $p = 0.015$; effect of pH significant at $p = 0.000$).

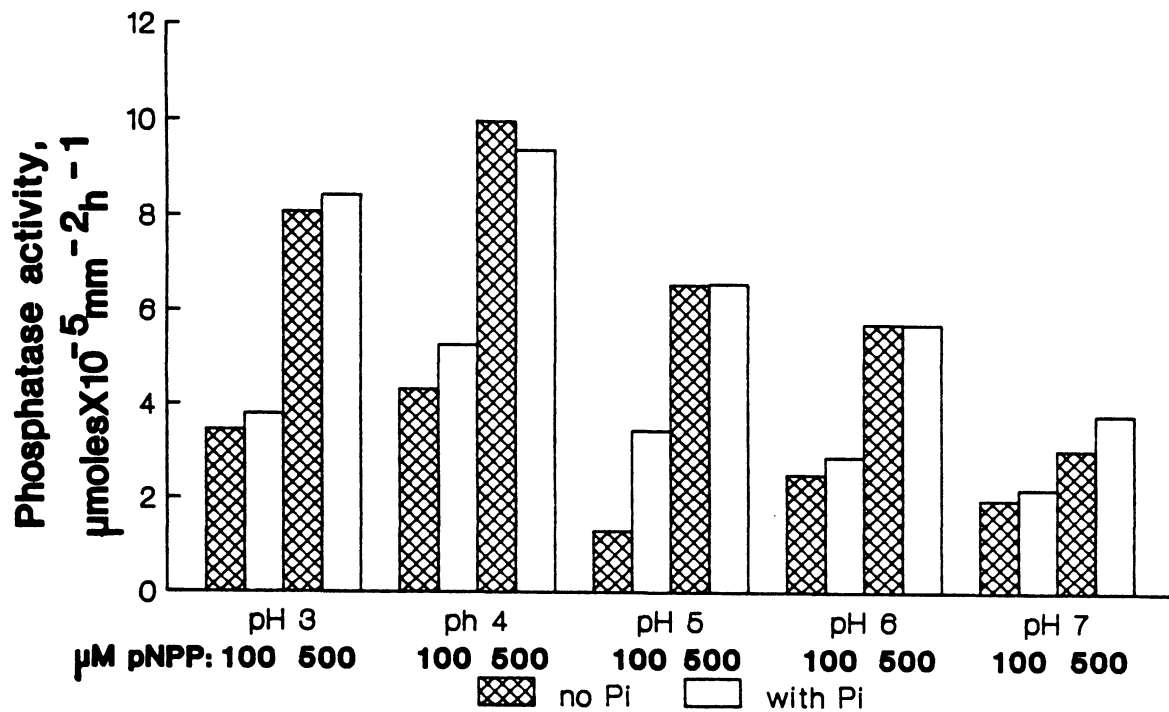


Figure 5. Inhibition of phosphatase activity by low phosphate levels: The effect of 100 and 500 μM KH_2PO_4 on root surface phosphatase activity ($\mu\text{moles} \times 10^{-5} \text{mm}^{-2} \text{h}^{-1}$) for roots incubated in equivalent concentrations of pNPP (effect of inorganic phosphate not significant, $p = 0.550$; effect of pNPP concentration significant at $p = 0.000$; effect of pH significant at $p = 0.000$).

Chapter 2. The Acid Phosphatase Activity of Eriophorum in Alaskan Tundra: Response to Vehicle Perturbation and Natural Groundwater Movement

Introduction

The effects of increased soil moisture and groundwater movement on plant productivity, community composition, and nutrient cycling in the arctic have been studied by a number of researchers. Chapin *et al.* (1979) noted that the distribution and robustness of Eriophorum vaginatum L. subsp. spissum (Fern.) Hult., a dominant species in tundra ecosystems, was positively related to groundwater flow. Wein and Bliss (1974) found production of E. vaginatum seed stalks 8 times as high along wet tracks created by vehicle passage and 44 times as high along drainage ditches. In a study of the effect of vehicle passage on tundra soils, it was found that plant productivity was higher at all track sites than at adjacent control sites (Challinor and Gersper 1975); this

increase was associated with increased bulk density, temperature, and depth of thaw in track soils (Haag and Bliss 1974, Gersper and Challinor 1975). Similar measurements have been made in areas of natural subsurface water movement ("water tracks") in Alaskan tundra. As was found at vehicle track sites, total production, plant nitrogen and phosphorus concentrations, and rates of nutrient cycling were found to be consistently higher for E. vaginatum tussocks in water tracks than in adjacent nontrack areas (Chapin et al. submitted).

Chapin and Shaver (1981) demonstrated that temperature effects on nutrient absorption, root growth, and organic matter mineralization could not account for the increased productivity and standing crop of nutrients in vehicle track plants. It is unlikely that increased moisture itself would cause such increases; water stress is rare in most tundra plants (Oberbauer and Miller 1979). Although low nutrient availability limits the growth of many tundra species (Chapin and Shaver 1985), the total amounts of nitrogen and phosphorus in the ecosystem are not unusually low. The limited availability of nitrogen and phosphorus is due to the slow rate of nutrient cycling in tundra ecosystems; the bulk of these nutrients is bound in slowly-decomposing soil organic matter (Chapin and Van Cleve 1978). Nutrient cycling occurs more quickly within tussocks of E. vaginatum than in adjacent non-tussock soil (Chapin et al. 1979), and tussock soils within naturally-occurring water tracks have much faster rates of decomposition and phosphorus cycling than in adjacent nontracks (Chapin et al. submitted).

In this paper we examine one class of enzymes involved in this decomposition and phosphorus cycling process, acid phosphomonoesterases. Phosphatase activity is associated with the mineralization of organic phosphorus compounds through the hydrolysis of phosphate ester bonds. Chapin et al. (submitted) concluded that the large differences in productivity and nutrient cycling observed between water track and

adjacent tundra were related primarily to water flow. One of the major benefits of flowing water in a nutrient-limited ecosystem is the reduced diffusional limitation to nutrient absorption. Likewise, limitation of organic phosphate hydrolysis by the rate of diffusion of substrate to the enzyme (see Ladd and Butler 1975) would be overcome by flowing water.

In this study, we looked at differences in the phosphatase activity of E. vaginatum with respect to track and adjacent tundra for both vehicle and water track sites. E. vaginatum is known to have root surface phosphatase activity (see Chapter 1); one objective of this study was to determine the contribution to phosphorus cycling made by the phosphatase activity of the dead and decaying roots, leaf sheaths, and soil which make up nearly all of the tussock-soil volume. In addition, we hypothesized that differences in phosphatase activity might account for some of the differences in phosphorus turnover, decomposition and thus productivity in both naturally-occurring water tracks and tracks caused by vehicle perturbation.

Methods

Study sites

Both the vehicle track site and the water track site are north of the Brooks Range in Alaska. The vehicle track studied was formed approximately 15 years ago at the base of Slope Mountain (68° 43'N, 149° 00'W, 655 m elevation). The track runs up a 5° north-facing slope, first through wet tundra and then into mesic tundra (Chapin and

Shaver 1981). The wet tundra site, referred to in this paper as wet track (and adjacent undisturbed tundra as wet control), is dominated by Eriophorum angustifolium and Carex aquatilis. The mesic site, referred to as mesic track and mesic control, is dominated by E. vaginatum. The natural water track system chosen for study is on a north-facing 5° degree slope approximately 7 miles north of Toolik Lake along the Dalton Highway (68° 45'N, 149° 34'W, 750 m elevation) and is dominated by E. vaginatum (Chapin et al. submitted). The field work reported in this paper was done during the summers of 1982-1986.

Preparation of samples

Six E. vaginatum tussocks, 3 from water tracks and 3 from adjacent nontracks, were collected in August 1982, shipped on ice, and stored frozen. E. vaginatum produces a new root system annually, and the dead roots from previous years make up much of the below-ground tussock volume (Wein 1973). For this study, thawed tussocks were picked apart and sorted into current year roots, dead roots (past season's and older), leaf sheaths, soil, and "other." The "other" material included E. vaginatum stems and stem bases as well as roots and stems of other plant species which had been growing on the tussock. Each component was then weighed, frozen in liquid nitrogen, and ground to powder using a flour mill. Water content and dry weights were determined for each component. Current season root, dead root, leaf sheath, and soil samples were stored frozen.

For the root surface activity assays, tussocks of E. vaginatum were collected from the water track and vehicle track sites and stored, moist and cool, until needed (not longer than two days). At the vehicle track site, clumps of E. angustifolium also were

collected. Current-year root tips approximately 1-2 cm in length were excised into 0.5 mM CaCl_2 (Epstein *et al.* 1963), where they were held until assayed (not longer than 4 hours). Vehicle track root samples were collected and assayed in 1982, 1983, 1985, and 1986; water track root samples were collected and assayed each growing season during the period 1982-1986.

Measurement of phosphatase activity

In all assays, p-nitrophenyl phosphate (pNPP) was used as substrate. For the homogenates, 2.0-mL aliquots from a suspension of the sample and pH 5.0 Modified Universal Buffer (MUB) (Ho and Zak 1979) were pipeted into 10-mL Nalgene reaction vials. Moisture content was calculated for these mixtures, and additional buffer was added to make a final volume of 4.0 mL liquid. One mL pNPP was added to each of three sample vials for each of the following final concentrations: 0.5, 1.25, 3.12, 6.25, 12.5, 25, and 50 mM pNPP. Samples were incubated at 20°C for 1 h. After incubation, 4.0 mL 0.5 N NaOH and 1.0 mL 0.5 M CaCl_2 were added to each sample and mixed. Samples were filtered using Whatman #2 paper. Blanks were prepared and incubated as samples but pNPP was not added until after the NaOH addition. Optical density of filtered samples was measured at 410 nm using a Gilford 260 spectrophotometer.

Intact root tips were assayed for surface phosphatase activity by a modification of the above method. Root tips were transferred from their CaCl_2 holding solution to 1.0 mL MUB in reaction vials. Three to six roots per vial were used; within any set of assays, the same number of roots per vial was used. A 1.0-mL aliquot of pNPP was added to each vial for final substrate concentrations of .05 to 100 mM pNPP (concentrations given in figures). Three vials of roots were incubated at each substrate

concentration in a temperature-regulated water bath at 10-12°C for 1-3 hours (temperature and incubation time were the same for each set of assays). After incubation, 0.5 mL from each reaction vial was added to 0.5 mL of 0.5 N NaOH and mixed. Optical density of the samples was measured at 410 nm with a Bausch & Lomb Spectrophotometer 88.

Calculations and data analysis

For homogenates, activity was expressed as $\mu\text{moles p-nitrophenol produced g}^{-1} \text{ dw h}^{-1}$. Root lengths and diameters of intact root tips were measured using a dissecting microscope. Approximate surface areas were calculated using the formula for a cylinder without ends. Enzyme activity was expressed as $\mu\text{moles product mm}^{-2} \text{ root h}^{-1}$.

Kinetic parameters (V_{max} , the maximum velocity of enzyme-catalyzed hydrolysis, and K_m , the substrate concentration at which velocity is half maximal) were calculated from Eadie-Hofstee transformations of the enzyme activity data.

From each of the homogenates for each tussock component, three subsamples were assayed. Apparent K_m and V_{max} values were determined using the activity measurements from each of the three subsamples at seven substrate concentrations. Single K_m and V_{max} values for each tussock component were determined in this way. K_m and V_{max} values for the six tussocks were then tested for significant differences using analysis of variance. Data for root surface phosphatases were analyzed in a similar way, and enzyme activity at each substrate concentration was also tested by a two-way factorial analysis of variance with substrate concentration and treatment (track or control) as factors. Where there was an unbalanced number of samples, differences between activities at each substrate concentration were tested by least squares means

analysis, using an alpha level determined by dividing .05 by the maximum number of pairwise comparisons to be made (Winer 1962).

Results

The measurement of phosphatase activity in homogenates of the live roots, dead roots, leaf sheaths and soil of E. vaginatum tussocks collected from the water track site in 1982 showed that live roots had the highest acid phosphatase activity on a dry weight basis (Figure 6). Differences among apparent V_{max} values determined for the four tussock components assayed were statistically significant ($p = .004$). Apparent K_m values were not significantly different among tissue and soil components (Figure 7). However, if the contribution of the phosphatase activity of each component to that of the total tussock is calculated (V_{max} times the dry weight of the component divided by the tussock dry weight), the activity of the live roots is seen to be the least significant (Figure 8). The difference between phosphatase activity for track and nontrack tussocks is not statistically significant for apparent V_{max} values ($p = .506$, Figure 6) or for K_m values ($p = .052$, Figure 7). However, when activity is expressed as percent contribution to the total tussock, track tussock components have significantly higher phosphatase activity than do components of nontrack tussocks ($p = .006$, Figure 8).

The root surface phosphatase activity of Eriophorum vaginatum was higher at every substrate concentration tested for tussocks growing in the mesic vehicle track at Slope Mountain than for those growing in adjacent control plots (Figure 9). This difference was statistically significant ($p = .002$) at every assay date from 1982 to 1985 (Figure 9a-d). An assay of the root surface phosphatases of E. angustifolium in the wet

vehicle track showed a similar response (Figure 9f) ($p = .01$). V_{max} values estimated from these data followed the same trend. V_{max} values for vehicle track roots were approximately 1.5 times higher than for control roots with the greatest difference (10 times greater) measured in 1982. Apparent K_m values showed no consistent trend. Only in 1986 was there no difference in phosphatase activity for track roots as compared to control roots (Figure 9e). Apparent V_{max} values estimated from the 1986 assay data were higher for track than for control tussocks, but differences were not statistically significant.

Differences in root surface phosphatase activity for *E. vaginatum* at the water track site were not clearcut (Figures 10 and 11). For several assay dates, no statistically significant differences were measured. Where differences were statistically significant, the direction of the difference varied with the date of assay. In a series of assays done throughout the growing season in 1983 (Figure 11), phosphatase activity was initially higher for track tussocks than for nontrack tussocks (Figure 11a). By the end of the season this had reversed (Figure 11d). The two intermediate date assays show the reversal, but differences were not statistically significant (Figure 11b,c). The early and late season results actually may reflect poor replication (one tussock from track and nontrack in June and one from nontrack and two from track in August). Except for 1982, when track tussocks had significantly higher root surface phosphatase activity (Figure 10a), differences were not detected at other assay dates (Figure 10b-d). Likewise, apparent V_{max} and K_m values showed few consistent differences.

Discussion

Chapin *et al.* (1979) suggested that the tussock growth form of E. vaginatum, with its earlier thaw, longer growing season, and higher rates of soil organic carbon and nutrient cycling, may be responsible for the high productivity of this arctic tundra species. The data presented here indicate that the phosphatase activity associated with this growth form may account for its high rate of phosphorus turnover. In a previous paper, we estimated that hydrolysis of soil solution organic phosphate compounds by the surface phosphatases of live E. vaginatum roots could provide approximately 65% of the plant's annual phosphorus demand (Chapter 1). Although the phosphatase activity of living roots is higher on a dry weight basis than the activity of the other tussock components assayed, the significantly greater amounts of dead roots, leaf sheaths, and soil in the tussock-soil volume result in a much greater contribution from these components to total phosphatase activity and potential organic phosphate hydrolysis.

Vehicle tracks may be man-made analogs of naturally-occurring water tracks in Alaskan tussock tundra in a number of characteristics, but the phosphatases of Eriophorum roots from the two systems do not behave in analagous fashion. Activities measured for root surface phosphatases from vehicle tracks were consistently higher than those for control plants; four years of data conform to this pattern. Natural water track plants, on the other hand, did not have higher activity than nontrack plants at all assay dates; differences were not always statistically significant, and at some dates nontrack roots showed higher activity than track plants.

The consistent results for vehicle track plants suggest that some change in synthesis of phosphatases has occurred in response to the changes associated with vehicle

perturbation. Higher activity may allow track plants to make use of increased substrate brought by water flow to the root surface; without such movement, diffusion probably limits the rate of enzyme-catalyzed hydrolysis (see Ladd and Butler 1975), and a lower activity would be sufficient to keep pace with the amount of organic phosphate substrate diffusing to the root surface. Several researchers have demonstrated that organic phosphorus moves more rapidly in soil than does inorganic phosphate (Hannapel et al. 1964ab, Rolston et al. 1975), and Martin (1970) found a lower adsorption by soil particles for organic than for inorganic phosphorus. If such is the case for these tundra soils, the increased movement of organic phosphorus substrate to the root surface in tracks may be more important than the movement of inorganic phosphate.

The differences in phosphatase activity observed between water track and adjacent tundra tussock components (live and dead roots, leaf sheaths, and soil) cannot in themselves explain the higher phosphorus turnover reported by Chapin et al. (submitted): phosphorus cycling rates were nearly 10 times higher in the track than in the nontrack. However, if the limitation to organic phosphorus hydrolysis is the movement of substrate to enzyme and not the enzyme activity itself, then the movement of water in tracks (compared to no movement in nontracks) may account for the difference in phosphorus turnover.

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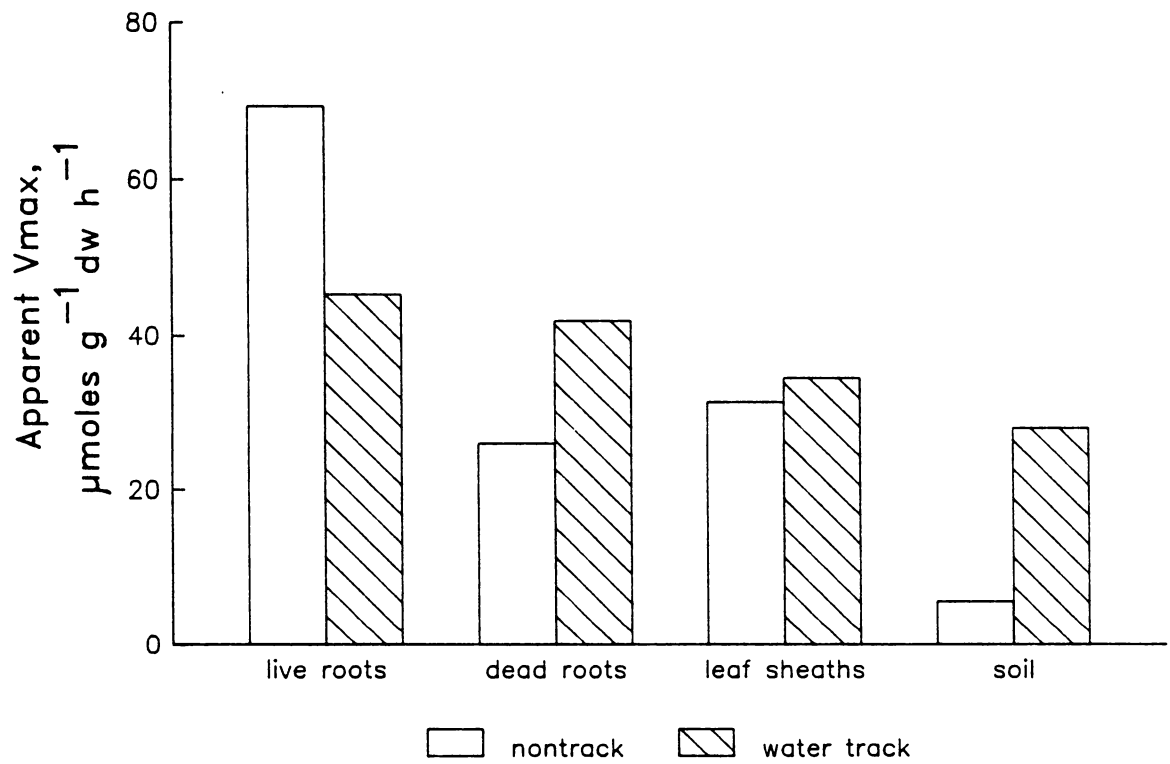


Figure 6. Tussock component apparent Vmax values, water track site: Acid phosphatase data for components of *Eriophorum vaginatum* tussocks collected in 1982 from the water track site; apparent Vmax values ($\mu\text{moles g}^{-1} \text{dw h}^{-1}$)

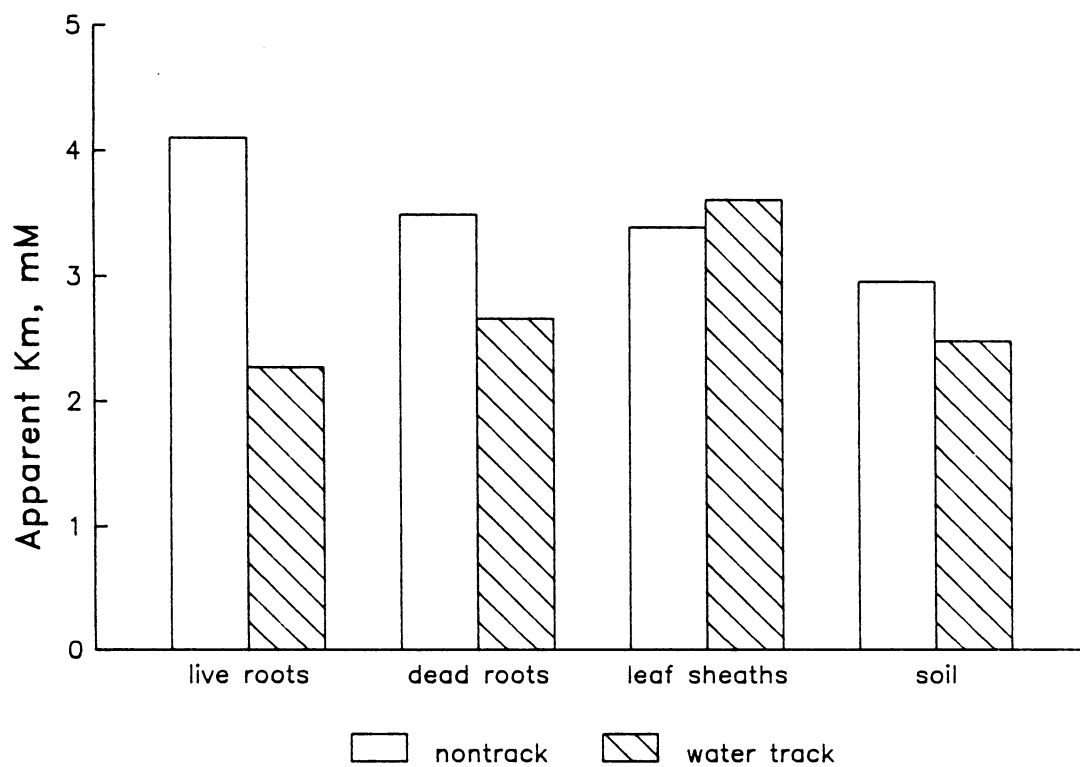


Figure 7. Tussock component apparent Km values, water track site: Acid phosphatase data for components of *Eriophorum vaginatum* collected in 1982 from the water track site; apparent Km values (mM).

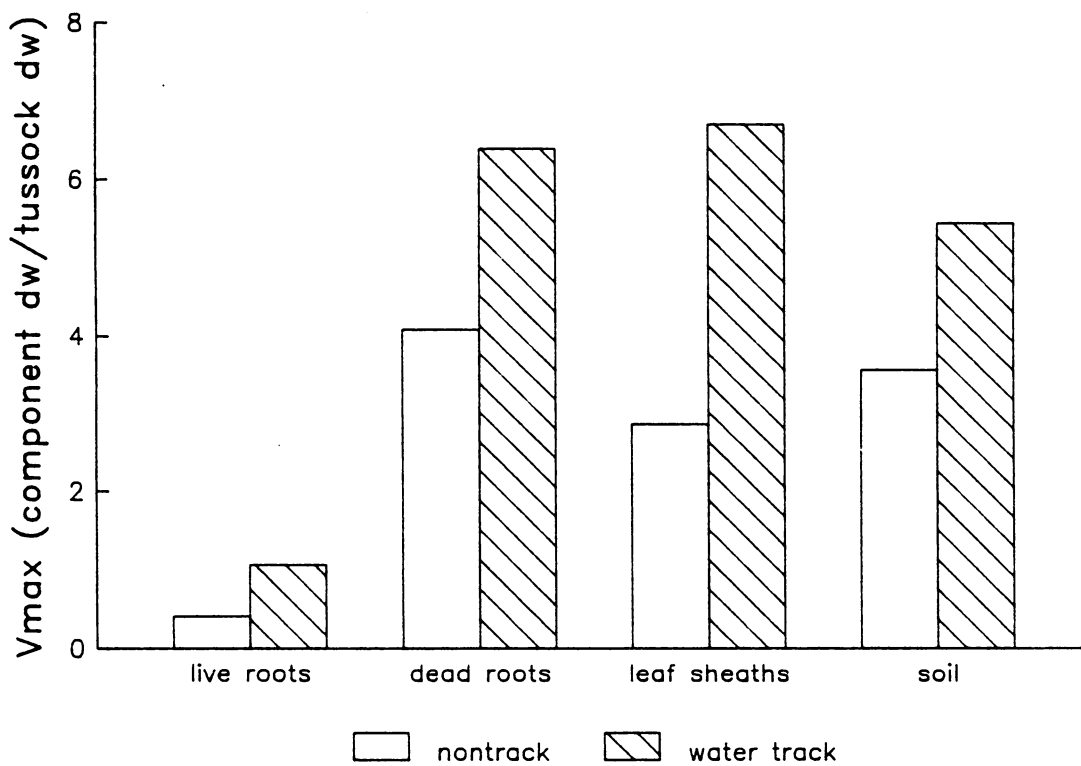


Figure 8. Tussock component Po hydrolysis index, water track site: Acid phosphatase data for components of *Eriophorum vaginatum* collected in 1982 from the water track site; the contribution of the phosphatase activity of each tussock component to phosphorus hydrolysis by the whole tussock: Po hydrolysis index = $V_{max} * (\text{dry weight of component} / \text{total dry weight of tussock})$.

Figure 9. Vehicle track root samples, surface phosphatase activity: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-4} \text{ mm}^{-2} \text{ h}^{-1}$) determined for tussocks from the vehicle track site at Slope Mountain (solid line = control, dashed line = track). Note: Y-axis scales are different for different sampling dates; X-axis scales are distorted so that comparisons can be made at each substrate concentration; symbols represent means \pm 1 std. dev. Figures a through e are for mesic track, E. vaginatum; Figure f is for wet track, E. angustifolium.

a) Late season, 1982 (3 subsamples of roots at each substrate concentration; 1 tussock per plot).

b) June 1983 (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).

c) August 1983 (3 subsamples of roots at each substrate concentration; 2 control, 1 track tussock).

d) Mid-season, 1985 (1 subsample of roots at each substrate concentration; 3 tussocks per plot).

e) Early season, 1986 (1 subsample of roots at each substrate concentration; 3 tussocks per plot).

f) Wet track, 1984 (3 subsamples of roots at each substrate concentration; 3 tussocks per plot).

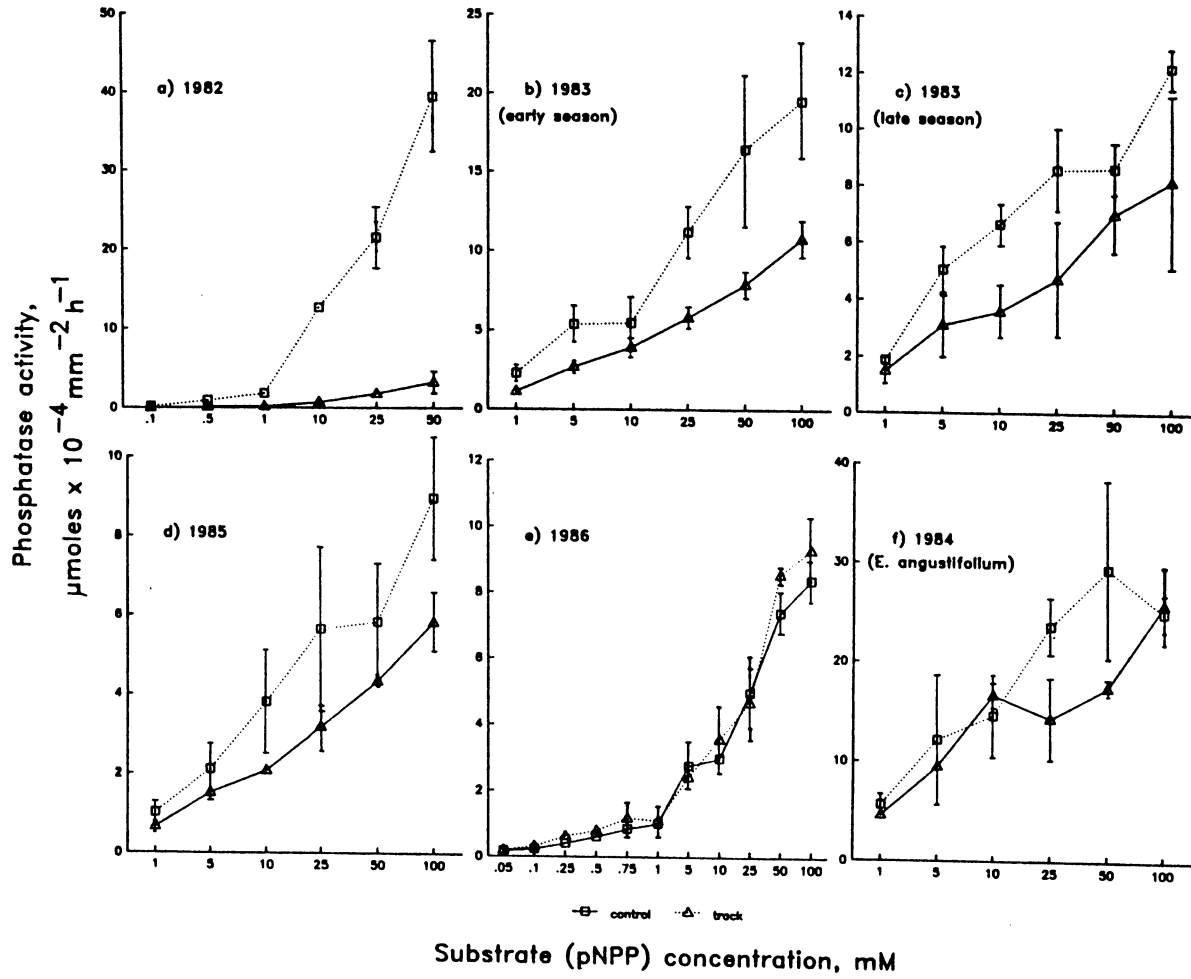


Figure 10. Water track root samples, surface phosphatase activity: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-4} \text{ mm}^{-2} \text{ h}^{-1}$) determined for tussocks from the water track site (solid line = nontrack, dashed line = track).
Note: Y-axis scales are different for different sampling dates; X-axis scales are distorted so that comparisons can be made at each substrate concentration; symbols represent means ± 1 std. dev.

- a) Late season, 1982 (3 subsamples of roots at each substrate concentration; 3 tussocks per plot).
- b) 1984 (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).
- c) Mid-season, 1985 (1 subsample of roots at each substrate concentration; 3 tussocks per plot).
- d) Early season, 1986 (1 subsample of roots at each substrate concentration; 3 tussocks per plot).

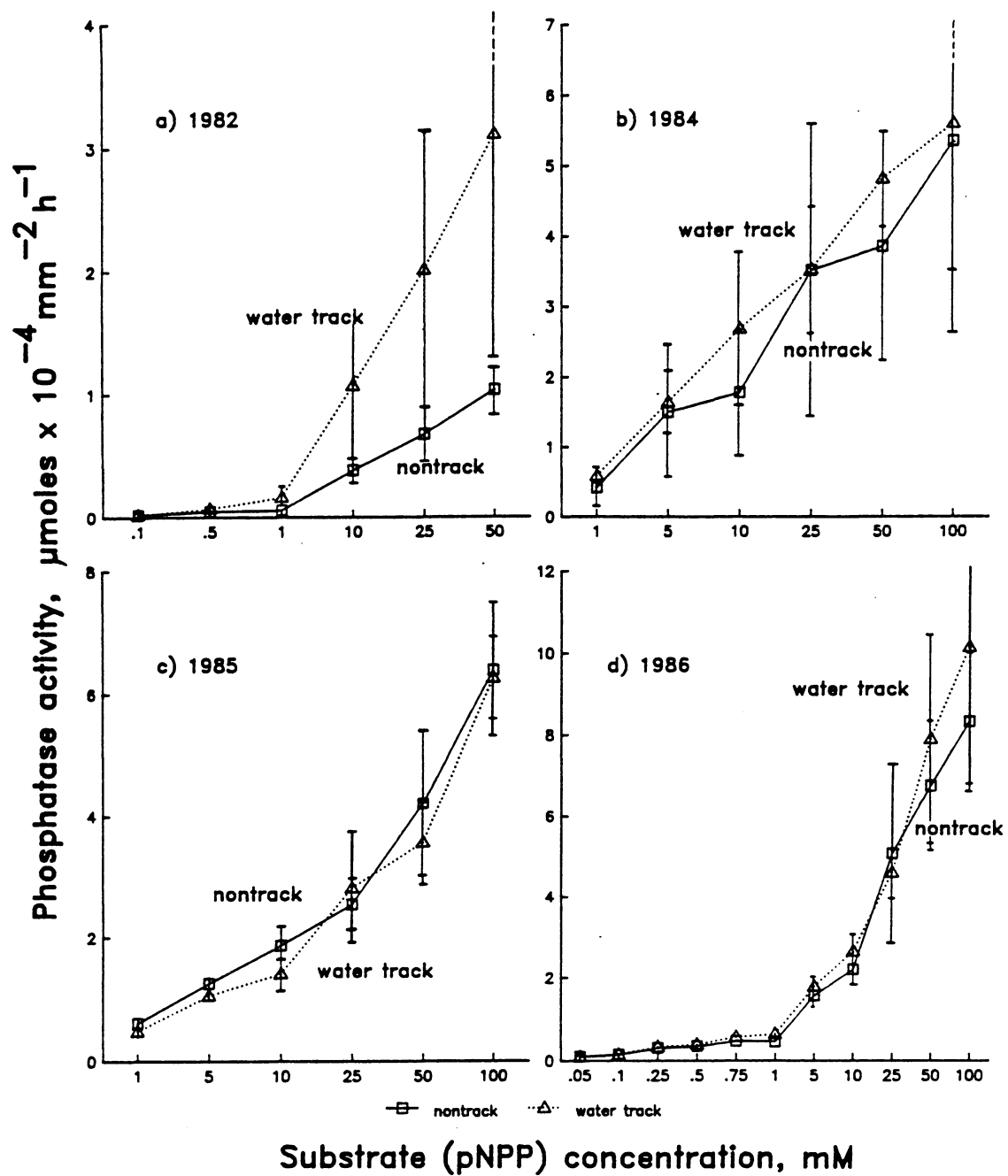
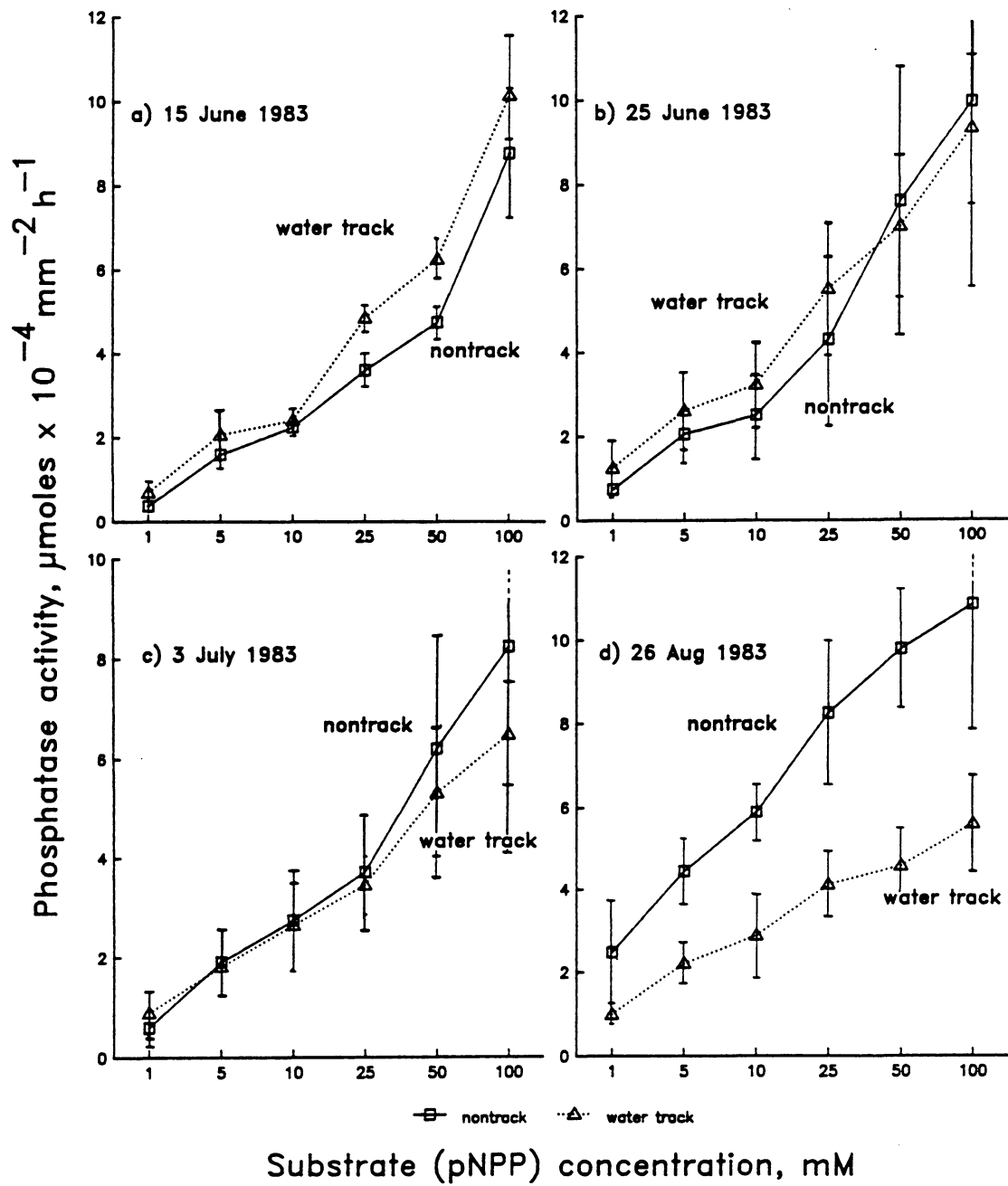


Figure 11. Water track, surface phosphatase activity over one season: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-4} \text{ mm}^{-2} \text{ h}^{-1}$) determined for tussocks from the water track site (solid line = nontrack, dashed line = track).
Note: Y-axis scales are different for different sampling dates; X-axis scales are distorted so that comparisons can be made at each substrate concentration; symbols represent means \pm 1 std. dev.

- a) 15 June 1983 (3 subsamples of roots at each substrate concentration; 1 tussock per plot).
- b) 25 June 1983 (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).
- c) 3 July 1983 (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).
- d) August 1983 (3 subsamples of roots at each substrate concentration; 1 nontrack and 2 track tussocks).



Chapter 3. The Effects of Fertilization on the Acid Phosphatases of *Eriophorum vaginatum*

Introduction

A considerable amount of evidence from laboratory and field studies supports the idea that plant phosphatases are adaptive enzymes, responding to phosphorus deficiency with increased activity or synthesis (Barrett-Lennard *et al.* 1982, Bielecki and Johnson 1972, Boutin *et al.* 1981, Chapman and Jones 1975, Dracup *et al.* 1984, McLachlan 1980ab, McLachlan and De Marco 1982, Reid and Bielecki 1970, Silberbush *et al.* 1981, Skujins 1976, Woolhouse 1969, Zink and Veliky 1979). Control of this adaptive response has been linked to phosphorus concentrations in the growth medium or in root cells, phosphorus status of the plant or its soil environment, or some combination of these factors (Boutin *et al.* 1981, Reid and Bielecki 1970, Skujins 1976). The adaptive nature of plant phosphatases has led to the suggestion that phosphatase activity might be used as an indicator of the phosphorus status of a plant or its soil environment

(Barrett-Lennard and Greenway 1982, Besford 1979, McLachlan 1980b, McLachlan and De Marco 1982, O'Connell and Grove 1985).

In these experiments, I use a low nutrient-adapted arctic tussock tundra species to "field test" the hypothesis that phosphatase activity provides a measure of the phosphorus status of a plant or of its soil environment. Eriophorum vaginatum (L.) subsp. spissum (Fern.) Hult., a dominant plant in arctic tussock tundra, has an annually-produced root system of unbranched, hairless, predominantly non-mycorrhizal roots (Wein 1973). These characteristics simplify interpretation of phosphatase activity measurements based on root surface area and reduce problems in comparing such measurements for different treatments.

Low nutrient availability limits growth in arctic ecosystems (Chapin et al. 1975, Shaver and Chapin 1980), and E. vaginatum has been shown to respond with increases in biomass and productivity to fertilization by nitrogen and phosphorus (Chapin et al. 1986, Shaver et al. 1986); by potassium, nitrogen, and phosphorus (Goodman and Perkins 1968ab); and by phosphorus alone (Tamm 1954). Nitrogen most strongly limits plant productivity in tussock tundra at Toolik Lake, Alaska (Shaver et al. 1986), where this work was done. Although fertilization resulted in increased plant biomass (Shaver et al. 1986), plant tissue phosphorus concentrations (Chapin et al. 1986), and phosphate availability in tundra soils (Chapin and Shaver 1985), I predicted that in a site where nitrogen was more limiting than phosphorus the phosphatases of E. vaginatum would not prove to be simple indicators of the phosphorus status of the plant or of the soil as suggested by other researchers for other species.

To investigate the effects of fertilization on the acid phosphomonoesterases of E. vaginatum, I used a variety of nutrient manipulation plots in arctic tussock tundra. Live and dead roots, leaf sheaths, and tussock soil were assayed to monitor changes in the phosphatase activity of all parts of the tussock involved in ground surface and

below-ground organic phosphorus mineralization. Phosphatase activity was measured for tussocks growing in plots fertilized with nitrogen, phosphorus, and potassium; nitrogen and phosphorus; phosphorus alone; and organic phosphorus.

Methods

Study site and fertilized plots

Field research was conducted during the summers of 1982-1986 at Toolik Lake (68° 38'N, 149° 34'W, 760 m elevation) located in the northern foothills of the Brooks Range in Alaska (see Shaver *et al.* 1986 for a more complete site description). Samples were collected from experimental plots in tundra dominated by tussock-forming Eriophorum vaginatum.

The first set of plots used in this study was fertilized in 1978 by F.S. Chapin and G.R. Shaver. These plots, described more completely elsewhere (Chapin and Shaver 1985), were fertilized with 25 g m⁻² N and P and 31 g m⁻² K ("NPK plots"). The second set, part of another study by Chapin and Shaver, received 10 g N m⁻² (NH₄NO₃) and 5 g P m⁻² (P₂O₅) annually beginning in 1980 (pers. comm.) ("NP plots"). The third set of plots was fertilized with phosphorus alone. Application rates were 10 g inorganic P (Pi) m⁻² (superphosphate) or 10 g organic P (Po) m⁻² (sodium inositol hexaphosphate). Fertilizer was applied in mid-June 1983. The last plot used in this study was fertilized with inositol hexaphosphate alone (10 g P m⁻²) in August of 1982.

Preparation of samples

Whole tussocks were collected from the 1978 NPK fertilization plots during the summers of 1981 and 1982, shipped on ice, and stored frozen. *E. vaginatum* produces a new root system annually, and the dead roots from previous years make up much of the below-ground tussock volume (Wein 1973). For this study, thawed tussocks were picked apart and sorted into current year roots, dead roots (past season's and older), leaf sheaths, soil, and "other." The "other" material included *E. vaginatum* stems and stem bases as well as roots and stems of other plant species which had been growing on the tussock. Each component was then weighed, frozen in liquid nitrogen, and ground to powder using a flour mill. Moisture content and dry weights were determined for each component. Current season root, dead root, leaf sheath, and soil samples were stored frozen.

For the root surface activity assays, tussocks of *E. vaginatum* were collected from each of the plots described above. Current-year root tips approximately 1-2 cm in length were excised within several hours of collection and held in 0.5 mM CaCl₂ (Epstein *et al.* 1963) until assayed (not longer than 4 hours).

Measurement of phosphatase activity

In all assays, p-nitrophenyl phosphate (pNPP) was used as substrate. For the homogenates, 2.0-mL aliquots from a suspension of the sample and pH 5.0 Modified Universal Buffer (MUB) (Ho and Zak 1979) were pipeted into 10-mL Nalgene reaction vials. Water content was calculated for these mixtures, and additional buffer was added to make a final volume of 4.0 mL liquid. One mL pNPP was added to each of three

sample vials for each of the following final concentrations: 0.5, 1.25, 3.12, 6.25, 12.5, 25, and 50 mM pNPP. Samples were incubated at 20°C for 1 h. After incubation, 4.0 mL 0.5 N NaOH and 1.0 mL 0.5 M CaCl₂ were added to each sample and mixed. Samples were filtered using Whatman #2 paper. Blanks were prepared and incubated as samples but pNPP was not added until after the NaOH addition. Optical density of filtered samples was measured at 410 nm using a Gilford 260 spectrophotometer.

Intact root tips were assayed for surface phosphatase activity by a modification of the above method. Root tips were transferred from their CaCl₂ holding solution to 1.0 mL buffer in reaction vials. Three to six roots per vial were used; within any set of assays, the same number of roots per vial was used. A 1.0-mL aliquot of pNPP was added to each vial for final substrate concentrations of .05 to 100 mM pNPP (concentrations given in figures). Three vials of roots were incubated at each substrate concentration in a temperature-regulated water bath at 10-12°C for 1-3 hours (temperature and incubation time were the same for each set of assays). After incubation, 0.5 mL from each reaction vial was added to 0.5 mL of 0.5 N NaOH and mixed. Optical density of the samples was measured at 410 nm with a Bausch & Lomb Spectrophotometer 88.

Calculations and data analysis

For homogenates, activity is expressed as $\mu\text{moles p-nitrophenol produced g}^{-1} \text{ dw h}^{-1}$. For intact root tips, root lengths and diameters were measured using a dissecting microscope. Approximate surface areas were calculated using the formula for a cylinder without ends. Enzyme activity was expressed as $\mu\text{moles product mm}^{-2} \text{ root h}^{-1}$.

Kinetic parameters (V_{max} , the maximum velocity of enzyme-catalyzed hydrolysis, and K_m , the substrate concentration at which velocity is half maximal) were calculated from Eadie-Hofstee transformations of the enzyme activity data.

We examined the variability among individual tussocks by collecting three tussocks from the 1978 fertilization plots and three from adjacent control plots in 1981 and again in 1982. From each of the homogenates for each component of each of these tussocks, three subsamples were assayed. Apparent K_m and V_{max} values were determined using the activity measurements from each of the three subsamples at seven substrate concentrations. Values of K_m and V_{max} for individual components of individual tussocks were determined in this way. K_m and V_{max} values for the six tussocks were then tested for significant differences using analysis of variance. Root surface phosphatase assays were done on live roots for tussocks collected from the 1978 NPK fertilization plots both early and late in the growing season in 1982 and again in 1984; from the 1980 NP fertilization plots in 1982, 1984, 1985, and 1986; from the 1983 inorganic and organic phosphate (P_i/P_o) plots 3 times in 1983 and again in 1984; and from the 1982 organic phosphate plot in 1983. Data for root surface phosphatases were analyzed in a manner similar to that for the tussock component homogenates, and differences in enzyme activity at each substrate concentration were also tested by two-way factorial analysis of variance with substrate concentration and treatment (fertilized or control) as factors. Where there was an unbalanced number of samples, differences between activities at each substrate concentration were tested by least squares means analysis, using an alpha level determined by dividing .05 by the maximum number of pairwise comparisons to be made (Winer 1962).

Results

The apparent V_{max} values for acid phosphomonoesterase activity measured in the homogenates of current season roots, dead roots, leaf sheaths, and tussock soil were significantly higher for *E. vaginatum* tussocks harvested in 1982 from plots fertilized in 1978 with nitrogen, phosphorus, and potassium than from control plots and tended to be higher for tussocks harvested in 1981 (Figure 12). The relative contribution of the enzyme activity of each component to phosphorus hydrolysis by the whole tussock was estimated by multiplying V_{max} values by the dry weight of each component divided by the total dry weight of the tussock (Figure 13). Current season roots had significantly higher V_{max} values than other components in the 1981 assay and were not significantly different from others in 1982. However, their contribution to the total amount of enzyme activity associated with the tussock was small in both years (Figure 13). Apparent K_m values also tended to be higher for fertilized tussocks than for control tussocks for each component, except for the 1981 leaf sheath sample (Figure 14). Again, this difference was statistically significant only for the 1982 samples.

Live intact root samples from the 1978 plots were assayed for root surface phosphatase activity early and late in the growing season in 1982 and again in 1984. Roots from fertilized plots had higher phosphatase activity at 5 of 6 substrate concentrations for both 1982 assays, and means were significantly higher at both dates (Figure 15a,b). Enzyme activity at the lowest substrate concentration (0.1 mM) was approximately 10 times higher for fertilized roots than for control roots. In 1984, six years after application of fertilizer, there were no significant differences between fertilized and control plots (Figure 15c). V_{max} estimates were higher and K_m lower for fertilized roots in 1982 but not different in 1984.

Root surface phosphatase activity was also determined for plants from the 1980 NP fertilization plots. These plots were fertilized at a lower application rate but on an annual basis. Assays done in 1982 indicated that fertilized roots had higher activity at every substrate concentration (Figure 16a); the mean for fertilized roots was significantly higher than that for control roots. Similar results were obtained in the 1984 assay (Figure 16b), but in 1985 and 1986 assay results were not significantly different (Figures 16c,d). In 1986, control roots incubated at the two highest substrate concentrations had significantly higher activity than fertilized roots and tended to have higher activity at lower substrate concentrations as well (Figure 16d). There were no significant differences between K_m and V_{max} values for plants from the two plots.

Root surface phosphatase activities determined for *E. vaginatum* fertilized only with phosphorus were, in most cases, not significantly different from control plant enzyme activities. Root surface phosphatase activity for tussocks harvested from plots fertilized with inorganic or organic phosphorus early in the growing season in 1983 tended to vary with treatment, length of time after fertilization, and time of year assayed (Figure 17), but differences were not statistically significant. One exception to this was the third growing season after fertilization, when roots from inorganic phosphate-fertilized plots had significantly lower enzyme activity than both control and organic phosphate-fertilized roots (data not shown). This assay was done at saturating substrate concentration only (100 mM pNPP). The root surface phosphatase activities determined for 3 tussocks one year after fertilization with inositol hexaphosphate (10 g m^{-2}) in the 1982 plot were not significantly different than those from control tussocks (data not shown).

Discussion

Plant phosphatase activity may provide a measure of the phosphorus status of a plant or of its soil environment as suggested by other researchers (Barrett-Lennard and Greenway 1982, Besford 1979, McLachlan 1980b, McLachlan and De Marco 1982, O'Connell and Grove 1985) in conditions where phosphorus is the only limiting nutrient. This study shows that in an ecosystem where another nutrient is more strongly limiting, *E. vaginatum* plants with higher tissue phosphorus concentrations growing in soils with higher available phosphorus had higher, not lower, phosphatase activity than did plants from unfertilized soils. The inverse relationship between phosphatase activity and soil phosphorus availability or plant phosphorus status documented by many researchers (Barrett-Lennard *et al.* 1982, Bielecki and Johnson 1972, Boutin *et al.* 1981, Chapman and Jones 1975, Dracup *et al.* 1984, McLachlan 1980ab, McLachlan and De Marco 1982, Reid and Bielecki 1970, Silberbush *et al.* 1981, Skujins 1976, Woolhouse 1969, Zink and Veliky 1979) does not appear to hold for this plant in its natural environment. The phosphorus status of both soil environment and plants improved significantly in the NP and NPK fertilization plots (Chapin and Shaver 1985; Chapin *et al.* 1986, Shaver *et al.* 1986), but phosphatases did not show the resultant decreased levels of activity that might be predicted based on the above studies.

Shaver *et al.* (1986) found that nitrogen was the most strongly limiting nutrient at the study site discussed in this paper. The effects of fertilization on the acid phosphatases of *E. vaginatum* can best be understood in this context. Fertilization with nitrogen in addition to phosphorus helps to ensure that the limiting effects of low nitrogen on phosphorus metabolism (Haag 1974) and enzyme production do not occur. The plots fertilized only with phosphorus in this study did not show marked increases in

plant phosphatase activity, as did the plants fertilized with both nitrogen and phosphorus. In the NP and NPK plots, fertilization resulted in increased total concentrations of plant phosphorus; however, by the end of the growing season this gain was diluted by the greater seasonal growth of the fertilized plants (Shaver et al. 1986).

This growth response to fertilization, causing increased demand for phosphate, may help to explain the increase in phosphatase activity found for all tussock components assayed. The enzyme activity of the dead roots and of the soil reflects the activity of the previous season's live roots, the amount of enzyme being excreted by roots and microbes, and microbial activity, all of which might be expected to increase with increased plant productivity, biomass, and demand for nutrients. An examination of specific plant parts showed that the major effect of fertilization on phosphorus fractions was to increase the soluble organic storage phosphate in stems (Chapin et al. 1986). If the additional phosphorus gained as a result of fertilization is used first to enhance storage reserves and second to support additional growth late in the growing season, increased phosphatase activity may serve to help meet the additional demand for phosphate.

The inverse relationship between soil or solution phosphate and phosphatase activity has led researchers to term phosphatases adaptive enzymes. In a phosphorus-poor environment, responding to potentially temporary increases in phosphate availability with decreased levels of phosphatase activity may not be "adaptive." Silberbush et al. (1981) concluded that ecotypes of Aegilops from phosphorus-poor soils were unable to regulate their phosphatase production and/or activity as well as did ecotypes from phosphorus-rich soils. A lack of inhibition of phosphatases by inorganic phosphate in the incubation medium (see Chapter 1) suggests that even the regulation provided by end-product inhibition is absent in E. vaginatum. Our data suggest that E. vaginatum produces phosphatases at a constitutive level related

to the concentration of available phosphorus in the tundra soils in which it grows. With fertilization, the amount of organic as well as inorganic phosphorus in the soil increases (Dalal 1977, Halstead and McKercher 1975) and diffusion of substrate to the root surface becomes less limiting, making higher enzyme activity advantageous. The decrease in root surface phosphatase activity relative to that of control plants that was observed after several years of fertilization may reflect a gradual acclimation to a high phosphorus environment.

The response of E. vaginatum phosphatases is not the only example of nitrogen fertilization affecting plant phosphatase activity. Corbean (1977) found that corn leaf phosphatase activity increased in nitrogen-fertilized plants, and O'Connell and Grove (1985) found that the leaf phosphatase activity of Eucalyptus increased when soil nitrogen supply was increased. Grinsted et al. (1982) suggested that the form of nitrogen provided to plants has an effect on the rhizosphere pH, which in turn may affect root phosphatase activity. Clearly, the phosphorus status of the plant or soil is not the only factor which can affect plant phosphatase activity.

The use of plant phosphatase activity as an indicator of plant or soil phosphorus status has been proposed by a number of researchers. The relative ease of collecting, assaying, and interpreting results for E. vaginatum roots made them seem a good candidate for such a "bio-assay." Yet the phosphatase activity of roots from different tussocks from the same study plot was quite variable. Likewise, tundra soils have an extremely high degree of variability in soil moisture, organic carbon, pH, various cations, and available phosphorus related to depth and microtopography (Everett 1978). Available phosphorus can vary according to soil depth, seasonality (Everett 1978), soil moisture and pH (Chapin and Shaver 1981), and lemming population fluctuations (Gersper et al. 1980). My results showed seasonal and yearly variations in phosphatase activity that, in some cases, were greater than responses to experimental manipulation.

Phosphatases may respond to changes in phosphorus availability in a clearly interpretable manner when all other factors are controlled. In tundra soils (and perhaps most other natural ecosystem) the interrelationships of a number of other variables makes measurement of phosphatases an unreliable indicator of the phosphorus status of a plant or its soil environment.

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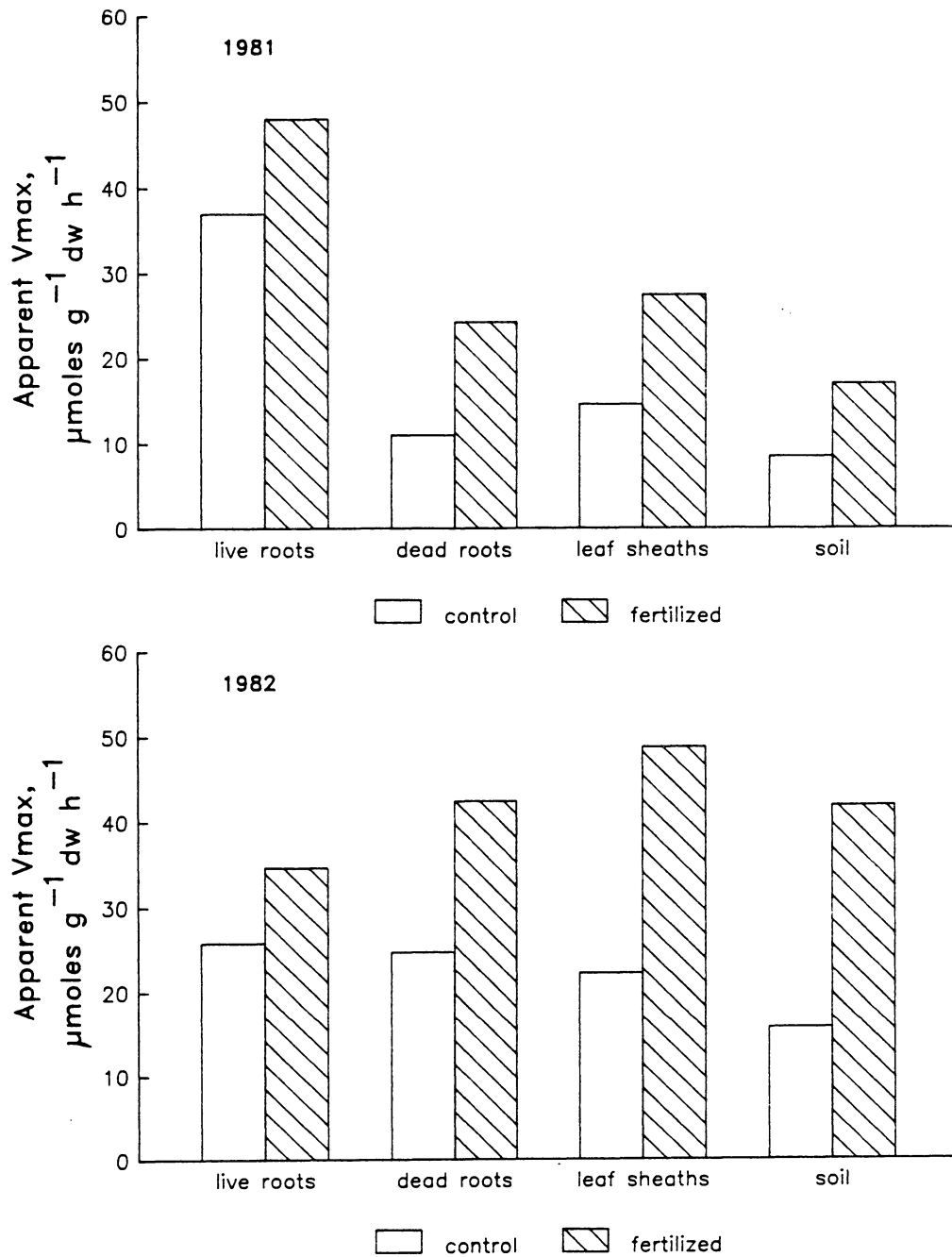


Figure 12. Tussock component phosphatase V_{max} values, 1978 NPK plot: Apparent V_{max} values ($\mu\text{moles g}^{-1} \text{dw h}^{-1}$) determined from Eadie-Hofstee transformation of the phosphatase activity of live roots, dead roots, leaf sheaths, and soil for *Eriophorum vaginatum* tussocks collected in 1981 and 1982 from plots fertilized in 1978 with 25 g m^{-2} N and P and 31 g m^{-2} K.

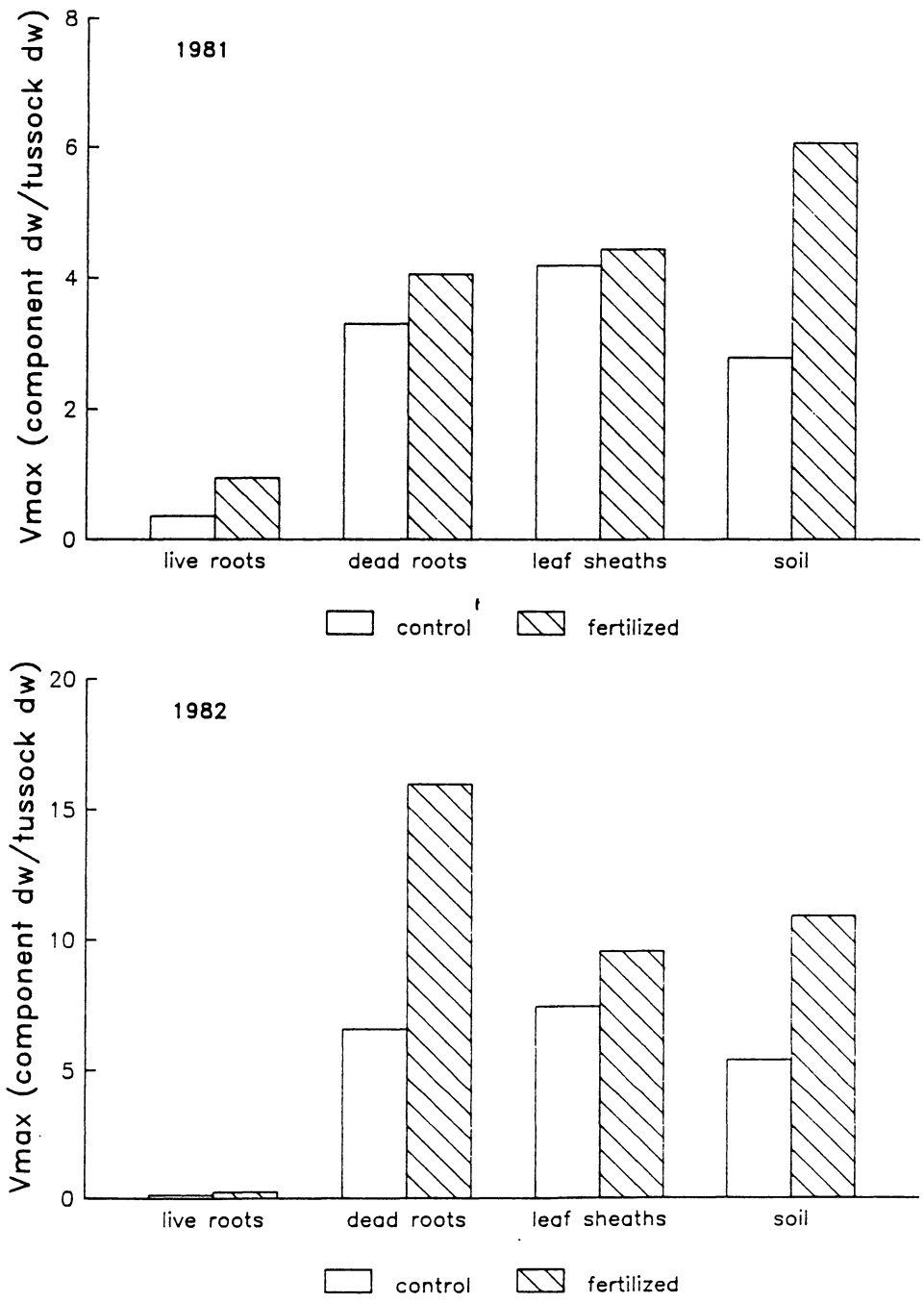


Figure 13. Tussock component Po hydrolysis index, 1978 NPK plot: The contribution of the phosphatase activity of each tussock component to phosphorus hydrolysis by the whole tussock, from plots fertilized in 1978 with 25 g m^{-2} N and P and 31 g m^{-2} K.
 index = $V_{\text{max}} \cdot (\text{dry weight of component} / \text{total dry weight of tussock})$.

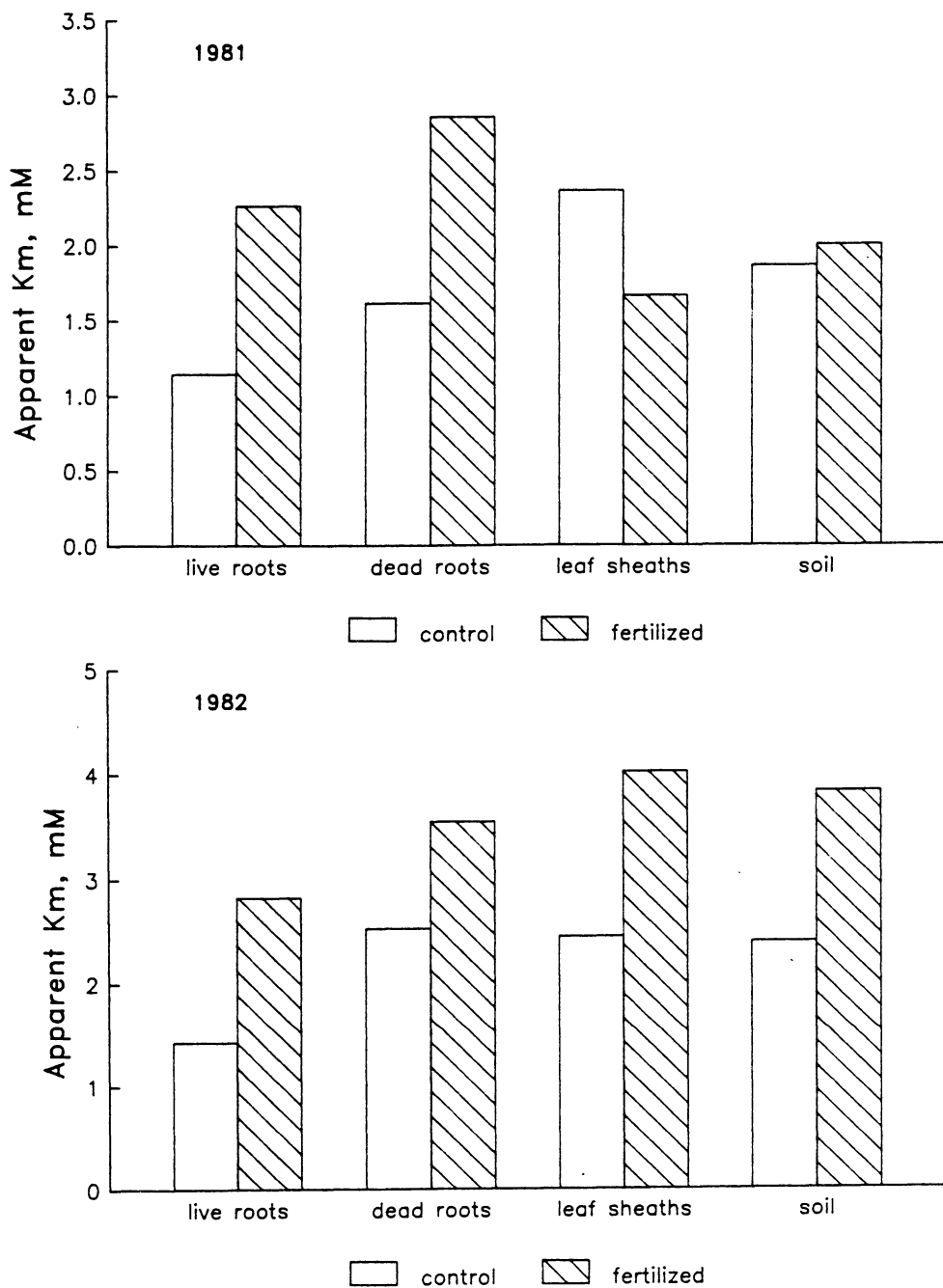


Figure 14. Tussock component phosphatase K_m values, 1978 NPK plot: Apparent K_m values (mM) determined from Eadie-Hofstee transformation of the phosphatase activity for *Eriophorum vaginatum* collected in 1981 and 1982 from plots fertilized in 1978 with 25 g m^{-2} N and P and 31 g m^{-2} K.

Figure 15. Root surface phosphatase activity, 1978 NPK plot: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-4} \text{ mm}^{-2} \text{ h}^{-1}$) determined for tussocks from plots fertilized in 1978 with 25 g m^{-2} N and P and 31 g m^{-2} K (solid line = control, dashed line = fert).

Note: Y-axis scales are different for different sampling dates; X-axis scales are distorted so that comparisons can be made at each substrate concentration; symbols represent means ± 1 std. dev.

a) Early season, 1982 (2 subsamples of roots at each substrate concentration; 1 tussock per plot).

b) Late season, 1982 (3 subsamples of roots at each substrate concentration; 1 tussock per plot).

c) Mid-season, 1984 (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).

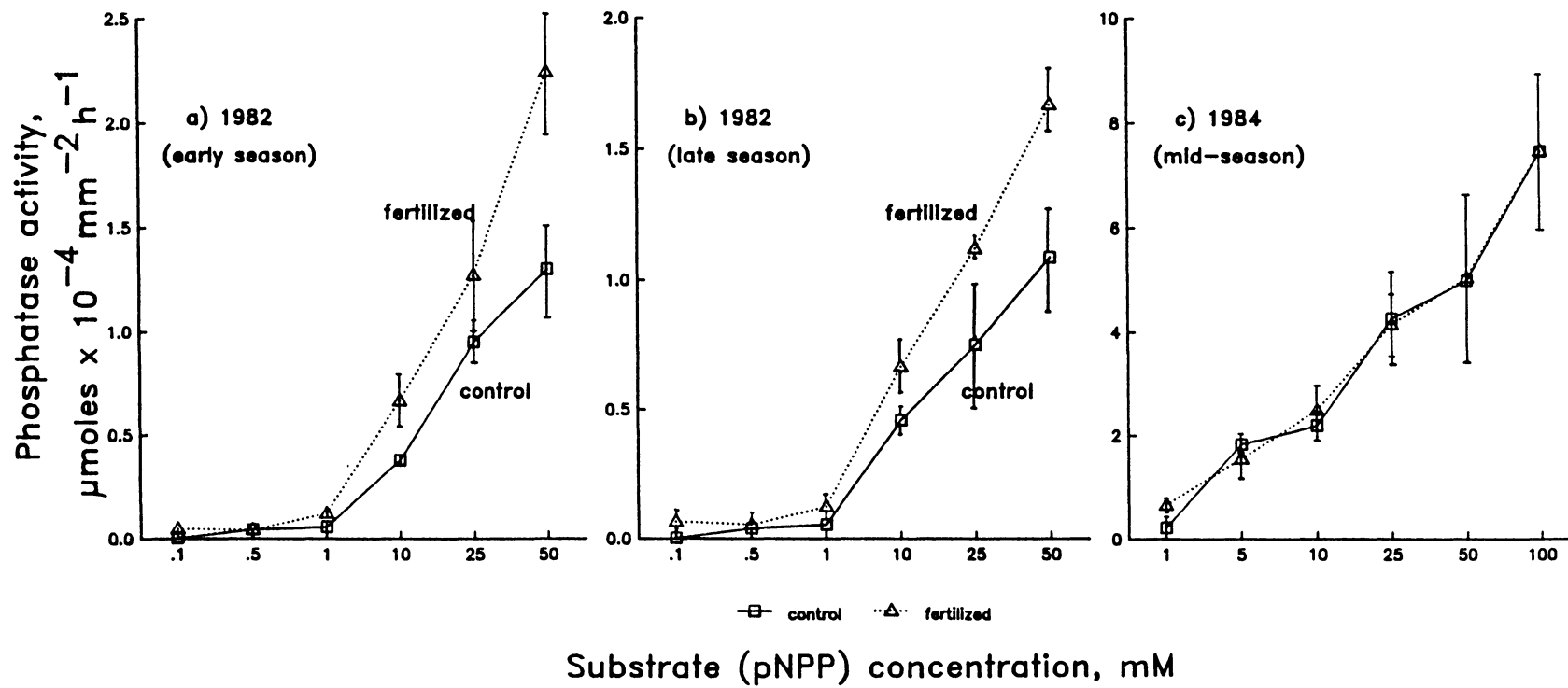


Figure 16. Root surface phosphatase activity, 1980 NP plot: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-4} \text{ mm}^{-2} \text{ h}^{-1}$) determined for tussocks from plots fertilized annually, beginning in 1980, with 5 g m^{-2} P and 10 g m^{-2} N (solid line = control, dashed line = fert).

Note: Y-axis scales are different for different sampling dates; X-axis scales are distorted so that comparisons can be made at each substrate concentration; symbols represent means ± 1 std. dev.

a) 1982 (3 subsamples of roots at each substrate concentration; 1 tussock per plot).

b) 1984 (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).

c) 1985 (1 subsample of roots at each substrate concentration; 3 tussocks per plot).

d) 1986 (1 subsample of roots at each substrate concentration; 3 tussocks per plot).

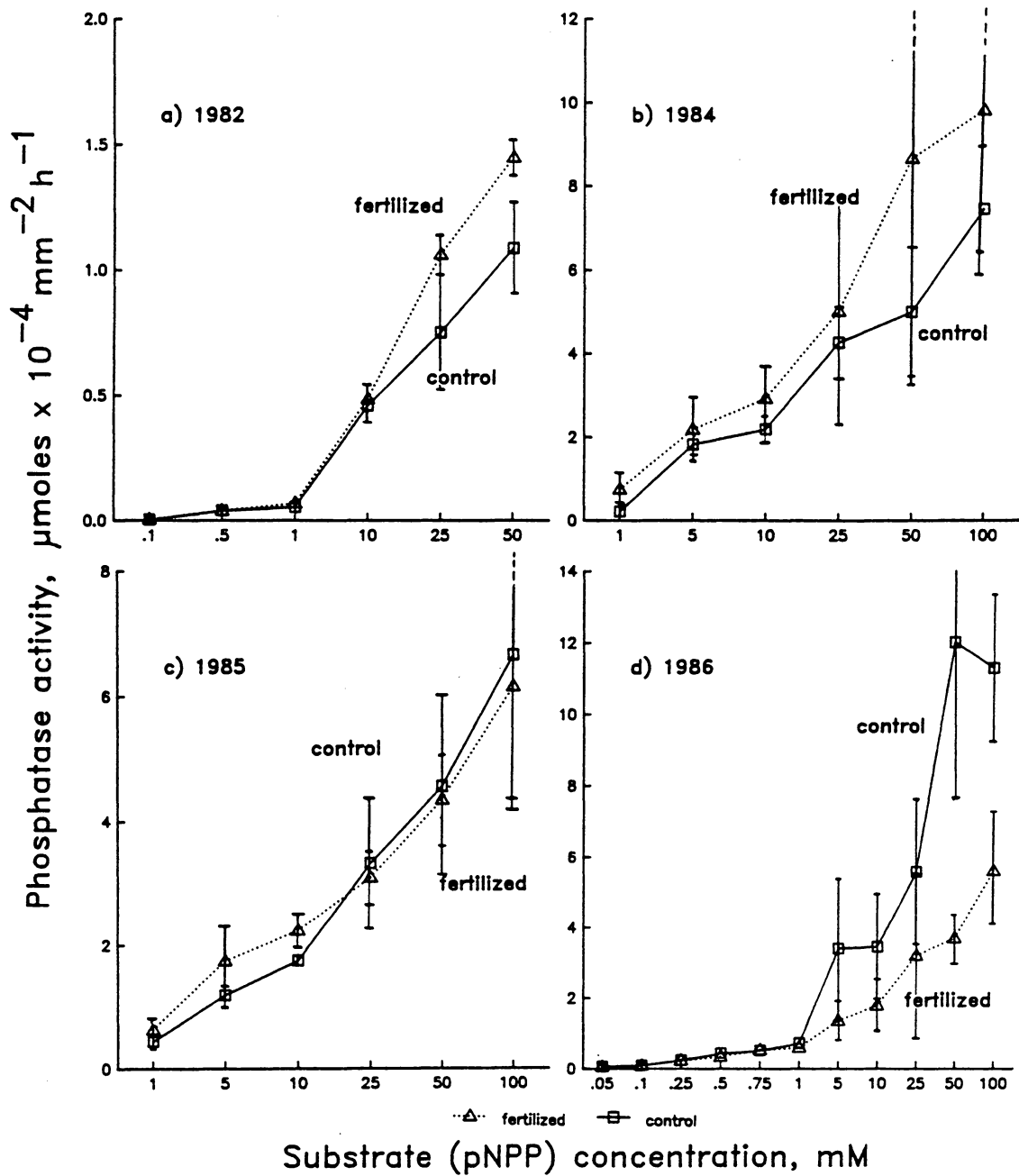


Figure 17. Root surface phosphatase activity, 1983 Pi/Po plot: Root surface phosphatase activity ($\mu\text{moles} \times 10^{-4} \text{ mm}^{-2} \text{ h}^{-1}$) determined for tussocks from plots fertilized early in the growing season in 1983 with 10 g m^{-2} inorganic phosphate (Pi) or 10 g m^{-2} organic phosphate (Po) (solid line = control, dashed line = Pi, dotted line = Po).

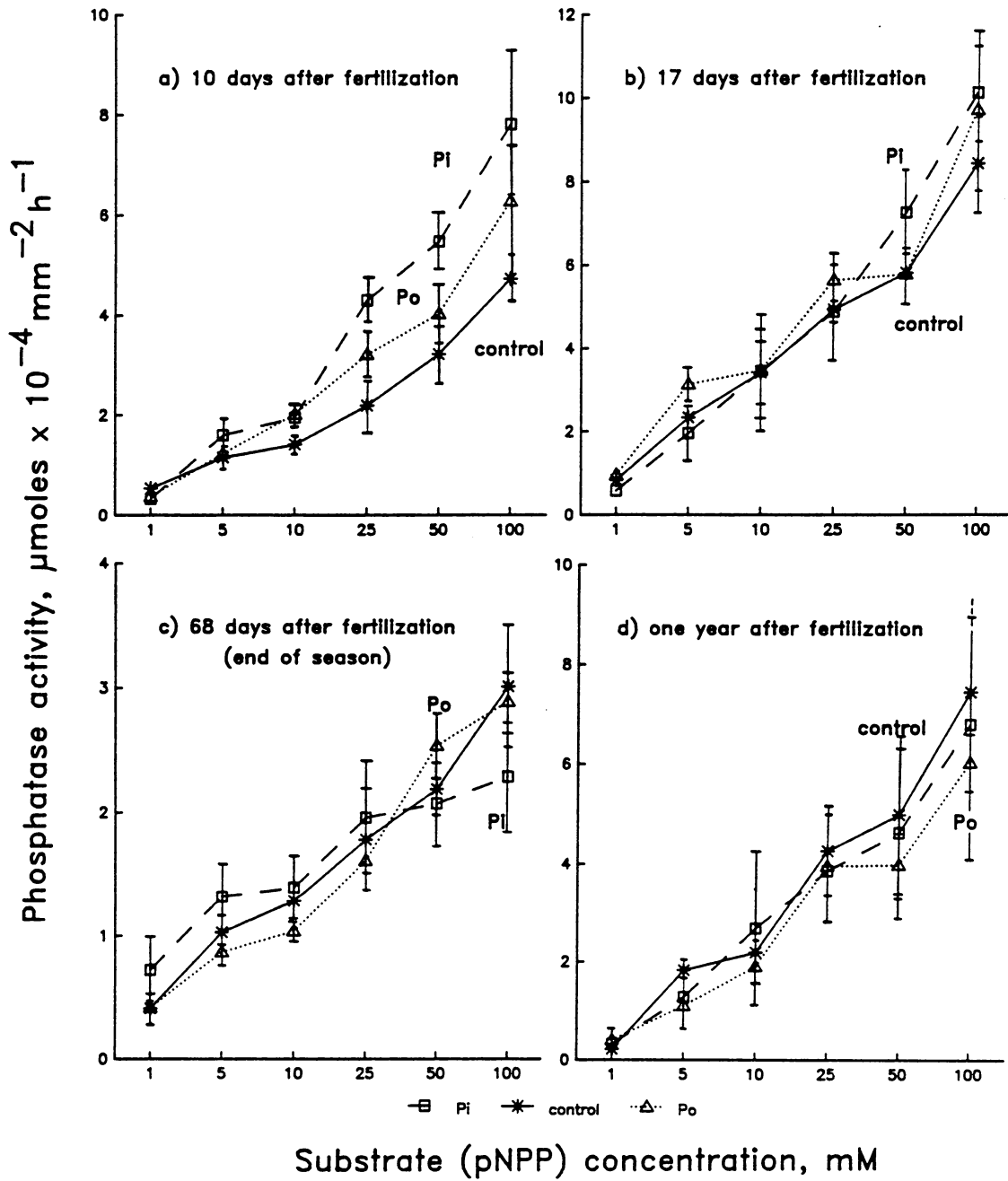
Note: Y-axis scales are different for different sampling dates; X-axis scales are distorted so that comparisons can be made at each substrate concentration; symbols represent means \pm 1 std. dev.

a) 10 days after application of fertilizer (3 subsamples of roots at each substrate concentration; 1 tussock per plot).

b) 17 days after application of fertilizer (3 subsamples of roots at each substrate concentration; 1 tussock per plot).

c) 68 days after application of fertilizer (3 subsamples of roots at each substrate concentration; 1 tussock per plot).

d) One year after application of fertilizer (3 subsamples of roots at each substrate concentration; 2 tussocks per plot).



Chapter 4. The Absorption of Inorganic Phosphate from ^{32}P -Labeled Inositol Hexaphosphate by *Eriophorum vaginatum*

Introduction

The importance of soil organic phosphorus as a source of available phosphate for plants is a subject of debate. The discovery of root surface phosphatases capable of hydrolyzing organic phosphorus compounds (e.g., Estermann and McLaren 1961, Dracup *et al.* 1984) suggested that soil organic phosphorus may contribute to the phosphorus nutrition of plants via root surface phosphatases. However, work demonstrating this direct contribution is scarce, and it is still generally accepted that the mineralization of organic phosphate by soil microbes is a critical intermediate step in the transformation of organic compounds to phosphate available for plant uptake.

In arctic tundra soils, up to 99% of the phosphorus in the rooting zone is organically bound (Chapin *et al.* 1980). Soil solution organic phosphate concentrations may be 23 times higher than soil solution inorganic phosphate (Barel & Barsdate 1978). Under these conditions of high organic and low inorganic phosphorus in soil solution, the contribution of root surface phosphatases to organic phosphate breakdown may be especially important.

Eriophorum vaginatum (L.) subsp. spissum (Fern.) Hult., a tussock-forming sedge which is a dominant species in much Alaskan tundra, has root surface phosphatase activity distributed along the length of its annually-produced roots (see Chapter 1). Comparisons with inorganic phosphate uptake rates indicate that enzymatic hydrolysis of organic phosphate could produce as much inorganic phosphate as can be absorbed by the plant (see Chapter 1). Phosphorus cycles 3-10 times more rapidly within the tussock soil region than beneath it (Chapin *et al.* 1979), suggesting that the presence of Eriophorum roots may be responsible for at least some of this organic phosphate mineralization.

The objective of this study was to determine whether the root surface phosphatase activity of E. vaginatum is associated with uptake of inorganic phosphate by the plant. We hypothesized that ³²P-phosphate from a ³²P-labeled organic phosphate compound would be absorbed after hydrolysis and that differences in uptake rates would parallel differences in root surface phosphatase activity. By measuring uptake rates and surface phosphatase activity of E. vaginatum roots from a number of tussock tundra sites differing in productivity, biomass, and soil and plant phosphorus concentrations, we examined the relationship between soil or plant phosphorus status and organic phosphate hydrolysis and uptake.

Methods

Field sites

Field research was conducted in Fairbanks, Alaska, and at Toolik Lake (68° 38'N, 149° 34'W, 760 m elevation) in the northern foothills of the Brooks Range in Alaska in tundra dominated by the tussock-forming sedge Eriophorum vaginatum. Samples were collected from natural and manipulated plots providing a range of soil and plant phosphorus concentrations. Three sites were used: a set of fertilized plots established by F.S. Chapin and G.R. Shaver; an area disturbed by vehicle passage; and an area of natural subsurface water movement.

Three tussocks were collected from each plot at each of the following sites and kept moist and cool until needed (not longer than 2 days) in late June and early July of 1986. Fertilization plots at Toolik Lake had been fertilized annually since 1980 by Chapin and Shaver (pers. com.) with 10 g N m⁻² (NH₄NO₃) and 5 g P m⁻² (P₂O₅). The vehicle track samples were taken from track and control plots at the base of Slope Mountain (68° 43'N, 149° 00'W, 655 m elevation) in a site disturbed by the passage of vehicles approximately 15 years previously (Chapin and Shaver 1981). Samples were collected from water tracks, areas of natural subsurface water movement, and from adjacent nontracks at a site 20 km north of Toolik Lake along the Dalton Highway (68° 45'N, 149° 34'W, 750 m elevation) (Chapin et al. submitted). At each of these sites, E. vaginatum in "treatment" plots (fertilized plot, vehicle track, water track) has higher production, biomass, and plant phosphorus concentrations than in adjacent control plots (Shaver et al. 1986, Chapin and Shaver 1981, Chapin et al. submitted).

Preparation of radiolabeled organic phosphate

The organic phosphorus compound used in this study, inositol hexaphosphate (phytate), was chosen primarily because of the large contribution of inositol phosphates to the organic phosphate pool of most soils (Halstead and McKercher 1975).

^{32}P -inositol hexaphosphate was prepared by a modification of the method of Martin (1970). Five grams of mung beans were allowed to germinate for 4 days with 400 μCi ^{32}P - NaH_2PO_4 and distilled, deionized H_2O (dH_2O). The germinating beans were then homogenized in a Waring blender (3 15-sec spurts) with 50 mL 0.2 N HCl at room temperature. Approximately 25 mL 0.2 N HCl was used to rinse the blender cup, and the homogenate was allowed to stand 10 min before centrifuging at 10,000 rpm for 15 min. Pellets were resuspended in 0.2 N HCl and recentrifuged; this was repeated with dH_2O . All supernatants were filtered through Whatman #2 filter paper, combined, and run on a 22x1 cm column of Biorad AG 1-X8 100-200 mesh (Cl^-) anion exchange resin.

The column was eluted with approximately 40 mL 0.2 N LiCl, 125 ml 0.4 N LiCl, and 125 mL 1.5 N LiCl (all LiCl solutions acidified). The peak eluting with 1.5 N LiCl was demonstrated to be phytate by autoradiographs of TLC plates run in 100:60 isobutyric acid:1 N ammonium hydroxide (Wood 1961) and comparisons with non-labeled standards of phytate and inorganic phosphate. The phytate fractions were lyophilized, desalted with 95% EtOH, lyophilized again, dissolved in 0.2 N HCl, and re-purified by anion exchange chromatography as above.

^3H -phytate was prepared in a similar fashion, germinating 5 g mung beans with 50 μCi ^3H -myo-inositol.

Purified substrates were stored as dry crystal or frozen slurry until needed and were then brought up to volume in 0.5 mM CaCl_2 . Concentrations given throughout the

paper refer not to the molarity of phytate, which has six phosphate groups, but to the molarity of phosphate itself.

Measurement of uptake

The terminal 8-12 cm of unsubsized first-year roots (Chapin et al. 1979) from tussocks were excised into 0.5 mM CaCl₂. Excised roots were put into cheesecloth "teabags" (Epstein et al. 1963) and held in 0.5 mM CaCl₂, aerated, at 11°C for 10-30 minutes prior to incubation with radiolabeled substrate. Each teabag contained an average of 57.4 mg dw.

The procedure for incubating roots was the same for all experiments and follows Procedure B of Epstein et al. (1963). Each teabag was incubated at 11°C for 10 min in 100 mL aerated 0.5 mM CaCl₂ solution made to the appropriate final concentration of ³²P-phytate or ³H-phytate (details given below). Teabags were transferred from the radioactive solution to 2 L aerated 0.5 mM CaCl₂, 50 μM NaH₂PO₄, and 50 μM P-phytate (non-radiolabeled), held for 1 min, transferred to 2 L of identical solution for 1 min, and then removed to drain on absorbent paper. Roots from each teabag were put in plastic scintillation vials, dried overnight at 80°C, weighed, and counted with 15 mL Biocount for 10 min on a BetaTrac 6895 scintillation counter. Two 1-mL samples from each radiolabeled solution used in uptake experiments were collected and counted in 15 mL Biocount to determine specific activity for each solution of ³²P-phytate or ³H-phytate. Post-incubation samples were taken from 180 μM P-phytate solutions to determine change in solution counts after uptake. Root and solution samples were counted a minimum of 3 times. Counts were corrected for quench and decay. Absorption

rates were calculated from the mean of at least 3 corrected counts and the specific activity of the solution used for each experiment.

Preliminary work to determine uptake kinetics and depletion of substrate from incubation solutions was done with 2 *E. vaginatum* tussocks collected from Goldstream Valley near Fairbanks, Alaska. Roots were excised and teabags prepared as described above. Roots were incubated in ^{32}P -phytate solutions of 1, 5, 10, 15, 20, and 60 μM concentrations for 10 min. Failure of cooling equipment during this experiment resulted in incubation temperatures ranging from 15 to 20°C. Solution samples were taken before incubation to determine specific activity and after root incubation to determine depletion of substrate. Eadie-Hofstee transformations of the uptake data were used to determine apparent K_m and V_{max} values.

Further experiments were done using roots from tussocks collected at Toolik Lake. Uptake experiments were done in the presence and absence of a commercial mixture of antibiotics to test for the contribution of rhizosphere microorganisms to hydrolysis and uptake (Gibco Laboratories Pen-Strep, .46 g L^{-1} for 50 μM P-phytate and .12 g L^{-1} for 10 μM P-phytate solutions). Possible inhibition of hydrolysis and absorption by inorganic phosphate was tested by doing uptake experiments in the presence of 50 μM NaH_2PO_4 . In a third set of assays, 2.7 and 27 μM ^3H -phytate (made from ^3H -inositol) was used instead of ^{32}P -phytate to determine whether *E. vaginatum* can absorb phytate intact. For each of these experiments, a total of 9 teabags was used for each treatment at each concentration of phytate, i.e. 3 teabags of roots from each of 3 tussocks.

For experiments using tussocks from the natural and manipulated plots (see Field Sites), six teabags of roots were made from each tussock. Uptake experiments were performed as described above using ^{32}P -phytate. Three teabags were incubated in 10 μM P-phytate and 3 in 50 μM P-phytate for roots from each plot.

Measurement of inorganic phosphate concentration

The concentration of inorganic phosphate left in each incubation solution was measured after each of four uptake experiments. Twenty-mL samples were taken from solutions of each concentration of labeled phytate prior to addition of roots and from the 9 individual incubation solutions after root incubation. These samples were assayed for inorganic phosphate according to Watanabe and Olsen (1965). Because phytate can interfere with this inorganic phosphate assay (Irving and Cosgrove 1970), standard curves done in the presence of phytate were used to determine absolute inorganic phosphate concentrations.

Root surface phosphatase activity measurement

Root surface phosphatase activity was measured using p-nitrophenyl phosphate (pNPP) as substrate (Woolhouse 1969) and modified universal buffer (Ho and Zak 1979). Roots were incubated at pH 4.0, 11°C for 1 hr in 50 μ M pNPP. Root tips for the assays were excised from the same tussocks used for uptake experiments.

Results and Discussion

This study provides the first evidence that Eriophorum vaginatum is capable of obtaining inorganic phosphate from an organic phosphate source through the action of enzymes produced by the plant itself. Previous work had suggested that root surface

phosphatases might contribute to the phosphorus nutrition of E. vaginatum (see Chapter 1); this study links enzyme-catalyzed hydrolysis of organic phosphate substrates with inorganic phosphate absorption.

Martin (1973) suggested that the high proportion of ^{32}P in wheat roots grown in ^{32}P -phytate may have been due to surface adsorption or passive diffusion of unchanged phytate into the free space of the roots. Experiments using ^3H -phytate (Figure 18) indicate that E. vaginatum roots do not absorb unhydrolyzed inositol hexaphosphate. The measurement of ^{32}P in E. vaginatum roots, then, is a measurement of that phosphate which is hydrolyzed from inositol hexaphosphate and then absorbed by the root.

Uptake experiments done with tussocks from Goldstream Valley near Fairbanks provided estimates of kinetic parameters (Figure 19). Measurement of ^{32}P in solution before and after root incubation demonstrated that only 7% depletion of substrate occurred during the 10-minute incubation period, indicating that substrate limitation was not increasing significantly during that time. The rest of the experiments were done using 10-minute incubations and concentrations from within the range tested with the Goldstream Valley root samples, 10 and 50 μM P-phytate. The apparent K_m determined by this assay, 2.6 μM , is lower by two to three orders of magnitude than K_m values determined using other substrates for E. vaginatum (Chapter 1), barley (Hall and Butt 1968), and clover (Dracup et al. 1984). The necessary use of artificially high substrate concentrations when using methods where product detection is less sensitive than with the use of radioisotopes may cause consistent overestimates of K_m .

Greaves et al. (1963) have estimated that 30 to 50 percent of the microorganisms present in soil and on plant roots can hydrolyze phytate to inorganic phosphate. However, uptake experiments done in the presence of a mixture of antibiotics in this study indicated that rhizosphere bacteria which continue to adhere to excised roots of

E. vaginatum do not make a significant contribution to phytate hydrolysis and inorganic phosphate absorption (Figure 20). Phosphatase activity or inorganic phosphate uptake measurements may actually be reduced by soil microbes. Uptake rates for roots incubated with 10 μM P-phytate were significantly higher in the presence of the mixture of penicillin and streptomycin than in its absence, indicating that microbes were successfully competing for inorganic phosphate once it was hydrolyzed from phytate. A similar effect was documented by Ridge & Rovira (1971) for wheat root phosphatases. For E. vaginatum roots incubated with 50 μM P-phytate, the antibiotics had no significant effect; at this higher concentration, the greater availability of inorganic phosphate may have masked any competition effect from microbial populations. Although we did not use specific fungicides in this study, there is evidence that E. vaginatum is not mycorrhizal (Wein 1973); Linkins (unpub) reports 3-5% infection of some roots with arbuscles. In general, our results agree with those of Martin (1973), who reported incorporation of inorganic phosphate from phytate by wheat roots to be unaffected by rhizosphere organisms.

The absorption of inorganic phosphate from phytate was not inhibited to any substantial degree by the presence of inorganic phosphate in the incubation medium (Figure 21). Earlier studies showed that root surface phosphatase activity (as measured by pNPP hydrolysis) is also insensitive to inhibition by inorganic phosphate (Chapter 1). ^{32}P uptake rates were reduced 15% in the presence of inorganic phosphate five times higher than the concentration of phytate P but were unaffected when concentrations of inorganic phosphate and P-phytate were the same. In arctic soils, solution organic phosphorus can be as much as 23 times the concentration of solution inorganic phosphate (Barel and Barsdate 1978); end product inhibition of organic phosphate hydrolysis is thus unlikely under field conditions.

Some differences in uptake rates were detected for roots from sites differing in productivity, plant nutrient status, and soil nutrient levels, but these differences did not parallel differences in phosphatase activity as measured by pNPP hydrolysis. ^{32}P uptake rates were of the same order of magnitude as root surface phosphatase activity measured by pNPP hydrolysis, but pNPP hydrolysis rates were consistently lower than uptake rates at comparable substrate concentrations. Uptake rates were significantly higher for E. vaginatum roots from control than for those from track plots at the vehicle track site for both 10 and 50 μM P-phytate incubations (Figure 22). Phosphatase hydrolysis rates measured with pNPP showed the opposite trend, but the difference was not statistically significant (Figure 22). There were no significant differences between uptake rates at either concentration for water track and nontrack roots, and the same was true for pNPP hydrolysis rates (Figure 23). However, uptake rates were significantly higher for fertilized tussock roots compared with control plot roots; again, there were no significant differences in pNPP hydrolysis rates (Figure 24).

The hydrolysis rates determined using roots incubated in pNPP were highly variable. The concentration of substrate used (50 μM) is a lower limit of the assay for this plant, and reliability of results is lower than at any higher concentration. The measurement of ^{32}P absorption is much more sensitive at low concentrations than is the measurement of pNPP hydrolysis. However, the results obtained using ^{32}P -labeled substrate are similar to those for pNPPase assays in which apparent V_{max} was determined for fertilized plots (Chapter 3) and water tracks (Chapter 2) but not for vehicle tracks (Chapter 2). There is some question about whether the two methods measure the same reaction at all; Tamura et al. (1982) found that high activity toward pNPP was not correlated with high activity toward phytate. Other researchers, however, have reported surface phosphatases active toward both pNPP and phytate (Bartlett & Lewis 1973).

The amount of inorganic phosphate in the incubation medium after root uptake experiments were completed was measured for the vehicle track and control samples, for the fertilization plot samples, and for "control" samples from a site near the fertilization plots. These results provided a measure of the difference between the rate of hydrolysis of phytate and the rate of inorganic phosphate uptake. With only one exception, the amount of inorganic phosphate measured in solution was greater than the amount of ^{32}P -phosphate absorbed (Table 2); the amount hydrolyzed and not absorbed exceeds that hydrolyzed and absorbed. Boutin *et al.* (1981) hypothesized that tomato cell wall phosphatases may function both to hydrolyze organic phosphate bonds and to transport the inorganic phosphate produced. The amount of inorganic phosphate hydrolyzed but not absorbed in this study suggests that this is not the case for *E. vaginatum*. Similarly, Bielecki and Johnson (1972) found evidence that absorption was not coupled to hydrolysis when they provided *Spirodela* with radiolabeled glucose-1-phosphate and found more labeled inorganic phosphate in the incubation medium than in the plant tissue. Dracup *et al.* (1984) have estimated that the surface phosphatases of subterranean clover can hydrolyze pNPP 600 times faster than the rate of phosphate absorption necessary for maximum growth. The fact that hydrolysis exceeds uptake in this study agrees with previous estimates that the root surface phosphatases of *E. vaginatum* have the capacity to hydrolyze more phosphate than can be used in a growing season (see Chapter 1). However, I hesitate to draw firm conclusions about the coupling between hydrolysis and uptake when those conclusions are based on a comparison of values determined by two different methods (radioisotope detection vs. the "molybdenum blue" phosphate assay).

This study provides evidence that the root surface phosphatases of *E. vaginatum* readily hydrolyze an organic phosphate compound such as inositol hexaphosphate. A proportion (6-99%) of the inorganic phosphate released through hydrolysis is rapidly

absorbed by the roots. Both inorganic and organic phosphate may be complexed with aluminum or iron and thus be rendered unavailable to plants under the acid conditions of most arctic soils (see Anderson et al. 1974). Martin & Cartwright (1971) have demonstrated that in soils of high phosphate retention, phosphorus from phytate is almost totally unavailable to ryegrass plants. In soils of low phosphate retention, however, phytate does provide a source of available phosphate. Some researchers have suggested that organic phosphates may move more rapidly than inorganic phosphate in soils (Hannapel et al. 1964), and this is an important characteristic for an element whose supply rate to plants is largely controlled by soil processes (Nye 1977).

E. vaginatum produces a new root system annually, and its root system is dense enough that nearly all the tussock soil volume is exploited by the end of the growing season (Chapin et al. 1979). An "average" tussock (23 cm diameter) has 550 cm² root surface area (J. Kummerow, pers. com.). Tundra soils average 11.5 $\mu\text{moles L}^{-1}$ soluble organic phosphorus (Barel & Barsdate 1978), which is 10-fold higher than the concentration of inorganic phosphate in tussock soils (K. Kielland, pers. com.). Calculations based on total root surface area and on the uptake rates determined here indicate that an E. vaginatum tussock could, at the pH and temperature conditions typical in tundra soils, absorb as much as 0.153 $\mu\text{moles min}^{-1}$ phosphate from organic phosphate sources, a substantial contribution to the total phosphorus requirement of the plant. Specific information concerning concentration and availability of phytates in tundra soils and the function of root surface phosphatases in organic phosphate hydrolysis and inorganic phosphate absorption under field conditions should be the focus of further research.

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Table 2. Fate of inorganic phosphate released by hydrolysis.

A comparison of the rates of inorganic phosphate absorption (measured by ^{32}P uptake) and release into the incubation solution (measured by methods of Watanabe and Olsen).

site	$\mu\text{M P-phytate}$	Rate of inorganic P released to soln.	Rate of inorganic P absorption	Percent of total hydrolyzed that is absorbed
$\mu\text{moles} \times 10^{-3} / \text{gdw} / \text{min}$				
control	10	.01	3.35	99.7
	50	1.6	9.56	85.7
fertil- izer plot	10	22	6.63	23.2
	50	64	18.49	22.5
vehicle control	10	175	33.92	16.2
	50	254	71.78	22.0
vehicle track	10	19	13.19	41.3
	50	343	22.27	6.1

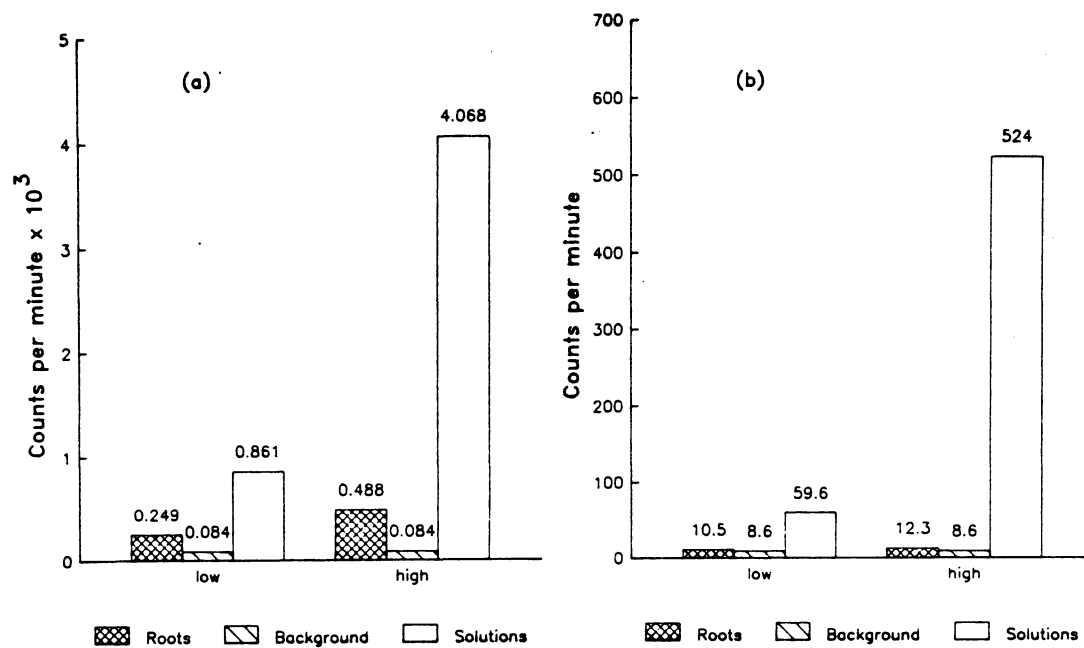


Figure 18. Uptake of unhydrolyzed inositol hexaphosphate: A comparison of uptake of label by roots from solutions of (a) ^{32}P -labeled inositol hexaphosphate (phytate) and (b) ^3H -labeled phytate. For ^3H -labeled phytate, counts of root samples incubated in radioactive solutions (counts shown) were not significantly different from background counts.

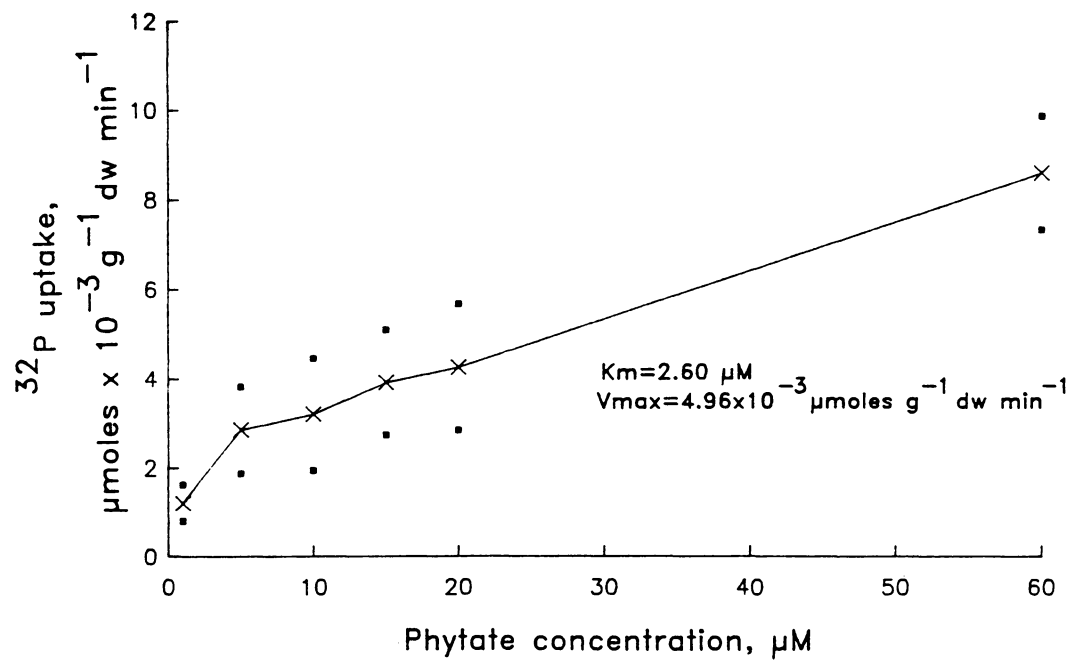


Figure 19. The kinetics of Pi uptake from phytate: The effect of phytate concentration on the rate of phosphate absorption from phytate. Absorption rate is $\mu\text{moles} \times 10^{-3} \text{g}^{-1} \text{dw min}^{-1}$.

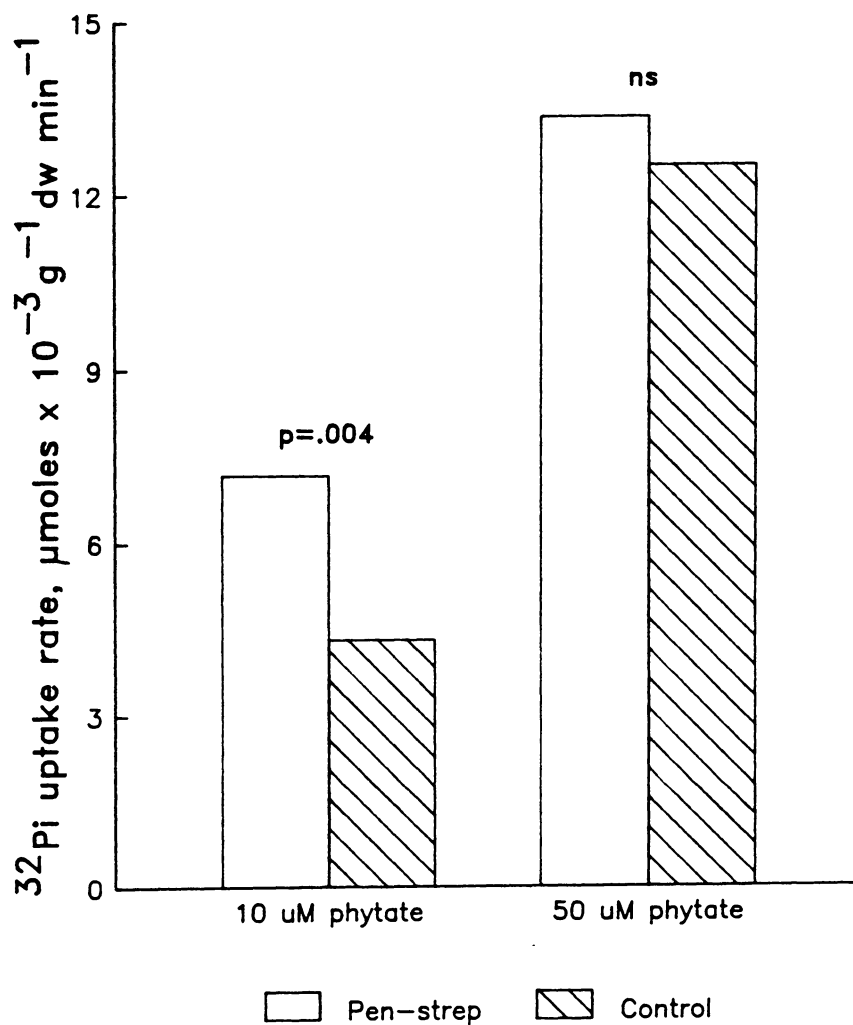


Figure 20. Effect of antibiotic on Pi uptake: ^{32}P -phosphate absorption rate in $\mu\text{moles} \times 10^{-3} \text{g}^{-1} \text{dw min}^{-1}$ at 10 and 50 μM P-phytate with and without antibiotic in incubation medium (see text for details).

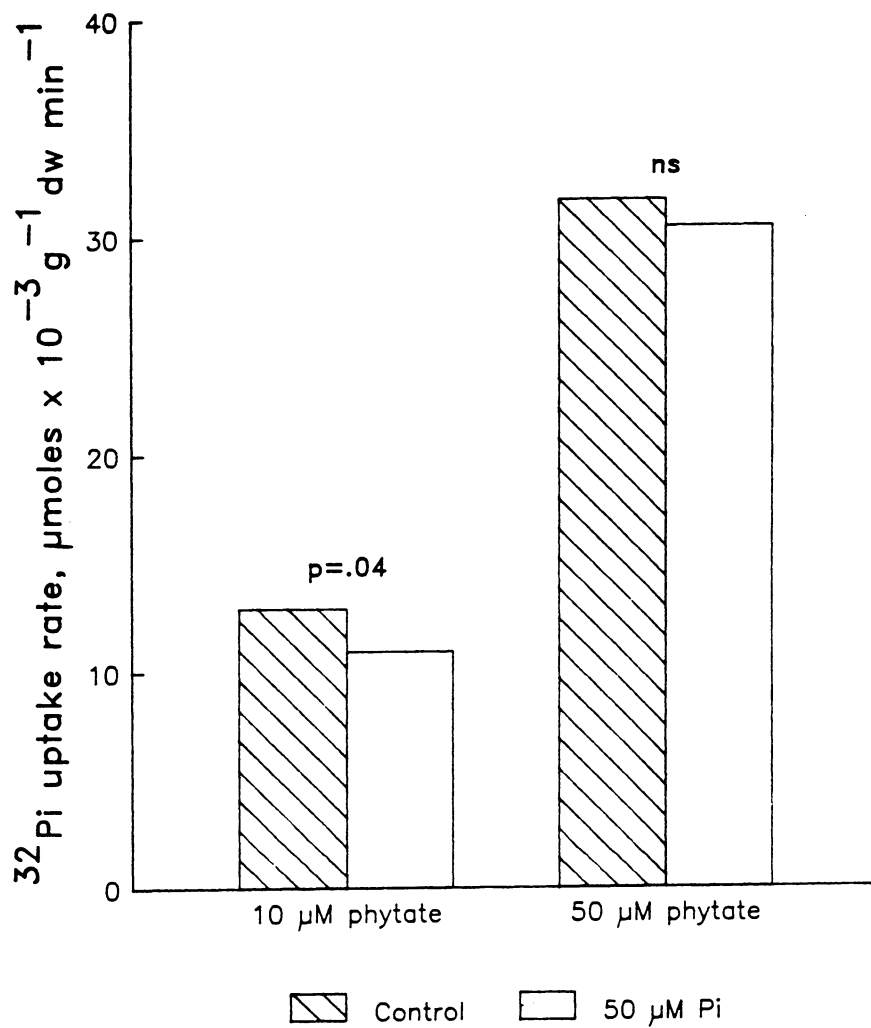


Figure 21. Effect of inorganic phosphate on phytate hydrolysis and uptake: ^{32}P -phosphate absorption rate in $\mu\text{moles} \times 10^{-3} \text{ g}^{-1} \text{ dw min}^{-1}$ at 10 and 50 μM P-phytate with and without inorganic phosphate in incubation medium (50 μM NaH_2PO_4 in both).

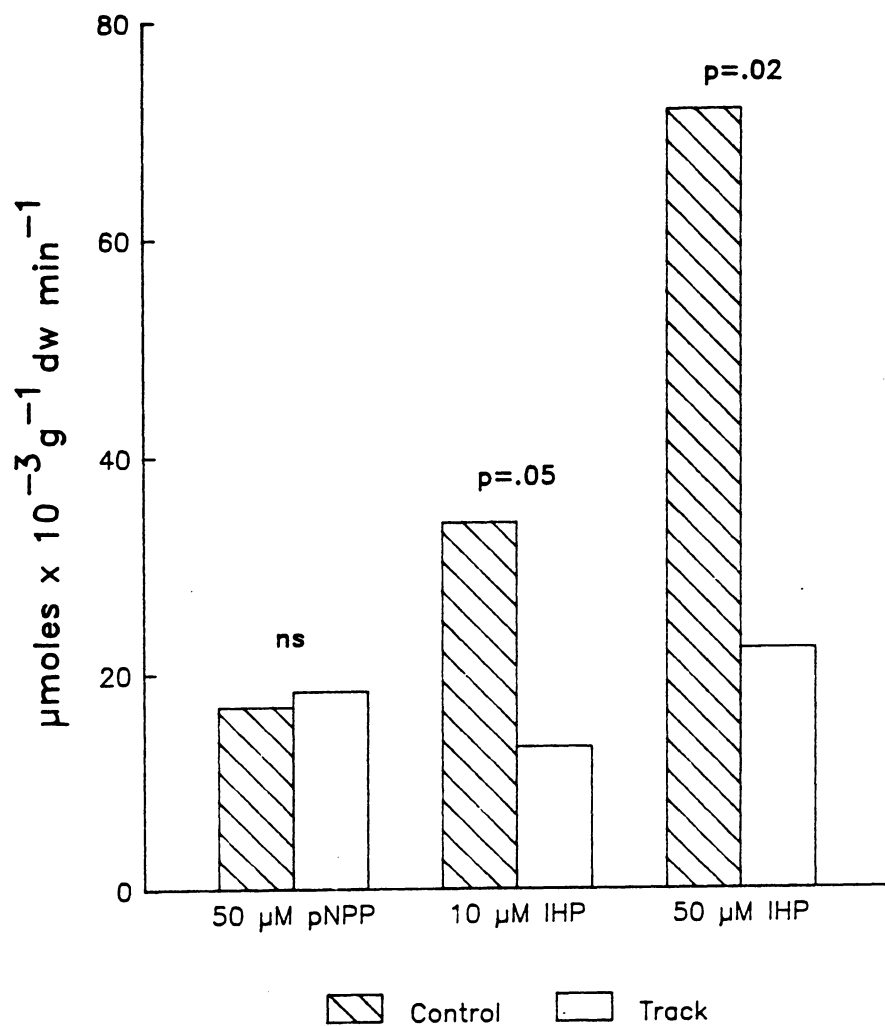


Figure 22. Comparison of absorption rates for plants at vehicle track site: ³²P-phosphate absorption rate in $\mu\text{moles} \times 10^{-3} \text{ g}^{-1} \text{ dw min}^{-1}$ at 10 and 50 μM P-phytate from vehicle track and control plots.

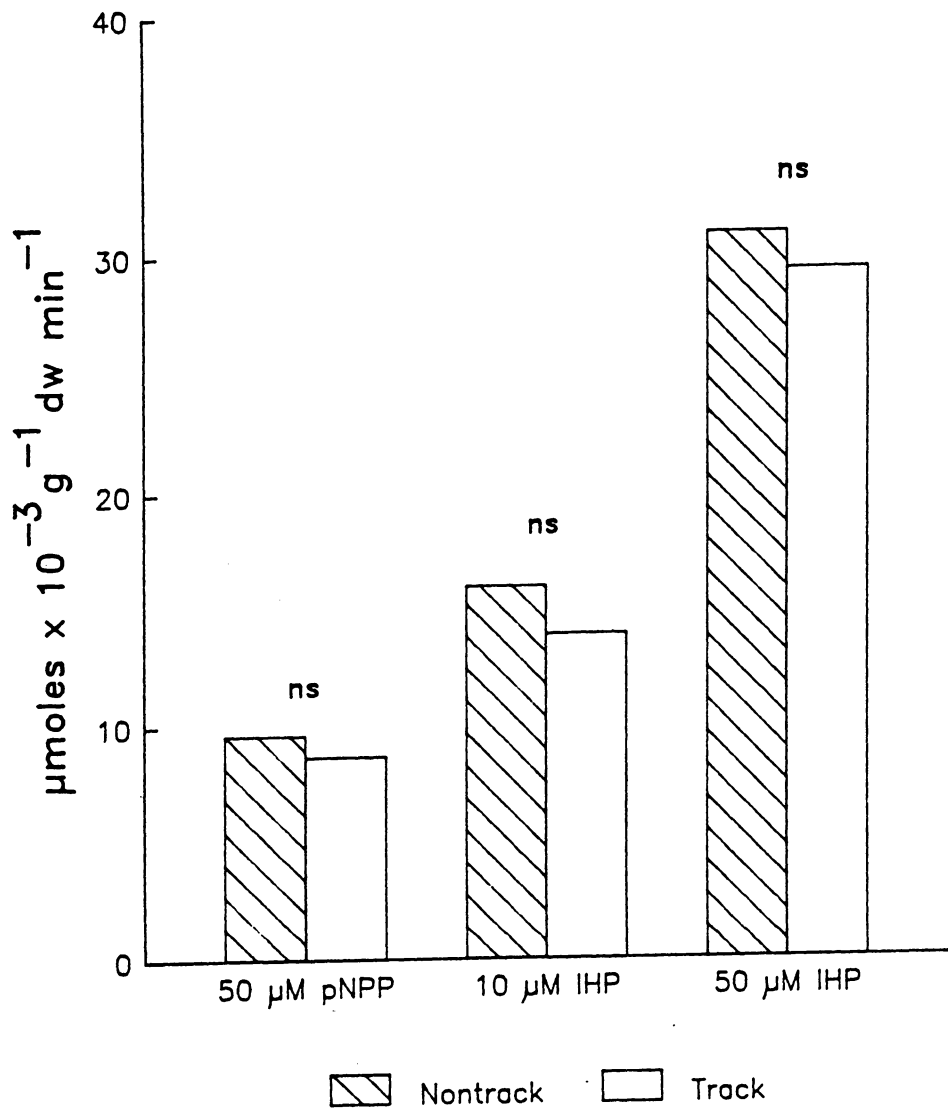


Figure 23. Comparison of absorption rates for plants at water track site: ³²P-phosphate absorption rate in $\mu\text{moles} \times 10^{-3} \text{ g}^{-1} \text{ dw min}^{-1}$ at 10 and 50 μM P-phytate from water track and nontrack plots.

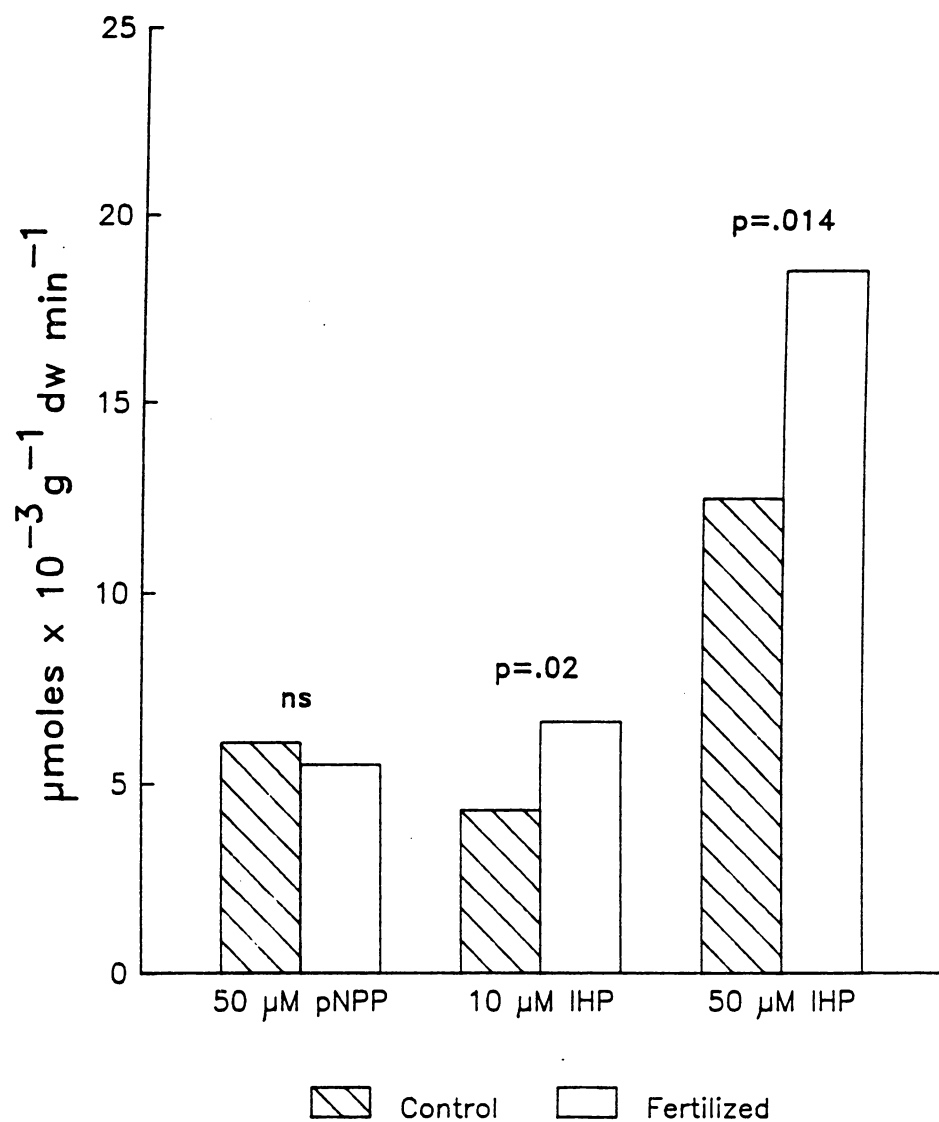


Figure 24. Comparison of absorption rates for plants at fertilizer plots: ³²P-phosphate absorption rate in $\mu\text{moles} \times 10^{-3} \text{ g}^{-1} \text{ dw min}^{-1}$ at 10 and 50 μM P-phytate from fertilized and control plots.

Chapter 5. The Effects of Organic and Inorganic Phosphorus Concentration on the Acid Phosphatase Activity of Ectomycorrhizal Fungi

Introduction

An increase in phosphorus accumulation and improvement of phosphorus status of higher plants caused by symbiotic association with mycorrhizal fungi has been well documented (Hatch 1937, Harley and McCready 1950, Rhodes and Gerdemann 1975). The principle factor limiting phosphorus uptake in most soils is thought to be the diffusion rate of inorganic phosphate to the root surface (Sutton and Gunary 1969, Nye 1977). Enhanced phosphorus uptake by mycorrhizae has been attributed primarily to mycorrhizal hyphae increasing the absorptive area of the root and expanding the total soil volume exploited for nutrient uptake (Sanders and Tinker 1971, Bowen *et al.* 1975).

The presence on ectomycorrhizal fungi of non-specific surface acid phosphatases capable of hydrolyzing a number of organic phosphorus compounds has invited speculation that mycorrhizae may contribute to the phosphorus nutrition of host plants in another way: this enzyme-catalyzed hydrolysis may produce inorganic phosphate from organic sources otherwise unavailable to the higher plant (Bartlett and Lewis 1973). The largest fraction of total soil phosphorus is organic, with much of that occurring as inositol phosphates (Dalal 1977); an organism's ability to use this phosphorus pool, especially in soils low in inorganic phosphate, might provide a considerable adaptive advantage.

The purpose of this study was to investigate the regulation of mycorrhizal acid phosphatases by inorganic and organic phosphate. The repression of acid phosphatases by inorganic phosphorus (or derepression by its absence) has been reported for Saccharomyces (Arnold and Garrison 1979), Candida utilis (Fernandez et al. 1981), Cryptococcus species (Greenwood and Lewis 1977), and Chlamydomonas reinhardi (Patni et al. 1977) as well as for the root surface phosphatases of higher plants (Woolhouse 1969, McLachlan 1980, Boutin et al. 1981, Dracup et al. 1984). Field studies by Alexander and Hardy (1981) showed an inverse correlation between extractable soil inorganic phosphorus and acid phosphatase activity on mycorrhizal roots of Sitka spruce. Some culture studies with ectomycorrhizal isolates, although done at phosphorus concentrations higher than those found in most soils, have corroborated these observations (Calleja et al. 1980, Lacaze 1983). However, Dighton (1983), who surveyed the phosphatase activity of eight ectomycorrhizal and two saprophytic fungal isolates grown in inorganic and organic phosphorus, concluded that phosphatase production was independent of the levels of inorganic or organic phosphate at which the fungi were grown.

Phosphorus is thought to be a limiting nutrient in many arctic tundra ecosystems, not because the total phosphorus capital is low but because much of it is tied up in organic forms (Chapin *et al.* 1978). Average solution inorganic phosphate concentrations may be as low as 0.5 micromolar, with soil solution organic phosphorus 23 times higher (Barel and Barsdate 1978). Mycelia of ectomycorrhizal fungi isolated from tundra soils, plants, or fruiting bodies have active surface acid phosphomonoesterases (Antibus *et al.* 1981), but whether these enzymes are produced or activated in response to the presence of organic phosphorus or absence of inorganic phosphorus is not known.

The objective of this study was to examine the acid phosphomonoesterase activity of ectomycorrhizal isolates of Cenococcum geophilum and Entoloma sericeum from phosphorus-poor arctic and temperate environments, looking specifically at the effects of providing inorganic and organic phosphorus at concentrations similar to those found in soils. We hypothesized that changes in either inorganic or organic phosphorus within the concentration range of natural soil solutions would affect both mycelial growth and the kinetic constants (apparent K_m and V_{max}) of surface and extracellular soluble acid phosphomonoesterases.

Materials and Methods

Four isolates of the ascomycete Cenococcum geophilum Fr. and one of the basidiomycete Entoloma sericeum (Bull. ex Merat) Quel. were grown in liquid medium of various organic or inorganic phosphate concentrations. Two of the C. geophilum isolates (one from Pinus virginiana in Maryland and the other from Salix rotundifolia

at Cape Simpson, Alaska) were assayed for surface and extracellular soluble phosphatase activity at 12-day intervals for 48 days. The Entoloma isolate (from Barrow, Alaska) and the other two C. geophilum isolates (from Barrow and Eagle Creek, Alaska) were assayed once: at 24 days for the Entoloma and Eagle Creek C. geophilum isolates and at 37 days for the slower-growing Barrow isolate. All isolates except the Maryland C. geophilum previously had formed typical ectomycorrhizae with the dwarf arctic willow Salix rotundifolia (Antibus et al. 1981). The effects of pH, temperature, and substrate concentration on these enzymes have been documented (Antibus et al. 1986).

Plugs 5 mm in diameter were cut from the edge of fungal colonies growing on plates of Hagem's medium (Antibus et al. 1986), divided in half, and transferred to 250-mL Erlenmeyer flasks containing 50 mL Hagem's medium amended as follows: a 4 mM citrate buffer (pH 5.6) was used in place of phosphate buffer, 3.8 mM KCl was added as a potassium source, and malt was omitted. Phosphorus was provided as orthophosphate (KH_2PO_4) or phytic acid (sodium inositol hexaphosphate) at concentrations of either 2 or 50 μM . Five flasks of each phosphorus treatment, each inoculated with five half-plugs, were analyzed at each sampling date.

The cultures were incubated at 20C in the dark. The pH of the medium after autoclaving was approximately 5.0 and declined with time after inoculation to a minimum of 4.5 at day 48.

Surface phosphatase activity was measured by removing individual mycelial mats from flasks, rinsing them three times with distilled water, blotting them on absorbent paper, and then incubating each mat in 2 mL of buffered p-nitrophenyl phosphate (pNPP) solution (pH 5.5) at 20C for three hours on a mechanical shaker (Ho and Zak 1979). Substrate concentrations used were 1, 5, 10, 25, and 50 mM pNPP. One plug from each of the five replicate flasks was assayed at each concentration. After incubation, 0.5 mL from each tube was added to 0.5 mL 0.5 N NaOH and vortexed.

The optical density at 410 nm was measured on a Gilford model 250 spectrophotometer. Assayed mycelial mats were rinsed in distilled water, frozen in liquid nitrogen, lyophilized, and weighed on a Cahn 26 electrobalance. Extracellular soluble acid phosphatase activity was assayed in a similar manner using aliquots of culture media. Residual inorganic phosphate in the medium at each sampling date also was measured (Bencini *et al.* 1983).

Phosphatase activity pH profiles were generated to determine whether across-treatment comparisons would be affected by pH optimum differences. Mycelial mats were incubated in saturating pNPP concentrations (50 mM); both substrate and buffer were adjusted to pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0.

Additional plugs of the Eagle Creek isolate of *C. geophilum* were grown in Hagem's medium (Antibus *et al.* 1986) for use in surface phosphatase inhibition experiments. Mycelial mats were incubated as described above in 5, 10, 25, 50, and 100 mM pNPP with 0, 5, 10, 25, 50, or 100 mM KH_2PO_4 in the incubation medium.

Enzyme activity was calculated and expressed in micromoles product (p-nitrophenol) per hour per mg dry mass of mycelia. This "surface activity" is comparable to the "accessible" activity of Calleja *et al.* (1980), which they found to be representative of cell wall phosphatase activity. Soluble activity was calculated and expressed in micromoles product per hour per mg dry mass of mycelia, based on total volume of culture medium and total dry mass of mycelia in each flask. Estimates of apparent K_m and V_{max} were determined from Eadie-Hofstee transformations of the enzyme activity data. Analysis of variance and Duncan's multiple comparison tests (SPSS Inc. 1983) were used to analyze the data. Apparent K_m and V_{max} were determined at each inorganic phosphate concentration for the inhibition experiments.

Results

Surface phosphatase V_{max} estimates for the two isolates assayed at 12-day intervals are presented in Figure 25. The isolates responded similarly to both inorganic (Pi) and organic (Po) phosphorus: V_{max} estimates were significantly higher for fungi grown in 2 μM Pi or Po than for those grown in 50 μM Pi or Po. V_{max} estimates increased first for 2 μM Po-grown fungi and continued to be higher through the end of the 48 days. The Maryland isolate of C. geophilum responded most to phosphorus deficiency; by day 24, the 2 μM Pi and Po treatments resulted in surface phosphatase V_{max} values at least six times higher than those of the 50 μM Pi and Po treatments, and by day 48 they were higher by 12 to 18 times.

Surface phosphatase V_{max} estimates for two of the three isolates assayed at one point in time also were higher for fungi grown in 2 μM Pi or Po than for those grown in 50 μM Pi or Po, although the differences for E. sericeum were not statistically significant (Table 3). E. sericeum, assayed at day 24, had V_{max} values between one and two orders of magnitude greater than did the C. geophilum isolates at day 24. V_{max} values determined for the Barrow isolate of C. geophilum at 37 days exhibited no pattern and no significant differences (Table 3). The poor growth exhibited by this isolate may account for the high variability in data.

The effect of phosphorus form and its concentration on growth rate for the Maryland and Cape Simpson isolates of C. geophilum is shown in Figure 26. For the first 24 days, there were few significant differences in growth for either isolate. After 48 days, dry mass of samples grown in 50 μM Pi or Po was approximately twice as high as for those grown in 2 μM Pi or Po. The Barrow isolate grew slightly faster in 50 μM Pi than in other treatments (Table 4). Differences for E. sericeum samples were not

statistically significant, and growth of the Eagle Creek isolate also was not affected by P treatments (Table 4).

Extracellular soluble phosphatase activity based on dry mass generally decreased with time. By day 48, activity was greater for the 2 μM Pi- and Po-supplied cultures than for the 50 μM Pi or Po cultures for both the Maryland and Cape Simpson isolates (Figure 27). For the other two isolates of *C. geophilum*, there were no clear trends (Table 3). Apparent V_{max} values for *E. sericeum* soluble phosphatases were significantly higher for 2 μM Po-grown samples than for the other three treatments (Table 3).

The relationship between extracellular soluble phosphatase activity and Pi concentration in either inorganic or organic phosphorus medium at each assay date is presented for the Maryland and Cape Simpson isolates grown at 50 μM P (Figure 28). Media prepared with inositol hexaphosphate had no measurable Pi at the beginning of the incubation period, but Pi concentrations increased with initial growth and soluble phosphatase activity increases. Inorganic phosphate concentration in the Pi treatment flasks, however, continued to decrease throughout the fungal growth period. Patterns of the level of soluble phosphatase activities were similar for both treatments.

We found no consistent relationship between apparent K_m estimates and P treatments. Values listed in Table 3 are representative of those found for all isolates assayed. K_m values determined for the Maryland isolate were significantly higher for mycelia grown in 2 μM Pi or Po than for those grown in 50 μM Pi or Po on day 48. For the Cape Simpson isolate, however, the reverse was true. Surface phosphatase K_m values of *E. sericeum* and the Eagle Creek and Barrow isolates of *C. geophilum* showed no significant differences (Table 3). There were no consistent trends in K_m estimates for extracellular soluble phosphatases for any of the isolates. All K_m estimates for soluble phosphatases were lower than those for surface phosphatases (see Table 3).

Surface phosphatase activities measured from pH 2.5 to 6.0 for the Maryland and the Cape Simpson isolates had optima at pH 5.0 or 5.5, with no statistically significant differences between the two pH values, regardless of the source or concentration of P.

Data from assays done with the Eagle Creek isolate to determine inhibition effects indicate competitive inhibition. Apparent K_m estimates for surface phosphatases of the Eagle Creek isolate of *C. geophilum* increased from 8.3 to 29.2 mM with increasing KH_2PO_4 concentrations in the incubation medium while V_{max} values remained the same.

Discussion

Both inducible and repressible phosphatases occur in a variety of plants and fungi. Though a number of compounds inhibit or activate the enzyme (Buchanan *et al.* 1979, Akiyama *et al.* 1981, Arnold and Garrison 1979), inorganic phosphate is probably the most important factor in the regulation of repressible acid phosphatases *in vivo* (Woolhouse 1969, Patni *et al.* 1977, Arnold and Garrison 1979, McLachlan 1980, Boutin *et al.* 1981, Fernandez *et al.* 1981, Dracup *et al.* 1984).

Our data indicate that inorganic phosphate is an important regulator of surface phosphatase activity for ectomycorrhizal fungi as well. The production of surface acid phosphatases by the isolates we examined was affected by changes in phosphorus concentration at the micromolar level. We found a consistent inverse relationship between phosphorus concentration in the medium and phosphatase activity. In some cases, the V_{max} values for isolates grown at 2 μM phosphorus were 18 times higher than those for isolates grown at 50 μM phosphorus. Our findings support those of earlier

culture studies done with different species and higher concentrations of inorganic phosphate (Calleja et al. 1980, Lacaze 1983).

We cannot draw conclusions about the effect of organic phosphate on surface or soluble phosphatase production. The isolates examined in this study responded to differences in phosphate concentration but not to differences in phosphate source. This result can be attributed to the presence of extracellular soluble phosphatases in the culture medium: the hydrolysis of inositol hexaphosphate to available inorganic phosphate by extracellular phosphatases prevented the mycelia in inositol hexaphosphate-supplied flasks from experiencing an inorganic phosphate deficiency. The presence of relatively large concentrations of inorganic phosphate in flasks to which only inositol hexaphosphate was added supports this hypothesis (see Figure 28); apparently hydrolysis rates were exceeding phosphate uptake rates. The early increase in surface phosphatase V_{max} values for the 2 μM Po-grown isolates of C. geophilum (Figure 25) may reflect a phosphate deficiency caused by increasing difficulty in the hydrolysis of lower inositol phosphates by extracellular enzymes.

The nearly identical levels of soluble phosphatase activity measured at 12 days suggest a constitutive enzyme, especially since early soluble phosphatase activity differed more among isolates than among treatments. Beaver and Burns (1980) suggest that the secretion of phosphatases into the external environment is an advantage only if the enzyme stays close enough to the cell to ensure that the inorganic phosphate released can be taken up. The extensive hyphal network formed by mycorrhizae may help to ensure that this condition is met.

The higher activities for surface and extracellular soluble phosphatases in the low-phosphorus flasks at later assay dates suggest that as phosphorus concentrations in either mycelia or media decrease, production of both surface and extracellular phosphatases increases. Apparently our "low P" treatment, 2 μM , was sufficient until

some of the original phosphorus supplied was depleted. The fact that dry mass differences due to phosphate source (inorganic or organic) were not significant at either concentration provides evidence that phosphatase production was induced before growth limitations began: surface and soluble enzyme activities increased significantly even when there were no significant differences between growth in organic and inorganic phosphate-supplied flasks. Analysis of internal metabolic and storage phosphorus pools might provide more definitive information about whether it is inorganic phosphate deficiency in the medium or in the fungus which is more important in the regulation of surface phosphatase production and activity.

The apparent K_m estimates determined in this study provide no clear evidence for the production of new phosphatase isozymes in response to phosphorus treatment. Estimates of K_m were higher by three orders of magnitude than typical soil solution organic phosphate concentrations. A K_m larger than the concentration of substrate usually available may allow for faster hydrolysis of occasional large additions of substrate (Price and Stevens 1982); in our study, high K_m values may be the result of using an artificial substrate (pNPP) at unrealistically high concentrations. The K_m values of extracellular soluble phosphatases were consistently lower than those for surface phosphatases, reflecting differences between bound and soluble enzymes. The increase in K_m estimates associated with increasing concentrations of KH_2PO_4 in the assay medium indicates a second level of regulation of surface phosphatases: competitive end-product inhibition by inorganic phosphate.

The difference in magnitude of response to phosphorus treatments between the Maryland and Cape Simpson, Alaska, isolates of C. geophilum may indicate adaptation to different edaphic conditions. The difference between the Cenococcum isolates and E. sericeum, which had surface V_{max} values two orders of magnitude larger than those

of Cenococcum, supports the suggestion that ectomycorrhizal fungi differ in their ability to enhance phosphorus uptake (Ho and Zak 1979).

The importance of organic phosphate as a phosphorus source for plants has been investigated by a number of researchers (Tate 1984). Anderson et al. (1974) suggested that the high stability of inositol hexaphosphate in soils and its resistance to decomposition were responsible for its relatively high concentration in many soils. Greenwood and Lewis (1977) found that species of soil yeasts able to utilize sodium and calcium salts of inositol hexaphosphate were unable to use the insoluble aluminum and iron salts; these insoluble forms may predominate in many soils. The demonstration that mycorrhizal fungi can utilize some forms of this organic phosphate compound as a sole source of phosphorus (Theodorou 1968, Mitchell and Read 1981, Dighton 1983) suggests that mycorrhizae may contribute to the decomposition of inositol hexaphosphate and that it in turn may contribute to the phosphorus nutrition of mycorrhizal plants. The fact that the activity and V_{max} values of surface and extracellular phosphatases change in response to phosphorus concentration at the micromolar level supports the hypothesis that available soil phosphorus plays a role in the regulation of ectomycorrhizal phosphatase production.

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Table 3. Mycorrhizal isolate phosphatase Vmax estimates.

Maximum velocity in $\mu\text{moles mg}^{-1} \text{hr}^{-1}$ and Michaelis-Menten constant (Km) in mM determined by Eadie-Hofstee transformations of acid phosphatase activity data for C. geophilum (Eagle Creek: Vmax $\times 10^{-2}$ for both surface and soluble values; Barrow: Vmax $\times 10^{-2}$ for surface, $\times 10^{-1}$ for soluble values) and for E. sericeum (soluble Vmax values $\times 10^{-1}$).

Treatment	Vmax, surface	Vmax, soluble	Km, surface	Km, soluble
<u>Entoloma</u>				
<u>sericeum:</u>				
2 μM Pi	4.69a*	8.31a	19.12a	1.98a
50 μM Pi	1.82a	3.95a	16.77a	1.51a
2 μM Po	5.39a	15.07b	20.66a	1.88a
50 μM Po	3.16a	6.67a	24.28a	1.54a
Eagle Creek:				
2 μM Pi	2.60a	6.78a	9.15a	4.90a
50 μM Pi	1.34b	6.68a	6.58a	5.01a
2 μM Po	2.43a	6.65a	10.11a	5.38a
50 μM Po	1.33b	7.87a	7.08a	5.36a
Barrow:				
2 μM Pi	3.55a	1.75a	10.60a	4.85a
50 μM Pi	3.24a	1.60a	12.80a	5.87a
2 μM Po	3.41a	1.86a	13.37a	5.40a
50 μM Po	3.09a	1.89a	12.57a	5.48a

*Numbers followed by the same letter within each column at each isolate are not significantly different ($p \leq .05$).

Table 4. Mycorrhizal isolate growth data.

Dry mass per flask in mg at two concentrations of Pi and Po for C. geophilum (Eagle Creek and Barrow isolates) and E. sericeum.

Treatment	Entoloma sericeum	C. geophilum, Eagle Creek	C. geophilum, Barrow
2 μ M Pi	16.92a*	4.96a	2.95a
50 μ M Pi	21.92a	4.83a	3.84b
2 μ M Po	15.31a	5.43a	2.93a
50 μ M Po	18.33a	4.61a	2.96a

*Numbers followed by the same letter within each column are not significantly different ($p \leq .05$).

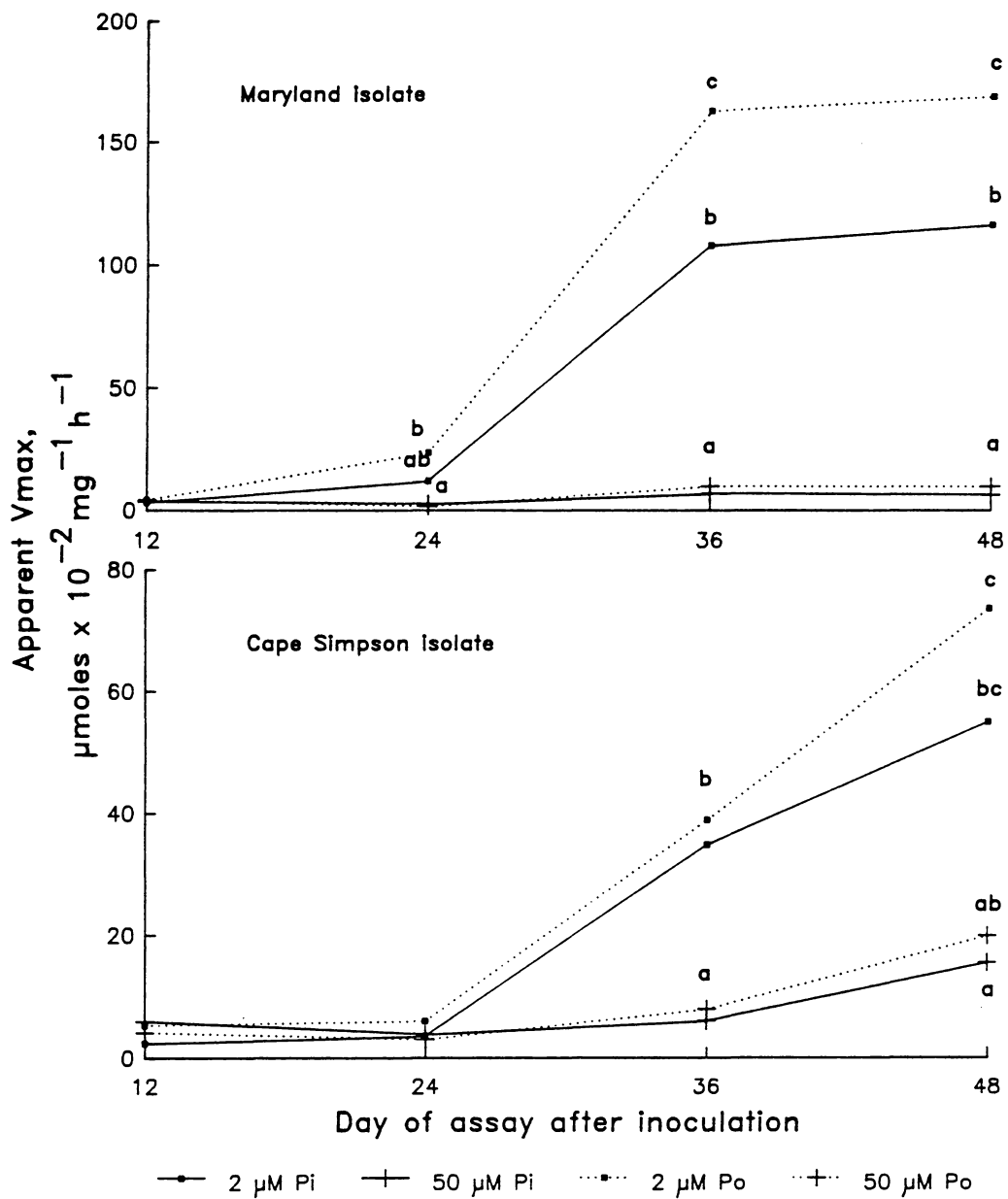


Figure 25. V_{max} estimates for surface phosphatases: V_{max} estimates in $\mu\text{moles} \times 10^{-2} \text{mg}^{-1} \text{hr}^{-1}$ determined by Eadie-Hofstee transformations of acid phosphatase activity data for *C. geophilum*.

a) Maryland isolate.

b) Cape Simpson isolate.

Points followed by the same letter within each assay date are not significantly different ($p \leq .05$).

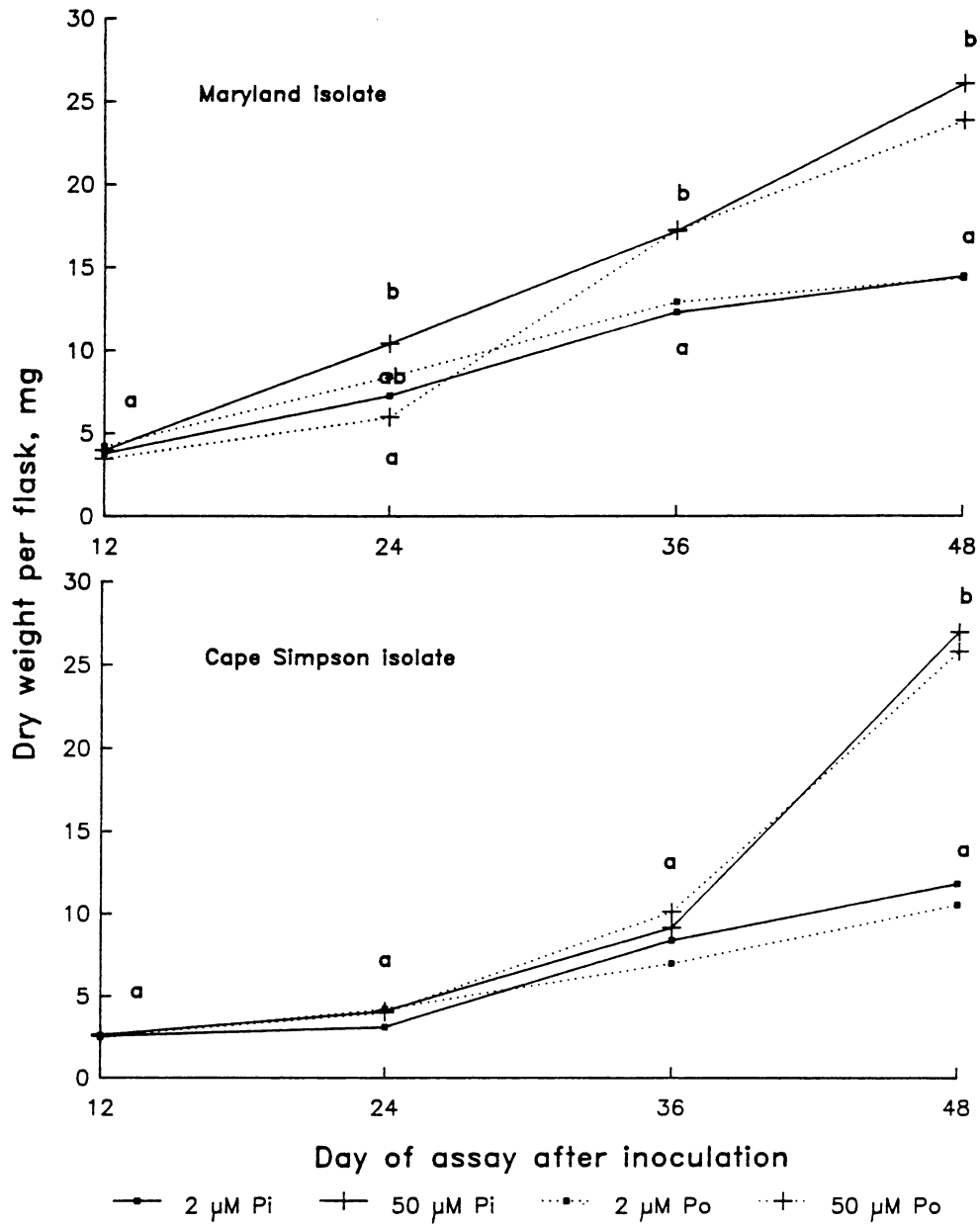


Figure 26. Growth of isolates: Total dry mass per flask, mg, for *C. geophilum*.

a) Maryland isolate.

b) Cape Simpson isolate.

Points followed by the same letter within each assay date are not significantly different ($p \leq .05$).

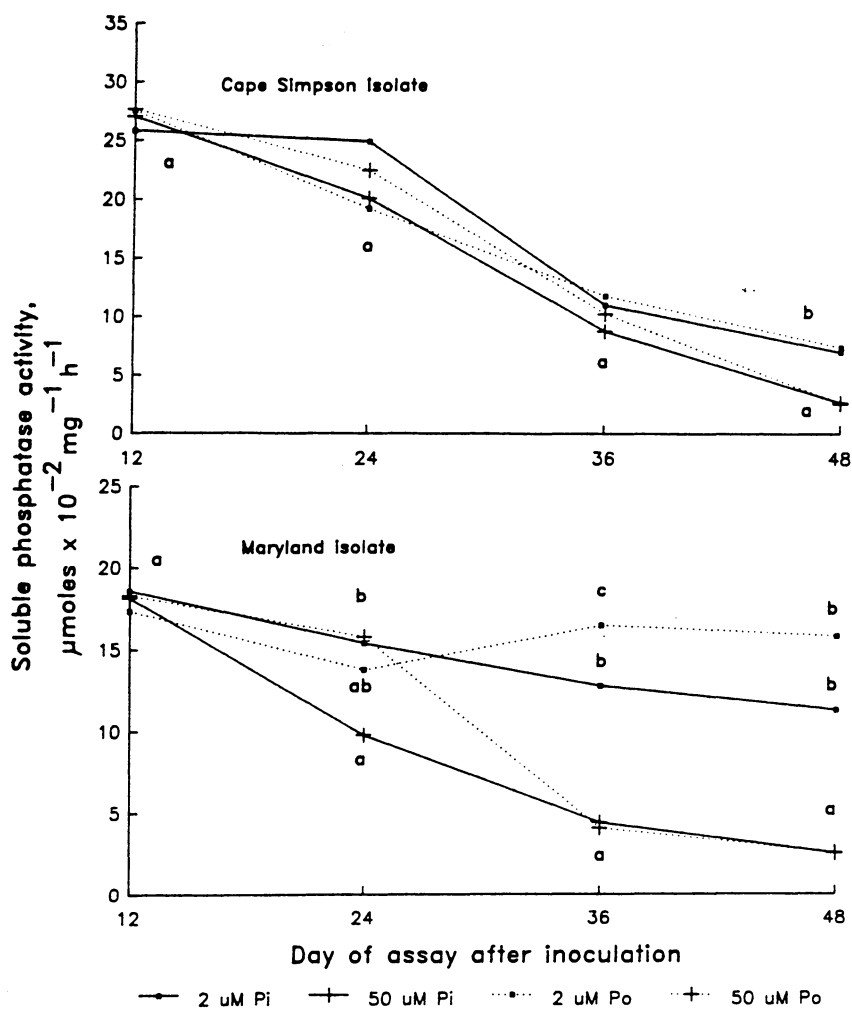


Figure 27. Extracellular soluble phosphatase activity: Extracellular soluble phosphatase activity determined at 50 mM pNPP for *C. geophilum*, based on total dry mass of mycelia in each flask ($\mu\text{moles mg}^{-1} \text{hr}^{-1}$).

- a) Maryland isolate.
 - b) Cape Simpson isolate.
- Points followed by the same letter within each assay date are not significantly different ($p \leq .05$).

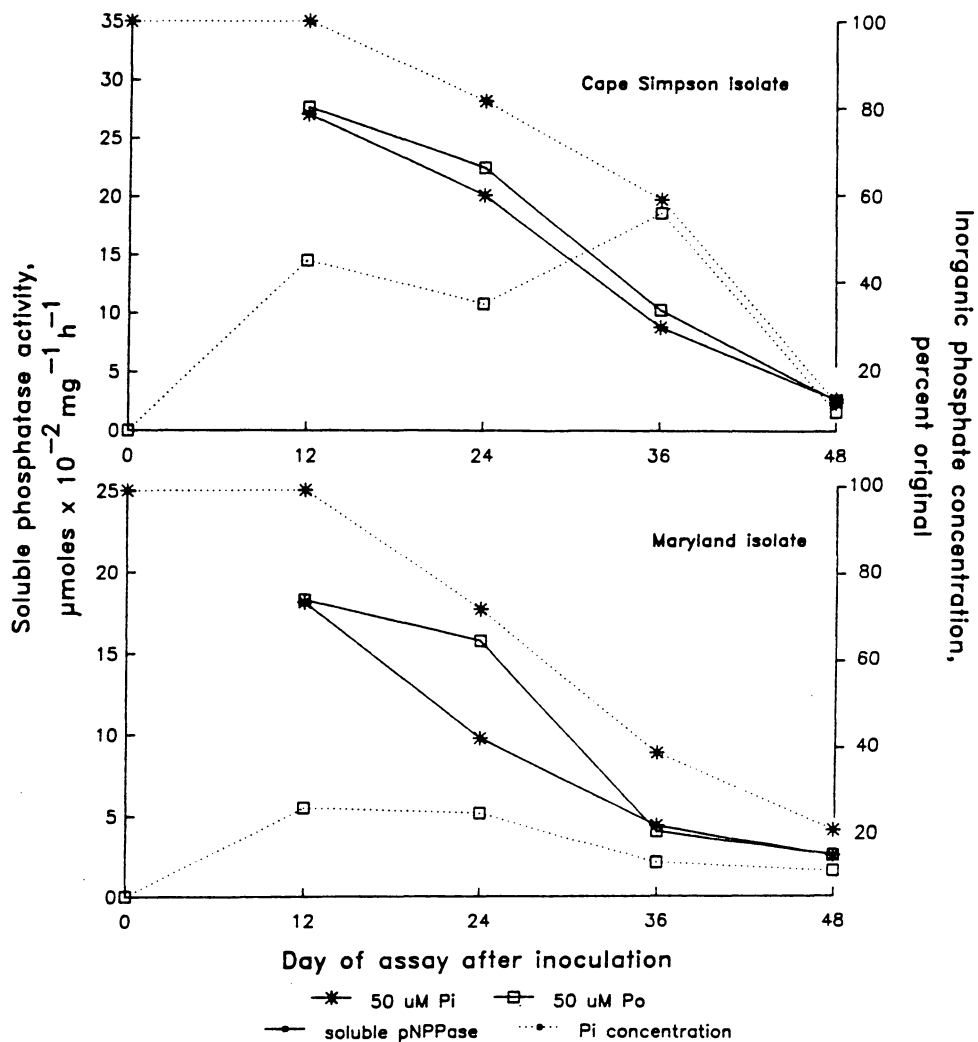


Figure 28. Extracellular phosphatase activity and inorganic phosphate: Inorganic phosphorus concentration (as percent of 50 μM)(broken line) and extracellular soluble phosphatases (solid line) in 50 μM Pi and Po treatment flasks for *C. geophilum*
 a) Maryland isolate.
 b) Cape Simpson isolate.

Chapter 6. A Survey of the Surface Acid

Phosphatase Activity of Ectomycorrhizal Roots of Arctic Willow and Birch

Introduction

The relationship between the production of acid phosphatases by ectomycorrhizal fungi and the levels of phosphate at which they are grown has been found to be inverse by some researchers (Alexander and Hardy 1981, Calleja *et al.* 1980, Lacaze 1983) and independent by another (Dighton 1983). In a previous study with isolates of the ectomycorrhizal fungi Cenococcum geophilum and Entoloma sericeum from arctic tundra ecosystems (Chapter 5), I found that fungi grown in low inorganic or organic phosphate media had higher surface phosphatase activity than those grown at higher phosphate levels. In this chapter, the surface phosphatase activity associated with the ectomycorrhizal roots of Salix and Betula species in northern Alaska are examined. My

objectives were to compare the phosphatase activity of the ectomycorrhizal roots of the two species 1) for different "color types" or fungal types on the same plant species and 2) of the same plant species and color types for individuals growing in areas differing in soil type, soil moisture, productivity, and rate of phosphorus turnover.

Methods

Field research was conducted during the summers of 1982 and 1983. Three sites were used for collecting Salix or Betula specimens for ectomycorrhizal roots:

- 1) Toolik Lake (68° 38'N, 149° 34'W, 760 m elevation) located in the northern foothills of the Brooks Range in Alaska, where Betula excilis plants were collected from both tussock tundra and heath sites and Salix phlebophylla was collected from the heath for a comparison of ectomycorrhizal root color types;
- 2) a natural water track system approximately 7 miles north of Toolik Lake along the Dalton Highway (68° 45'N, 149° 34'W, 750 m elevation) on a 5° north-facing slope (see Chapin *et al.* submitted for a complete site description), where Betula was collected to compare phosphatase activity for plants growing in the water track with that for those growing in adjacent nontrack tundra; and
- 3) Eagle Creek in central Alaska (65° 26'N, 145° 30'W, 760 m elevation), where Salix roots were collected from three sites differing in slope angle, drainage, soil composition, organic soil depth, and vegetation cover ("fellfield" refers to the dry, rocky top of the slope; "cassiope" to a zone part way down the slope and intermediate in moisture and drainage; and "snowfield" to a zone at the bottom of the slope which is covered by a snowbank until later in the season and thus wetter than the other zones).

Once plant samples were collected, ectomycorrhizal roots were excised into 0.5 mM CaCl₂ (Epstein *et al.* 1963) and held until enough roots were found to begin an assay (not longer than 4 hours). Surface acid phosphomonoesterase activity was measured using p-nitrophenyl phosphate (pNPP) as substrate (Woolhouse 1969). Roots were transferred from the CaCl₂ solution to a 2.0-mL buffered pNPP solution (Ho & Zak 1979) and incubated at pH 5.5 and 10-12°C. Incubation times varied but were the same for each set of assays to be compared. Substrate concentrations fell within the range 0.1-150 mM and are given on figures.

After incubation, 0.5 mL from each reaction vial was added to 0.5 mL 0.5 N NaOH and mixed. Optical density at 410 nm was measured on a Bausch & Lomb Spectrophotometer 88. Root lengths and diameters were measured using a dissecting microscope. Approximate surface areas were calculated using the formula for a cylinder without ends. Enzyme activity was expressed as μmoles p-nitrophenol produced mm⁻² h⁻¹. Enzyme activity differences were tested by a two-way factorial analysis of variance with substrate concentration and "treatment" (color type or site, depending on the question) as factors or at each substrate concentration with color type and site as factors.

Results and Discussion

At the three sites examined in this study, the major differences in surface acid phosphatase activity of ectomycorrhizal roots were related to color type differences and not to site differences.

Betula ectomycorrhizal roots of the same color type (white) collected from a dry heath site and a wet tussock site at Toolik Lake had nearly identical surface acid phosphatase activity (Figure 29). Salix ectomycorrhizal roots of different color types (white, brown, black) collected at the heath site showed much more variability in surface phosphatase activity (Figure 30).

At the water track site, Betula ectomycorrhizal roots had significantly different phosphatase activity in the track as compared to adjacent nontrack samples, but this difference was in opposite directions for the two color types assayed (Figure 31).

Similarly, Eagle Creek samples showed some differences according to the sites from which they were collected, but this differed with color type. There were no significant differences among black roots from the three sites (Figure 32a), but white roots had significantly different activities at some substrate concentrations (Figure 32b). Analysis of variance for all data, using site and color type as factors, showed a significant color type effect and no site effect at each substrate concentration. However, as at the water track site, the responses of color types were different at different sites (Figure 33): for snowfield samples, surface phosphatase activity was significantly higher for black roots than for white roots, but for fellfield and cassiope samples the opposite tended to occur, at least at higher substrate concentrations.

Although the specific levels of available soil phosphate were not measured at each of these sites, other soil characteristics related to phosphate availability such as moisture, organic matter content, depth of thaw, water movement, and nutrient turnover rates differ considerably. Despite these differences, phosphatase activity did not seem to be related to site characteristics. Where site differences were significant, there was no consistent trend in the relationship between site and surface phosphatase activity. In fact, this relationship tended to reverse itself for the different color types assayed. It appears that the extent of infection by various fungal types would be more important in

determining the total phosphatase activity of an ectomycorrhizal root system than are changes in the enzyme activity of any one type in response to soil microsite differences.

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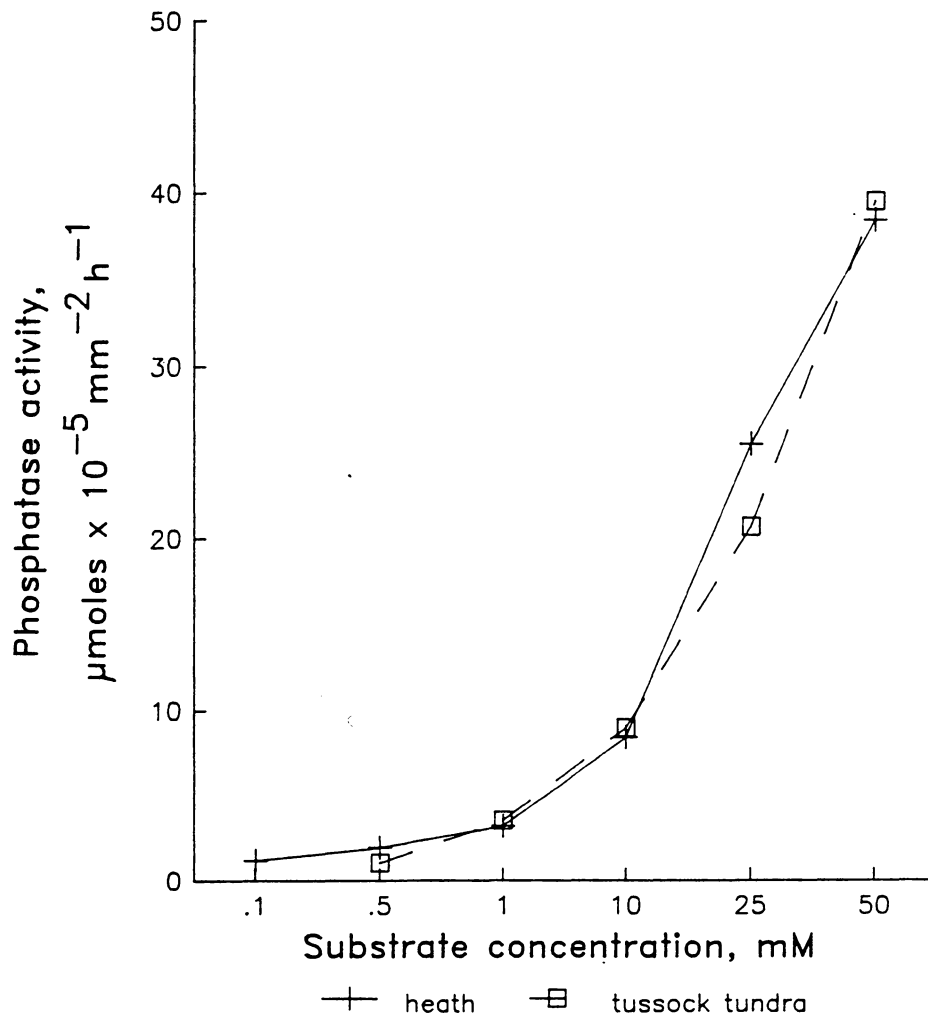


Figure 29. Birch surface acid phosphatase activity, Toolik Lake: Phosphatase activity ($\mu\text{moles} \times 10^{-5} \text{mm}^{-2} \text{h}^{-1}$) for *Betula* ectomycorrhizal roots collected at a dry heath site (solid line) or wet tussock site (broken line) at Toolik Lake.

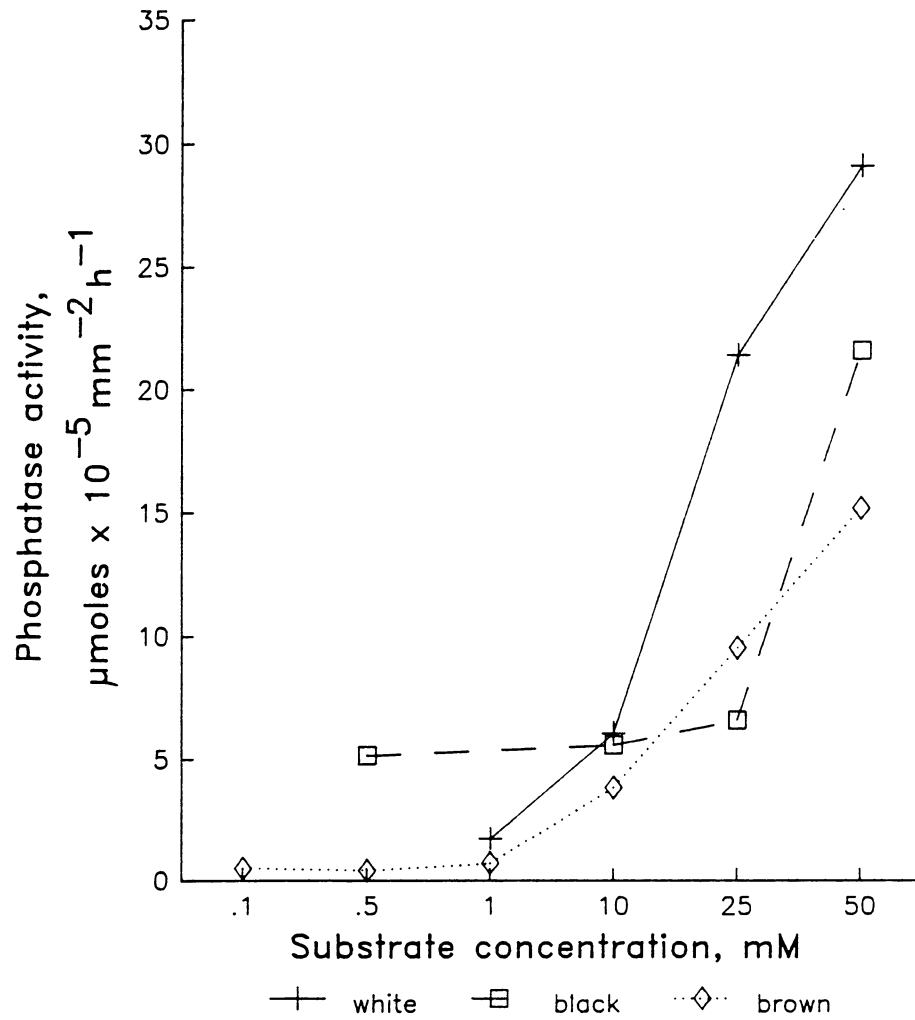


Figure 30. Willow surface acid phosphatase activity, Toolik Lake: Phosphatase activity ($\mu\text{moles} \times 10^{-5} \text{mm}^{-2} \text{h}^{-1}$) for *Salix* ectomycorrhizal roots of different color types (white, black, brown) collected from heath site at Toolik Lake.

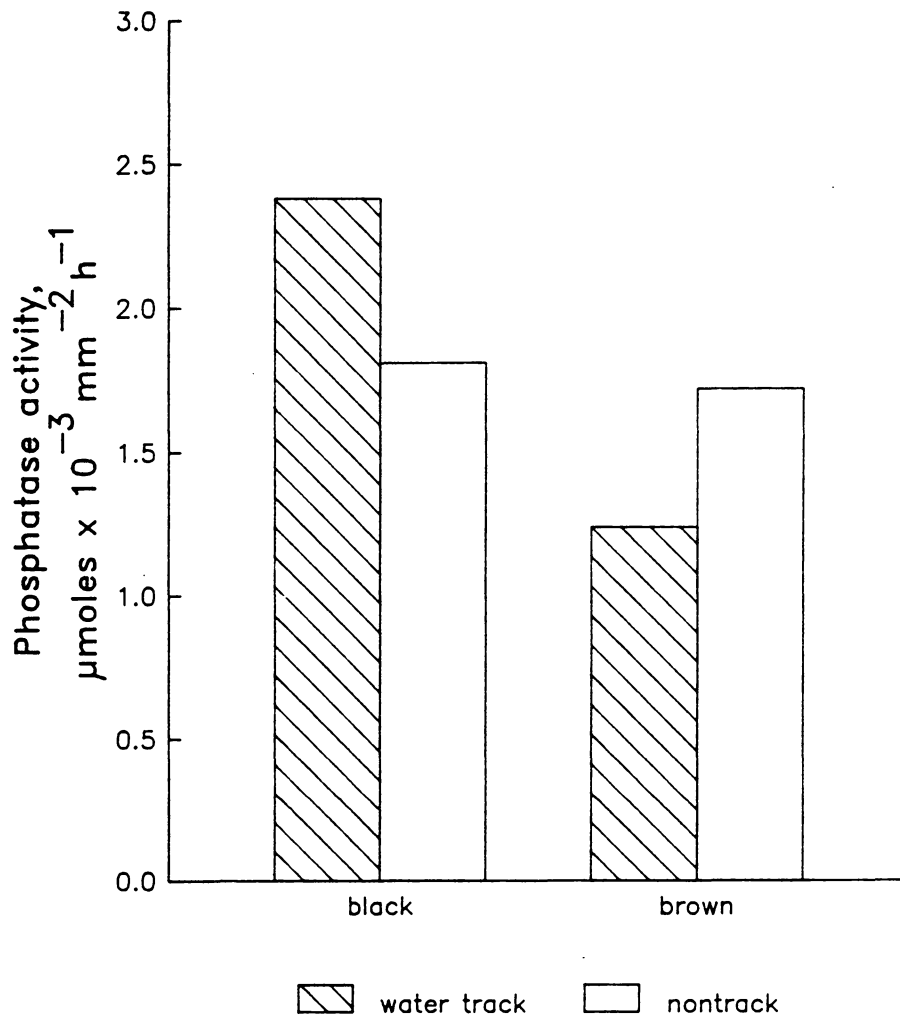


Figure 31. Birch surface acid phosphatase activity, water tracks: Phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{ mm}^{-2} \text{ h}^{-1}$) for *Betula* ectomycorrhizal roots of different color types (black, brown) collected from water tracks or from adjacent nontrack tundra.

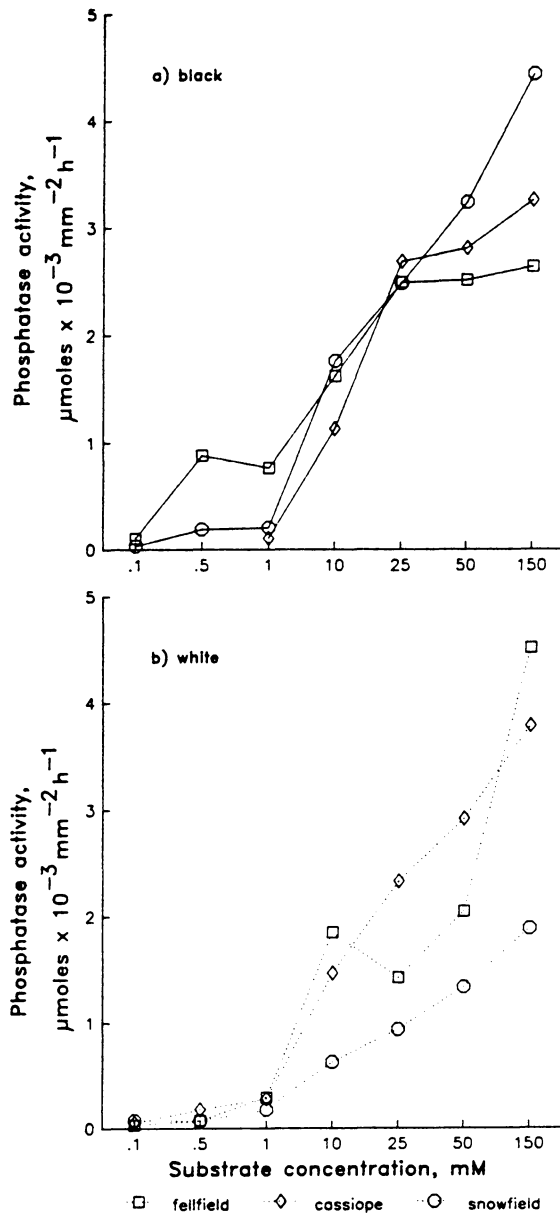
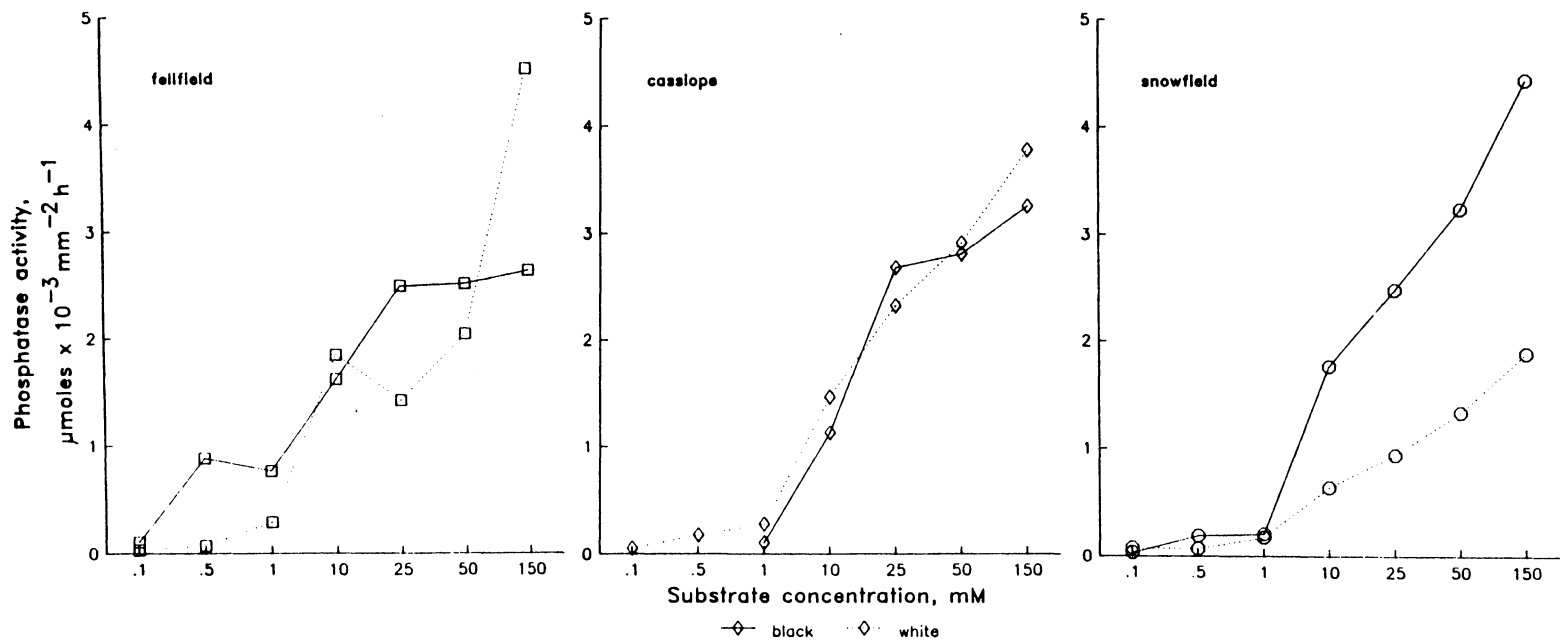


Figure 32. Willow phosphatase activity by sites, Eagle Creek: Surface acid phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{ mm}^{-2} \text{ h}^{-1}$) for *Salix* ectomycorrhizal roots collected from fellfield, cassiope zone, and snowfield at Eagle Creek: a) black ectomycorrhizal roots; b) white ectomycorrhizal roots.

Figure 33. Willow phosphatase activity by color types, Eagle Creek: Surface acid phosphatase activity ($\mu\text{moles} \times 10^{-3} \text{ mm}^{-2} \text{ h}^{-1}$) for Salix ectomycorrhizal roots of different color types (white, black) at Eagle Creek fellfield, cassiope zone, and snowfield.



Summary

Eriophorum vaginatum L. subsp. spissum (Fern.) Hult., a dominant plant in arctic tundra ecosystems, has root surface acid phosphatase activity evenly distributed from the root tip to a distance at least 16 cm from the tip. These root surface phosphatases have optimal activity from pH 3.5 to 4.0; mean soil pH for soil samples collected with roots was 3.69. Apparent energy of activation and Q_{10} values ($14.0 \text{ kcal mol}^{-1}$ and 2.2, respectively) do not provide evidence for temperature acclimation, but substantial phosphatase activity was measured at 1°C . Kinetic parameters determined for this root surface phosphatase were as follows: $K_m = 9.23 \text{ mM}$, $V_{\text{max}} = 1.61 \times 10^{-3} \text{ } \mu\text{moles mm}^{-2} \text{ h}^{-1}$. The presence of inorganic phosphorus in the assay medium did not inhibit root surface phosphatase activity except at very high concentrations (100 mM); even then, only slight inhibition was detected (7 to 19%). A comparison of hydrolysis rates with inorganic phosphate assimilation rates measured for E. vaginatum indicates that organic phosphate hydrolysis may occur as rapidly as inorganic phosphate absorption. Calculations show that inorganic phosphate produced by root surface phosphatase activity may satisfy approximately 65% of the annual phosphate demand of E. vaginatum. Since arctic tundra soils are typically higher in dissolved organic phosphorus

compounds than in inorganic phosphate, this root surface phosphatase activity may make a considerable contribution to the phosphate nutrition of E. vaginatum.

In addition to its root surface phosphatases, E. vaginatum has acid phosphatase activity associated with all the below-ground and ground-surface tussock components: dead roots from previous seasons, leaf sheaths, and tussock soil. Although live roots have the highest phosphatase activity on a dry weight basis, the significantly greater mass of the other tussock components results in a significantly greater contribution to the total phosphatase activity (and potential organic phosphate hydrolysis) of the tussock. As the tussock-soil volume is composed primarily of previous seasons' roots in various stages of decomposition, the phosphatase activity associated with these components may make a sizeable contribution to the efficient recycling of nutrients within the tussock.

The root surface phosphatases of Eriophorum growing in vehicle tracks (areas disturbed by vehicle passage) and water tracks (areas of natural sub-surface water movement) did not behave in analagous fashion despite many other similarities between the sites. Activity measured for root surface phosphatases from vehicle track plants were consistently higher than those for control plants. Water track plants, however, did not have higher root surface phosphatase activity than non-track plants at all assay dates, and differences were not always statistically significant.

Phosphatase activities for E. vaginatum growing in plots fertilized with nitrogen, phosphorus, and potassium were higher for every tussock component (live roots, dead roots, leaf sheaths, soil) assayed. Root surface phosphatases responded similarly to the same treatments and to lower levels of phosphorus and nitrogen applied annually, but activity was not significantly affected by fertilization with inorganic or organic phosphate alone. Because nitrogen is the most strongly limiting nutrient at the study site, fertilization caused increased growth which in turn caused increased demand for

phosphate. These results are in opposition to those reported for other species, where it was found that soil or plant phosphorus is inversely related to plant phosphatase activity. In this study, Eriophorum plants with higher tissue phosphorus concentration growing in soils with higher available phosphorus levels had higher, not lower, phosphatase activity. In addition, seasonal and yearly variation in results was greater in some cases than differences among treatments plots. The phosphatases of E. vaginatum would not provide a reliable measure of plant or soil phosphorus levels as other researchers have proposed for other species.

Experiments with a radiolabeled organic phosphate compound showed that E. vaginatum is able to hydrolyze and absorb inorganic phosphate from an organic phosphate source, inositol hexaphosphate, in solution. This uptake of inorganic phosphate from an organic source is neither enhanced by rhizosphere microorganisms nor inhibited to any substantial degree by the presence of inorganic phosphate in the incubation medium. A comparison of these assimilation rates with those determined for inorganic phosphate shows that E. vaginatum can obtain inorganic phosphate from inositol hexaphosphate at rates similar to those for inorganic phosphate assimilation. Extrapolations based on tundra soil solution phosphate concentrations, estimates of annual phosphate demand, and the rates measured in this study suggest that the assimilation of inorganic phosphate from organic phosphate sources could provide a substantial contribution toward the annual demand of E. vaginatum for phosphorus.

Kinetic constants (K_m and V_{max}) were determined for surface and extracellular soluble acid phosphatases produced by two ectomycorrhizal fungi (Cenococcum geophilum Fr. and Entoloma sericeum (Bull. ex Merat) Quel.) grown in axenic culture at 2 or 50 micromolar KH_2PO_4 or sodium inositol hexaphosphate. Results were similar for both inorganic and organic phosphorus-supplied cultures: surface V_{max} estimates were significantly greater for 2 micromolar- than for 50 micromolar-grown isolates. The

presence of constitutive extracellular soluble phosphatase activity resulted in the appearance of inorganic phosphate in media initially supplied only with organic phosphorus, suggesting substrate hydrolysis in excess of phosphate uptake. No consistent relationship was found between apparent K_m estimates and phosphorus treatments. The two species had surface phosphatase V_{max} values differing by as much as two orders of magnitude. The size of the response to phosphorus treatment differed among isolates. The response of phosphatases to changes in phosphorus at concentrations comparable to soil solution phosphorus supports the hypothesis that levels of available soil phosphorus may control ectomycorrhizal phosphatase production or activation.

Despite the response of fungal isolates to levels of phosphate in solution, the surface acid phosphatase activity of field-collected ectomycorrhizal roots of arctic Betula and Salix species differed more among "color types" or fungal types than it did among sites differing in soil organic matter, moisture, and other characteristics affecting phosphate availability. The results of assays done for several color types and several sites for both plant species suggests that the extent of infection of roots by various fungal types probably affects the total potential for organic phosphate hydrolysis more than does any change in phosphatase activity in response to soil conditions by any one type.

The surface acid phosphatase activity measured for E. vaginatum roots was of the same order of magnitude as that of the ectomycorrhizal roots of Betula and Salix. In addition to the phosphatase activity associated with the fungal mantle, which is reflected by my measurements, ectomycorrhizae have an extensive network of hyphae increasing the volume of soil "exploited" by surface phosphatases. Eriophorum also has an additional mechanism for organic phosphate hydrolysis; in addition to its root surface phosphatase activity, the nonfunctional roots from previous seasons have significant

phosphatase activity. Both the predominantly non-mycorrhizal roots of E. vaginatum and the ectomycorrhizal roots of Salix and Betula have phosphatase activity with the potential to hydrolyze far more organic phosphate than is measured in tundra soil solution.

The measurement of enzyme activity using an artificial substrate under controlled conditions limits the interpretation of data in terms of the "real world." The challenge to biochemical ecologists is to bridge the gap between laboratory studies and field work. The use of a radiolabeled compound which does occur in soils, inositol hexaphosphate, provided evidence that phosphate released by hydrolysis is absorbed by the plant root. Future studies focused on the types of organic phosphate compounds available for hydrolysis in tundra soils, the relationship between "pNPPase" activity and activity toward naturally-occurring organic phosphate substrates, and the coupling of hydrolysis with inorganic phosphate absorption will take another step toward elucidating the role of phosphatases in the phosphorus nutrition of arctic plants.

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