NUTRIENT LIMITATION AND PLANT AND SOIL MICROBIAL GROWTH IN AN ARCTIC COASTAL SALT MARSH

by

Sarah K. Hargreaves

A thesis submitted in conformity with the requirements
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Abstract

In order to assess the nutrient limitation of plants and soil microorganisms in the Arctic coastal salt marsh at La Pérouse Bay, Manitoba, a nitrogen (N) and phosphorus (P) fertilization experiment was conducted. The partitioning of carbon (C), N and P was measured from June 2003 to July 2004. Soil solution inorganic N and P results indicate that the system is strongly limited by N and, to a lesser extent by P. Results indicate that strong N-limitation and high concentrations of inorganic P compared to dissolved organic P moderate the release of phosphatases. Primary productivity is co-limited by N and P, while soil microbial biomass is limited by carbon. Plants and microbes compete for N, but not P, during the summer. Overall, there was a seasonal shift in the allocation of nutrients within the community, from a microbial dominated system in the winter to a plant-dominated system in the summer.

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Chapter 1: General Introduction

The extent of the availability of nutrients in an ecosystem limits primary and secondary productivity and, ultimately, controls many aspects of ecosystem function (White 1993, Schlesinger 1997). Nutrient availability within an ecosystem depends, not only on the overall abundance of nutrients, but also on rates of biogeochemical cycling, anthropogenic disturbances, and effects of ecosystem management (Aerts and Chapin 2000). Nitrogen (N) and phosphorus (P) cycles are particularly important as these two elements are often most limiting for primary productivity in terrestrial environments. Nutrient availability to individuals within a system often depends on species-specific (and trophic-specific) nutrient limitation and on the ability of individuals to compete effectively for access to nutrients. An element is considered limiting if an increase in its availability increases the productivity of individuals, and at larger ecological scales, an entire ecosystem (Chapin et al. 1986). In northern terrestrial ecosystems, including coastal zones such as salt-marsh ecosystems, it is well known that primary production is limited mainly by nitrogen (N) and then by phosphorus (P) (Vitousek and Howarth 1991, Vitousek and Farrington 1997, Aerts and Chapin 2000). N and P limitation is especially apparent in systems where nutrient cycling is retarded at low temperatures, such as in Arctic soils (Nadelhoffer 1992). In this Introduction, factors affecting plant and microbial access to N and P will be reviewed, specifically in the context of Arctic ecosystems.

1.1 Cycling of nitrogen (N) and phosphorus (P)

Free diatomic nitrogen gas, N_2, accounts for 78% of the atmosphere by volume (Epstein 1972), but it must first be fixed as ammonia ions before it is available in the biosphere. Atmospheric nitrogen is fixed by specialized prokaryotic bacteria, which are either free-living or are symbiotically associated with the roots of plants, that convert N_2 to ammonia using the enzyme nitrogenase. The ammonium ions liberated through nitrogen fixation are used in the synthesis of amino acids and proteins by these free-living prokaryotic bacteria or by symbiotic prokaryotic cells associated with other organisms. When the free-living microorganisms and host plants die, decomposers convert organic nitrogen to inorganic nitrogen as a result of nitrogen mineralization (ammonification and nitrification). Ammonification is a process that releases ammonium ions (NH_4^+) as a result of the breakdown of organic nitrogen in necromass. Ammonium ions that are released into the soil solution can be taken up by plant roots and other microorganisms which do not fix N_2 or, under aerobic conditions, ammonium may be converted to nitrite and then nitrate by nitrifying microorganisms. Nitrate is also taken up freely by most plant roots. Once in the biosphere as combined nitrogen, N may cycle between several labile

pools within a given environment without returning to the atmospheric pool of free nitrogen gas. Global nitrogen cycling is maintained by denitrifying bacteria that discharge fixed nitrogen into the atmosphere as nitrous oxide or gaseous N_2. Nitrogen may also be lost from a system through leaching.

In contrast to nitrogen, which is distributed as an atmospheric gas, phosphorus comprises 0.12% of the lithosphere as apatite ($Ca_5(PO_4)_3OH$) (Epstein 1972). Unweathered phosphorus is made labile by chemical weathering, mostly by the action of carbonic acid in rainwater. Although chemical weathering is a slow process, labile P is quite abundant in many soils yet it is largely unavailable for uptake because it is bound to mineral and organic soil surfaces (Vance et al. 2003). The magnitude of the fluxes between exchangeable and soluble inorganic orthophosphate (P_i) in soils is regulated by chemical reactions that are pH-dependent, and include the adsorption-desorption of P_i on soil surfaces, the dissolution-precipitation of P-bearing minerals (particularly iron, aluminum and calcium), hydrolysis of organic matter and overall rates of chemical weathering (Hinsinger 2001, Vance et al. 2003). The speciation of P in solution is determined by solution pH. In the range of pH values that is relevant to most soils, P_i in forms HPO_4^{2-} and $H_2PO_4^-$ are the most dominant orthophosphate ions (Mengel 1982, Vance and Chapin 2001). $H_2PO_4^-$ is dominant from around pH 2.8 to 6.8, both $H_2PO_4^-$ and HPO_4^{2-} are present in fairly equal proportions at pH 7, and HPO_4^{2-} dominates in soil solution above pH 7.2 and below pH 12 (Hinsinger 2001). Phosphorus is lost from a system by erosion of sediment and organic particulate matter, which is commonly followed by transport in river systems and precipitation in ocean sediments (Epstein 1972).

1.2 Acquisition of nitrogen and phosphorus by plants and soil microorganisms and the role of enzymes in the environment

Total soil N can be partitioned into total organic N and total mineral or inorganic N. Of the total organic N, soluble organic N (SON) usually accounts for only 0.3 to 1.5% (Brady and Weil 2002), and is comprised of organic monomers such as amino acids and amino sugars. Dissolved inorganic nitrogen ions (ammonium and nitrate ions) have a pool size similar to that of SON so that the great bulk of soil N (95-99%) is in organic compounds, such as proteins or as part of humic compounds (Brady and Weil 2002). In most soils, concentrations of inorganic nitrogen (ammonium and nitrate ions) in the soil solution are low and range from 10^{-5} to 10^{-4} M (Mengel 1982). Rates of N mineralization by soil microorganism are dependent on litter quality, particularly the C:N ratio of litter (Chapin 1993). When the C:N ratio is relatively high (greater than 25-30), microorganisms immobilize most of the mineralized N. At lower C:N ratios

microbial demand for N compared to C is lower so that net mineralization is increased and inorganic N is released into the soil solution for plant uptake. However, low temperatures in Arctic soils also control net mineralization so that soil mineralization rates range only from 0.05 to 0.5 g m^{-2} $year^{-1}$ compared with values of 1.1 to 20 g m^{-2} $year^{-1}$ in temperate and tropical biomes (Nadelhoffer 1992). When net N mineralization rates are low, N can be taken up by plants and microbes in inorganic and low molecular weight organic forms (Schimel and Chapin 1996, Schiller et al. 1998, Henry and Jefferies 2003).

The organic fraction of phosphorus in soil generally comprises 20 – 80 % of the total soil P (Brady and Weil 2002). In the organic P pool alone, inositol phosphates, which interact with high molecular weight humic compounds, are the most abundant and constitute 10 – 50% of this pool. Dissolved organic P (DOP) makes up a large portion of the total phosphorus in the soil solution because inorganic P is so readily absorbed by organic clays in the soil. However, because DOP is so mobile, it is easily leached (Brady and Weil 2002). Concentrations of orthophosphate in the soil solution are usually even lower than concentrations of inorganic N and range between 10^{-6} to 10^{-8} M (Hinsinger 2001). Phosphorus is taken up by plants and soil microbes only as inorganic orthophosphate (P_i), thus the availability of P to plants and microorganisms is mainly controlled by the degradation of organic forms of P (Schlesinger 1997).

In terrestrial environments where there are relatively low and often limiting N and P supplies in the soil solution, plants are able to enhance acquisition of nitrogen and phosphorus in a number of ways. Plants adapted to infertile soils typically have high capacity (i.e. high V_{max}) to take up mobile ions such as potassium and inorganic N, but do not have the capacity to increase absorption of immobile ions such as phosphate (Aerts and Chapin 2000). Biomass allocation to roots increases in response to N and P deficiency, although the effect of a deficiency of N is usually stronger (Andrews et al. 1999). Plants are able to increase the amount of surface area available for nutrient absorption by promoting new root growth, modifying root architecture, developing root hairs, and promoting mycorrhizal symbioses (Marschner 1995, Vance et al. 2003, Hodge 2004). New roots of the dominant Alaskan tundra species, *Eriophorum vaginatum*, closely follow the retreating boundary of frozen soil as the active layer develops at spring thaw (Chapin 1974b, a), thus benefiting from the large release of nutrients from thawing plant and microbial necromass. Mycorrhizae are thought to be advantageous in nutrient uptake by plants because they acquire nutrients at a lower carbon cost than roots, due to their smaller diameter and greater surface: volume ratio (Aerts and Chapin 2000). Nutrient exploitation under low concentrations is especially important for P acquisition

(Smith 1997), since P is relatively immobile and because inorganic P is taken up mostly by diffusion, not mass flow (Epstein 1972). For example, when mycorrhizal activity is suppressed in N-limited grasslands, plant production can become P-limited, suggesting that effective P uptake by mycorrhizas may be an important factor explaining the widespread N-limitation of plant growth within temperate ecosystems (Grogan and Chapin 2000). However, in some cases plants do not benefit from these mutualisms (Klironomos 2002), so it is clear that plant-fungal mutualisms do not affect all plants in the community equally. Moreover, plants are able to enhance the solubility of calcium phosphates by secreting organic acids such as citrate and malate that acidify the rhizosphere (Hinsinger 2001). Riley and Barber (1971) found that the concentration of P in shoots increased linearly with decreasing pH of the soil solution adjacent to the root.

Another factor regulating the supply of biologically-available P to plants and microbes is the activity of a group of enzymes called phosphomonoesterases, or phosphatases, that are both intra- and extracellular. In plants, the intracellular enzymes catalyze many processes involved in internal remobilization of P and are ubiquitous in the vacuole and cytoplasm throughout development (Duff et al. 1994). Extracellular phosphatases are localized in the cell wall, outer surface of the root epidermis cells, and the root apical meristem. From there, the enzymes are secreted into the soil solution and catalyze the hydrolysis of phosphomonoesters and the release of orthophosphate in the rhizosphere. Phosphatases are also released by soil microorganisms in the soil system.

Phosphatases are complexes of enzymes that typically have maximum hydrolyzing capacity at different pH values, so they are divided into acid and alkaline categories. Alkaline phosphatases express maximum activity well above pH 7, while acid phosphatases have an optimum pH generally between pH 4 and 6 (Jansson et al. 1988). Plant roots exude only acid phosphatases (Duff et al. 1994, Vance et al. 2003), but soil microorganisms secrete phosphatases which show maximum activity in both the acid and alkaline pH ranges (Jansson et al. 1988). Nevertheless, the fact that different enzymes have maximum rates at different pH values does not mean that they are totally inactive at other pH values (Jansson et al. 1988). Along with pH, phosphatase activities also depend on temperature and the concentration of metal ions in the soil solution. In particular, alkaline phosphatases have been characterized as metallo-enzymes with an essential metal ion, usually Zn^{2+}, present at the active site of the enzyme (Jansson et al. 1988).

Because of their role in P mineralization, phosphatases are thought to play an important role in plant and soil microbial P uptake, especially in Arctic soils, where the availability of

inorganic phosphate is very low (Nadelhoffer 1992). One of the first studies to examine the importance of phosphatases in plant-root P uptake in Arctic soils was conducted by Kroehler and Linkins (1988). Using the artificial substrate *p*-Nitrophenyl phosphate (*p*-NPP), they verified that *Eriophorum vaginatum* has acid phosphatase activity evenly distributed along its root surface, and, that like many enzymatic reactions, the product in the phosphatase catalysis (P_i) is an inhibitor of the enzymatic activity, but only at high concentrations of P_i (Kroehler and Linkins 1988, Johnson et al. 1999). Later, they demonstrated more definitively the ecological importance of phosphatases in P uptake in Arctic plants. Using radioactive ^{32}P-labelled inositol hexaphosphate, Kroehler and Linkins (1991) provided evidence that *E. vaginatum* is able to hydrolyze and absorb phosphate from a naturally occurring organic P compound (not just artificial *p*-NPP) at concentrations as low as those found in soil solution (micromolar concentrations) and at rates comparable to the plant's ability to absorb inorganic phosphate as estimated by Chapin and Tryon (1982).

1.3 Role of nitrogen and phosphorus in plant tissues

As macronutrients, nitrogen and phosphorus are required in relatively large amounts, they are directly involved in plant metabolism and their function cannot be replaced by another mineral element (Marschner 1995). Within plants and microorganisms, nitrogen is incorporated into amino acids and proteins in enzymes, structural and storage proteins, and nucleic acids (Mengel 1982). Phosphorus is used in many processes, including energy transfer in the cell, nucleic acid synthesis, signalling, carbohydrate metabolism, and nitrogen fixation (Vance et al. 2003).

In plants, a concentration of 1.5% N and 0.2% P per dry weight is considered adequate for plant maintenance (but not necessarily growth) (Epstein 1972), but these values vary greatly from species to species (Aerts and Chapin 2000). Given their relative importance in cell cycling and maintenance, it is not surprising that a shortage of either N or P causes a reduction in the rate of biomass accumulation in plants (Marschner 1995) and a reduction in activity of soil microorganisms (Sundareshwar et al. 2003).

1.4 Nutrient limitation in plants and soil microorganisms

Amounts of N and P in plant biomass are determined by the balance of N and P uptake, carbon assimilation, and the loss of these nutrients through turnover, leaching, exudation, herbivory and parasitism (Aerts and Chapin 2000, Güsewell 2004). The average mass N:P ratio of terrestrial plant species at their natural field sites is 12 -13 (Elser et al. 2000), but these

values vary widely both within and among species. Within a single species, plant tissue N:P ratios may vary 50-fold depending on the nutritional status of the soil and on the plasticity of the species in response to N and P in the soil (Phoenix et al. 2003, Güsewell 2004).

Nutrient limitation in plants is commonly assessed in controlled and replicated fertilization experiments and the process is regarded as nutrient-limited if there is a significant difference in growth ($P<0.05$) between fertilized and unfertilized samples (Vitousek and Howarth 1991). The type of nutrient limitation is typically gauged by examining nutrient ratios. Mass ratios are less than atomic ratios by a factor of 1.17 for C:N, 2.58 for C:P and 2.21 for N:P. The 'Liebig law of the minimum' suggests that, in order to grow, plants require essential mineral nutrients in specific proportions. When N and P supplies are sub-optimal there is a 'critical N:P ratio' at which plant growth is co-limited by N and P, below which growth is limited by only N, and above which growth is limited by only P (Güsewell 2004). Similarly, Koerselman and Meuleman (1996) recently proposed the use of the N:P mass ratio in tissues, not the absolute tissue nutrient content, as an indicator of the type of nutrient limitation. They plotted P content and N content of above-ground biomass in samples taken from unfertilized plots from 40 fertilization experiments conducted in European wetland systems. Overall, they found that for N:P mass ratios > 16 the community biomass production was P-limited, for N:P ratios < 14 the community biomass production was N-limited, and the community biomass was co-limited by N and P if the N:P ratio was between 14 and 16. This approach of Koerselman and Meuleman (1996) is meaningful and it is easy to determine the ratios in assessing nutrient limitation (Aerts and Chapin 2000), but other considerations must be made when interpreting N:P ratios this way. First, the use of N:P ratios for the determination of nutrient limitation assumes that the growth of the plant species or vegetation is always limited by either N and/or P. Moreover, it is necessary to differentiate between nutrient limitation at the community level and limitation at the species level, since the N:P ratio of individual species within a community can be very different from that of the community as a whole (DiTomasso and Aarssen 1991).

In microorganisms, carbon to nitrogen ratios are not consistent. For example, C:N ratios in fungi can vary widely. Carbon content in these microbes is usually relatively constant (45% C) but percent N can range from 3 -10%, resulting in a range of C:N ratios from 15:1 to 4.5:1 (Paul and Clark 1996). In contrast, bacteria have N in their cytoplasm and in peptidoglycan of their cell walls so C:N ratios in these microorganisms typically range from 3:1 to 5:1 (Paul and Clark 1996). As a result of the variation between functional groups of microorganisms, there are problems when interpreting the C:N ratios of microbial communities, since a change in the suite of microbes in the soil community will change the C:N ratio of the microbial biomass.

It is known that there is a 'critical N:P ratio' at which plant growth is co-limited by N and P. However, the 'law of the minimum' from which this concept is taken is an idealization of N and P requirements in plants (Sinclair 1993), because the supply of one nutrient affects the acquisition of the other (Treseder and Vitousek 2001). Although simple relationships between N and P exist, for example P-deficient plants increase P uptake and reduce N uptake, and *vice versa* (Aerts and Chapin 2000), the functional relationships of the two elements within an individual are also intimately connected. For example, as proteins, phosphatases have relatively high N concentrations (between 8% and 32%) (Treseder and Vitousek 2001) so an increase in phosphatase activity in response to P-limitation increases the export of N as a consequence of enzyme loss to the external environment. The secretion of H+ from plants that take up NH_4^+ ions induces acidification of the rhizosphere, thereby increasing P uptake (refer to section 1.2) (Gahoonia et al. 1992).

In terms of phosphorus limitation, the level of phosphatase activity is widely used as an indicator of P-limitation of some component of the system. Goldstein et al. (1988a, 1988b) have shown that the expression of acid phosphatases is regulated by P limitation which triggers inducible excretion of acid phosphatases in both tomato plants (*Lycopersicon exculentum* cv VF 36) and suspensions of cultured cells under conditions of phosphate starvation. However, the importance of phosphatase activities in leading to enhanced orthophosphate supplies to plant roots is not well understood. Some studies have shown strong relationships between acid phosphatase activities (AcidPases) and P uptake (Kroehler and Linkins 1988, Helal 1990), while the results of other studies have been inconclusive (McLachlan and Demarco 1982). Phosphatase activities have also been used as an indicator of microbial P-limitation. Sundareshwar et al. (2003) studied the differential nutrient limitation between trophic levels by comparing plant and microbial responses to fertilization. Higher pore-water phosphatase activities in unfertilized samples suggested that soil microorganisms in the system were P-limited.

Nutrient limitation within a community also can change over time and the change in nutrient limitation is due to differences in the sources of nutrients (refer to section 1.1). The results of most studies looking at changes in nutrient limitation during plant succession indicate that N is limiting to primary productivity on newly-formed sites, but nitrogen levels increase over time as a result of microbial activity, nitrogen fixation and the build up of organic matter in the soil (Wardle 2002). In contrast, P becomes limiting gradually over time as the P from the primary soil materials (rock) is depleted.

1.5 The effects of intraspecific and interspecific interactions on nutrient cycling

Plant species create positive feedbacks that affect patterns of nutrient cycling in natural ecosystems so that, in general, differences among plant species in nutrient uptake and loss reinforce existing patterns of soil nutrient availability (Hobbie 1992). In nutrient-poor systems, such as in the Arctic, plants grow slowly, use nutrients efficiently and produce nutrient-poor litter that decomposes slowly. In contrast, in nutrient-rich systems, plants grow rapidly and produce readily-degradable litter (Hobbie 1992). Species effects, like the above-mentioned patterns in nutrient cycling, as well as nutrient interactions of individuals and their environment and interactions between different trophic levels, all contribute to overall ecosystem fertility.

The outcome of interactions between different species in response to the availability of N and P can be positive or negative for one or both species. A well-known interspecific interaction that is positive, and which has been applied by farmers for millennia, is the practice of intercropping agricultural crops with nitrogen-fixing legumes to enhance overall soil fertility and ecosystem productivity (Reynolds et al. 1994, Wardle 2002). In contrast, Wardle (2002) documented an example of an allelopathic interaction in a series of experiments that examined the effect of the leaf litter from the shrub *Calluna vulgaris* on the growth of *Betula pendula* and found that tannin-protein complexes in the leaf litter retarded soil nitrogen mineralization and N uptake in the birch seedlings (Handley 1961).

Interactions within and between microbial groups are also evident. Competition for nutrients within fungal communities (often comprising much of the microbial biomass) is well-documented on artificial and natural substrates (Widden and Hsu 1987, Wardle 2002). In some cases, one fungal species can reduce the competitive saprophytic ability (litter colonization) of another (Widden and Hsu 1987). There are also reports that fungal species can reduce the biomass production of each other in soil (Wardle et al. 1993). On an individual basis, it was previously thought that soil microorganisms were limited by carbon availability, but recently, however, Schimel and Weintraub (2003) have linked demands for C and N by challenging the classic model of the decomposition of soil organic matter (SOM) described by first-order kinetics with exoenzyme-catalyzed decomposition. They argue that the exoenzyme system used to break down SOM is C limited, but that microbial growth is N limited, so that adding N to the N-limited system decreases respiration, and C is redirected from respiration into microbial growth.

Between trophic levels, plant species differ in their effects on the activity of soil microorganisms as a consequence of their differences in net primary productivity, the amounts of resources returned to the soil and by influencing the quality of these resources available belowground (Wardle 2002). Soil microbial biomass is generally enhanced by the presence of

actively growing plants, largely through the addition of relatively easily degraded carbon sources that are released into the rhizosphere by plants (Wardle 2002). Similarly, plant litter with a low C:N ratio (below 30) leads to a decrease in the C:N ratio of soil organic matter. This facilitates net N mineralization that occurs below a C:N ratio of about 30, and the increased availability of inorganic N often results in increased plant N uptake (Kaye and Hart 1997). Soil nutrient status affects the relative success of mycorrhizal and non-mycorrhizal plant genotypes under different soil nutrient conditions. In fertile soils, the growth rate of non-mycorrhizal genotypes is higher than mycorrhizal ones, but non-mycorrhizal genotypes grow slowly in low-P soils (Koide 1991).

There is strong theoretical and experimental support that, in many ecosystems the growth of plants and soil microorganisms is limited by N availability (Kaye and Hart 1997, Schimel and Bennett 2004). Competition occurs when two organisms are limited by the same resource and utilize the same pools of that resource (Tilman et al. 1982). It has long been thought that soil microbes out-compete plants for access to nutrients because of their faster uptake kinetics (due to favourable surface: volume ratio) and their mobility within the soil solution. In the case of nitrogen, however, it is now known that plants (and microorganisms) are able to take up organic forms of N (free amino acids) (e.g. Schimel and Chapin 1996, Henry and Jefferies 2003). This means that plants are able to access sources of organic N thereby short-circuiting the N cycle and directly taking up organic monomers (e.g. amino acids, amino sugars, nucleic acids) before microbial mineralization and potential immobilization can occur. Plants may lose N to microbes at a microsite scale because of uptake kinetics, but ultimately the ability of plants to take up organic N allows them to compete effectively with soil microbes at the macro-scale, because they are able to intercept organic N (Schimel and Bennett 2004). Because plants retain N (and P) for extended periods, as opposed to the rapid turnover of these elements that occurs in soil microbial communities, plants are able to immobilize nutrients over time more effectively.

1.6 Seasonal restraints on nutrient cycling in Arctic and Alpine soils

In addition to the effects of biotic interactions on nutrient cycling mentioned above, abiotic effects can also strongly influence nutrient cycling. Two features distinguish Arctic ecosystems: they are dominated by cold winter conditions and are strongly nutrient-limited (Shaver and Chapin 1980, 1986). In these systems, constraints on nutrient availability are amplified, because cold, wet conditions limit chemical weathering and rates of decomposition (Mengel 1982, Nadelhoffer 1992). This means that one of the main mechanisms by which species affect the dynamics of soil cycling is by influencing the temporal availability of nutrients

(Wardle 2002). Mineralization rates can vary among Arctic sites, but *net* mineralization is generally lower than in soils in warmer regions because of the low rates of decomposition. Mineralization studies of soils using the buried-bag technique (Eno 1960), which prevents plant nutrient uptake, have often shown that net N and P mineralization during the summer months is negative in wetlands of the sub-Arctic (Rosswall and Granhall 1980) and Arctic (Chapin 1988, Giblin et al. 1991). In contrast to summer months, Giblin et al. (1991) found that net mineralization in a wet sedge tundra was positive during the autumn, winter and at spring thaw. In another tundra ecosystem, nearly half the annual phosphorus return to the soil was estimated to occur during the period of snowmelt (Chapin et al. 1978). The supply of nutrients to plants may therefore depend on competitive interaction for nutrients between plants and soil microbes during the growing season (Harte and Kinzig 1993), or on the ability for plants to sequester nutrients at times when flushes of nutrients are released from soil microbes (Jonasson et al. 1999).

In a well-cited study conducted in northern Sweden during the summer months, Jonasson et al. (1996) found that only microbial biomass C increased after sugar additions, and that microbial immobilization of N and P, but not C, increased after NPK-fertilization. From these results, the authors concluded that microbial biomass production was stimulated by labile carbon and that microbial activity was stimulated by labile C and by nitrogen and phosphorus additions. The significance of these results is two-fold. First, the data were the first direct evidence that, although microbial biomass may be mainly C-limited, microbial activity (measured as nutrient immobilization) increases in response to fertilization with N and P additions in Arctic soils. Secondly, the strong increase of microbial N and P after fertilization was the first direct evidence of a high potential for soil microbes to act as strong nutrient sinks in these soils (Jonasson et al. 1996).

The strong microbial sink strength observed by Jonasson and colleagues (1996) has proven to be particularly important in mobilization-immobilization cycles and seasonal dynamics of microbial populations. Work over the past decade has shown that microbial populations can be active under the snow, so that plants and soil microbes are most active in their rates of nutrient (N) uptake at different times of the year (Jaeger et al. 1999, Lipson et al. 1999). From studies in the Colorado alpine, plant uptake of N usually peaks mid-growing season in summer, whereas microbial biomass is highly variable during the growing season for plants. In contrast, microbial biomass and microbial immobilization of N increases, and microbial C:N ratio decreases, throughout the fall and winter as plant and microbial litter inputs at the end of the growing season provide a pool of nutrients for the growth of cryogenic microorganisms. The

microbes that dominate in fall and winter can grow under a snow pack at temperatures around the freezing point (-5 to + 3°C). Microbial metabolism under snow pack is thus an important biogeochemical sink for nutrients (N) and the paradigm suggests that nutrients immobilized by microorganisms throughout the winter are available to plants in the spring after microbial die-backs caused by freeze-thaw cycling and community turnover as the soil warms (Lipson et al. 1999).

Other studies of soil microbial communities, which have focused on the seasonality of microbial population dynamics, reveal that the microbial community undergoes a shift in function, and the genetic structure of the community changes, between winter and summer (Lipson et al. 2002, Schadt et al. 2003). In 2002, Lipson et al. measured and compared fungal: bacteria ratios in the summer and winter using reciprocal hybridization of community DNA. The results of bacterial and fungal counts and substrate-induced respiration experiments revealed that microbial biomass is higher in soils in winter than in summer, and that fungi make up most of the biomass during the winter while bacteria dominate in the summer months.

1.7 Nutrient cycling in an Arctic salt marsh at La Pérouse Bay, Manitoba

Most studies of plant and microbial nutrient immobilization in Arctic ecosystems have focused on nitrogen cycling during the summer months, since it is the nutrient mainly limiting primary productivity, and because of the role of microbial mineralization in N-cycling and the relative ease of using the ^{15}N stable isotope to study these processes. This is true of the coastal salt marsh at La Pérouse Bay, Manitoba (58° 4'N, 94° 3'W), located at the sub-Arctic/Arctic boundary on the southwest coast of the Hudson Bay, Canada, where studies of plant-herbivore and plant-microbe interactions in recent years have focused on N acquisition.

The marshes around La Pérouse Bay, like numerous coastal marshes of the eastern Arctic of North America, are summer breeding grounds for a colony of lesser snow geese (*Chen caerulescens caerulescens* A.O.U.) that is part of the Mid-Continent Population of this species. Over the past thirty years, the Mid-Continent Population of lesser snow geese has shown a near geometric increase in numbers, and the increase has been largely attributed to increased foraging on agricultural crops on the wintering grounds and along the flyways (Jefferies et al. 2003). The geese arrive in May to nest, rear their broods, moult, and migrate south again in September, all the while foraging extensively on low-lying salt marsh vegetation. The effects of goose foraging on primary productivity at La Pérouse Bay have been extensively studied and reviewed recently (Jefferies et al. 2003). It is known that, at low herbivore densities of approximately 3500 pairs at La Pérouse Bay in 1978 (Cooke 1995), a positive feedback actually

increased net primary productivity (NPP) and the turnover rate of nitrogen, despite goose grazing. The mechanism behind this stimulatory effect was that goose faecal matter provided inorganic and organic N fertilization to the salt-marsh swards that were N-limited, thus reducing the sink strength of the feeding geese and actually increasing net above-ground primary productivity in grazed areas compared with ungrazed areas (Cargill and Jefferies 1984b, Bazely and Jefferies 1985). A stimulatory effect of grazing has also been reported in Fennoscandian Arctic-alpine tundra heaths, where reindeer grazing increased gross N mineralization rates, thus indicating that reindeer grazing leads to increased rates of nutrient cycling in this system (Stark et al. 2002).

A second positive feedback occurs at higher goose densities that have occurred in the last two decades and this has led to the degradation of the coastal salt marshes at La Pérouse Bay (Srivastava and Jefferies 1996, Jano et al. 1998). The local snow goose population at La Pérouse Bay had reached approximately 44,500 pairs at the time of the last census in 1997. The second positive feedback is initiated after thaw in spring, when the birds grub in the ground for roots and rhizomes of their preferred salt-marsh species. Sites that are grubbed become dominated by exposed sediments that dry out in summer and become hypersaline - almost 3 times the salinity of sea water (Iacobelli and Jefferies 1991). Along with hypersalinity, grubbed sites are characterized by high compaction, low soil N, low redox potentials, and depletion of a soil seed bank (Jefferies et al. 2003, McLaren and Jefferies 2004), and they represent an alternative stable state (Hik et al. 1992). Under these conditions, plants of the intertidal salt marsh and graminoids and willows in the supratidal marsh are unable to re-establish and, eventually, the organic surface layers are eroded by wind and water (Iacobelli and Jefferies 1991, Srivastava and Jefferies 1996).

From previous research at La Pérouse Bay, it is known that the dominant form of available N for plant growth is NH_4^+, but that some nitrogen may be present as nitrate ions and as soluble organic nitrogen in the soil solution (Wilson and Jefferies 1996, Henry and Jefferies 2003). Although few small (5m x 5 m) patches of intact vegetation remain, the intertidal salt marsh is presently a vegetative mosaic dominated by degraded sites devoid of vegetation. Loss of vegetation leads to loss of N from the system (McLaren and Jefferies 2004), further limiting the soil nutrient supply.

Previous fertilization experiments on the intertidal salt marsh have shown that primary productivity is first limited by N but exhibits a strong response to P when N is readily available (Cargill and Jefferies 1984a, Ngai and Jefferies 2004) This response is similar to that in other Arctic ecosystems (Chapin et al. 1986). However, in a coastal salt marsh on the eastern

seaboard of the U.S.A., which was studied by Sundareshwar et al. (2003), soil microbial populations were P-limited, in contrast to N-limited plant productivity. It was suggested that differential nutrient limitation could alleviate competition for nutrient resources. Furthermore, they indicate that differential nutrient limitation between trophic levels is a consequence of ecosystem development that acts to maximize overall resource conservation within the system. Overall resource utilization by different groups of organisms should be particularly important in nutrient-limited systems, such as the intertidal salt marsh at La Pérouse Bay.

1.8 Outline of thesis

Economic-based theories suggest that plants optimize growth by allocating more resources to the acquisition of nutrients that most limit growth (Bloom et al. 1985). Plants may use resources that are available in excess to capture those that are not. As such, it is important to consider the availability of C, N and P simultaneously in order to adequately assess the regulation of N and P acquisition by plants and microbes (Treseder and Vitousek 2001). Following previous research on primary productivity on the intertidal salt marsh (Cargill and Jefferies 1984a, Ngai and Jefferies 2004) and studies conducted in other coastal salt marshes (Sundareshwar et al. 2003), the focus of this study was based on determining the extent of nutrient limitation in plant and microbial growth in these salt-marsh soils and examining seasonal variation and the effect of goose herbivory in the partitioning of N, P and C among plants, microbes and the abiotic soil compartment. A fertilization experiment, with additions of N and P to soil, was set-up in order to compare the responses of plant and microbial growth to nutrient additions. In addition, acid and alkaline phosphatase activities in soils of the different treatments were assayed in order to determine whether activities were higher in unfertilized plots compared with fertilized plots and the results were compared with the overall N and P contents of plants, microorganisms and the soil. This study took place in Wapusk National Park, where the use of radioactive isotopes is prohibited. The rational for the study of phosphorus dynamics in this system is as follows. It is known that N plays an important role in plant and microbial growth in the system, but it is also apparent that P quickly is co-limiting when N is added (Ngai 2004). If differential nutrient limitation between trophic levels (microorganisms and plants) is occurring with respect to N and P, then the additions of N and P should alleviate the limitation and this should result in increased microbial biomass (measured as carbon), immobilization of N and P, and increased plant growth measured as above-ground plant biomass. As an indicator of P-limitation, phosphatase activities should be higher in unfertilized or N-alone fertilized plots compared with soil from plots that received P. If strong

competition for nutrients is occurring, the result should be an increase in either microbial biomass and the immobilization of N and P or an increase in plant growth and possibly higher N and P contents in plant tissue. In addition, total pool sizes of N and P may be expected to rise in these two compartments in response to the nutrient additions. In order to determine whether soil microbial growth is carbon-limited, additions of glucose were made to plots and microbial biomass and the N and P contents of the biomass were measured over a period of 6 days. If there is an increase in microbial biomass, it suggests that the soils are C limited for microbial growth. Overall, the results also should indicate the sink strength of plants and microbes and whether there are strong seasonal differences in microbial activity between winter and summer leading to nutrient release at the spring thaw.

1.9 Research questions examined in this study

1. What is the role of soil phosphatase mineralization in plant and soil microbial demand for P at La Pérouse Bay, Manitoba?

2. What limits plant growth and microbial immobilization of C, N and P on the western intertidal salt marsh?

3. How do the allocation of C, N and P to plant, microbial and soil compartments vary seasonally?

4. What is the effect of goose grazing on microbial immobilization of C, N and P?

Chapter 2: General methods

2.1 Site description

The study was conducted at La Pérouse Bay (58° 4'N, 94° 3'W) in Wapusk National Park, approximately 30 km east of Churchill, Manitoba, on the southwest coast of Hudson Bay (Figure 2.1). The area, which is part of the Hudson Bay Lowlands, experiences isostatic rebound of about 0.8 cm y^{-1} (Hansell et al. 1983). As a consequence of the uplift, the Bay can be subdivided along a coastal gradient (1 meter rise per 3 km at La Pérouse Bay) into intertidal, supratidal and freshwater marshes. The entire intertidal zone, which is closest to the sea, is tidal only in the late summer and autumn. The older supratidal zone is rarely flooded (less than two times every three years). Beyond the reach of tides, approximately 1.8km inland, lie the freshwater marshes.

Small tributaries extend from the Mast River and flow through the supratidal and intertidal marshes. At the outflow of these channels, fresh water mixes with the seawater that results in brackish inshore water with salinity of less than 12 g of solutes L^{-1} (Srivastava and Jefferies 2002). Landscape heterogeneity across the three marshes is influenced by frost-heaving and is characterized by ephemeral ponds in the freshwater marsh, and both ponds and streams in the supratidal marsh and upper intertidal marsh. In all three habitats, frost-heaving also results in raised hummocks, 15 to 25 cm high, on which the less salt-tolerant vegetation grows (Jefferies et al. 1979). In addition, the topography of the intertidal marsh is influenced by debris deposited by tidal activity.

The salt-marsh soil is classified as a Regosolic Static Cryosol (Agriculture Canada Expert Committee on Soil Survey 1987) and consists of approximately 88% sand, 10% silt and 2% clay (Jefferies et al. 1979). Permafrost lies below the active layer, which is up to 30 cm deep. Soils in the supratidal zone are characterized by a thin humified mineral layer where the vegetation has been lost as a result of goose foraging and where the humified mineral layer is still present. The exposed sediments in spring are typically covered by ephemeral algal mats that eventually dry-out and blow away in summer (McLaren and Jefferies 2004). This leads to further erosion of the organic-rich layer, ultimately exposing mineral sediments very low in organic matter. On the wetter intertidal marsh especially, hydrogen sulphide is generated by bacteria carrying out hydrogen oxidations and CHO oxidations under anaerobic conditions. The soil in the freshwater marsh is a Lithic-Mesic Organic Cryosol (Agriculture Canada Expert Committee on Soil Survey 1987) with an upper layer of soil composed of highly humified peat (Jefferies 1988).

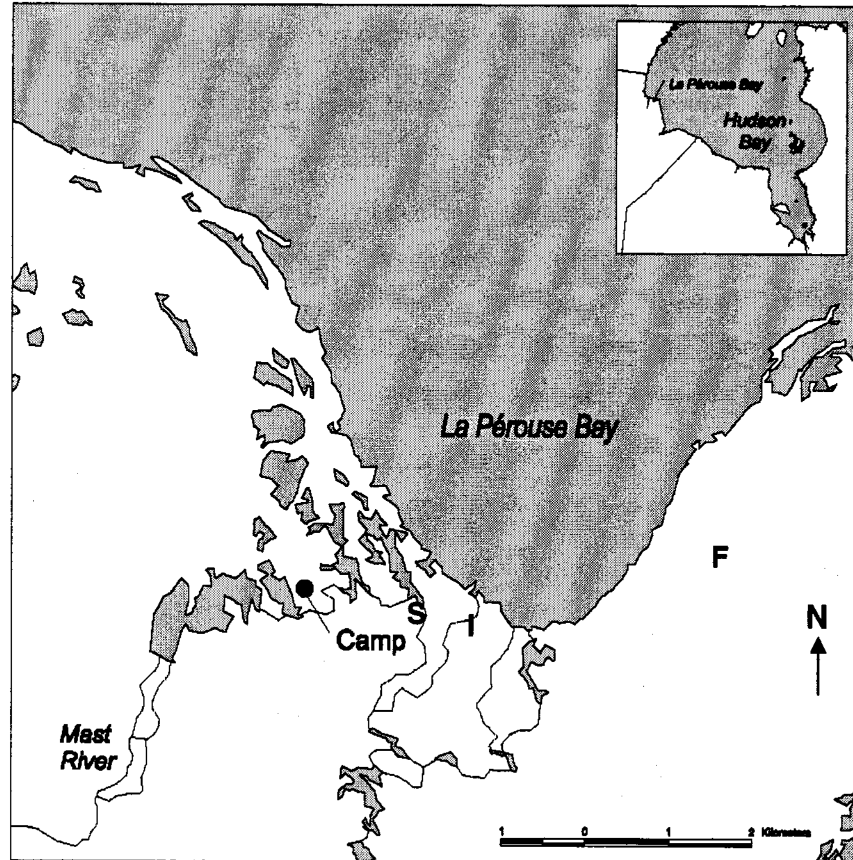


Figure 2.1 Map of La Pérouse Bay, Manitoba and surrounding marshes. The main fertilization experiment was conducted on the western intertidal salt marsh (I). Comparative studies also were conducted on the western supratidal marsh (S) and the eastern freshwater marsh (F). The field laboratory is located in the camp, indicated on the map.
Scale: 1.1cm = 1km.

The intertidal marsh is dominated by discontinuous swards of two low-lying graminoids, *Puccinellia phryganodes* and *Carex subspathacea*. Also present are *Potentilla egedii*, *Plantago maritima*, and *Ranunculus cymbalaria* (Jefferies et al. 1979) (Figure 2.2). *Puccinellia phryganodes* and *C. subspathacea* also dominate low-lying areas of the supratidal marsh, whereas woody vegetation, including *Salix brachycarpa*, *Myrica gale*, *Betula glandulosa*, and grasses *Festuca rubra* and *Calamagrostis deschampsiodes* dominate the frost-heave hummocks. Moss (*Drepanocladus spp.* and *Aulacomnium spp.*) and *Carex aquatilis* characterize low-lying freshwater patches in the freshwater marsh, while low shrubs, such as *Rhododendron lapponicum* and *Salix actophila*, grow on hummocks. Nomenclature follows Porsild and Cody (1980).

The main herbivores in the snow-free season in all three habitats are lesser snow geese (*Chen caerulescens caerulescens* A.O.U.). The birds arrive in mid-late May to nest and they leave with their broods in early September. Historically, the geese have fed extensively on *P. phryganodes* and *C. subspathacea* in the intertidal and supratidal salt marshes. As a result of intense grazing and grubbing by geese, the intertidal and supratidal zones are now characterized by increasingly smaller patches of intact vegetation amidst extensive mudflats devoid of vegetation. More recently, the geese have had to move from the degraded intertidal and supratidal marshes, to the freshwater marshes to feed on the lower-quality forage species, *Carex aquatalis* (Jefferies 1988).

In 2003, spring melt started in late April, and the growing season started at the beginning of June. From the Environment Canada weather station at Churchill (Table 2.1), air temperatures from June to August 2003 ranged from -2.5 to +30.5°C and total rainfall was 229.7 mm. Bird counts from the observation tower on the intertidal marsh estimate the number of broods foraging to be approximately 30 per day. By mid-October 2003, the ground was frozen and the total snowfall for the entire month was 57.9 cm. Air temperatures ranged from -43.0 to +11.0°C from September 2003 to May 2004, and remained below freezing from December to February 2004. Persistently low temperatures and an above-average amount of snow maintained a deep and dense snow pack into May 2004. The snow began to melt in mid-May and the soil underwent a series of spring freeze-thaw cycles. Due to the late spring melt in 2004, the growing season started in mid-June, about two weeks later than in 2003, and the geese nested in late June but did not forage extensively on the intertidal or supratidal marshes.

Figure 2.2 Photograph of the western intertidal salt marsh at La Pérouse Bay, Manitoba in July 2003. Photograph taken by the author.

Table 2.1 Weather data from Environment Canada, Churchill, Manitoba. Monthly averages from June 2003 - June 2004 were recorded at station Churchill UA, Climate ID 5060606 (58°43' N, 94° 4' W), from which total snowfall and total rainfall are available. Climate normals (*) are from 1971 - 2000 and are only available from Churchill A, Climate ID 5060600 (58°44' N, 94° 3' W). Maximum temperature and minimum temperature refer to extreme maximum and minimum temperatures, respectively. [empty] = No data available, T = trace amounts.

	Jun 2003	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2004	Feb	Mar	Apr	May	Jun	Annual Normal*
Mean temperature (°C)	+ 8.0	+13.5	+14.3	+ 8.1	- 0.5	-12.5	-17.6	-31.7	-23.3	-21.5	-13.5	- 8.0	+ 4.2	- 6.9 +/- 1.3
Maximum temperature (°C)	+26.0	+30.0	+30.5	+29.0	+11.0	+ 2.0	- 3.0	-16.0	-10.3	+ 8.5	+ 6.0	+ 5.0	+20.0	
Minimum temperature (°C)	- 2.5	+ 2.0	+ 4.0	-10.5	-12.5	-25.5	-34.5	-43.0	-39.5	-38.5	-26.0	-21.0	-4.0	
Total rainfall (mm)	93.3	68.1	68.3	74.8	5.2	0.0	0.0	0.0	0.0	0.0	0.0	T	14.8	264.4
Total snowfall (cm)	0.0	0.0	0.0	5.5	57.9	98.4	40.9	16.8	17.0	10.4	8.3	14.2	0.4	191.0
Total precipitation (mm)	93.3	68.1	68.3	80.3	63.1	98.4	40.9	16.8	17.0	10.4	8.3	14.2	15.2	431.6
Monthly normal*	+ 6.6	+12.0	+11.7	+ 5.6	-1.7	-12.6	-22.8	-26.7	-24.6	-19.5	- 9.7	- 0.7		
Standard Deviation	2.1	1.7	1.5	1.8	2.1	3.3	3.5	3.4	3.7	3.1	2.9	2.4		

2.2 Experimental design

In order to study the effect of nutrient addition on plant and soil microbial nutrient cycling, goose-exclosures were set-up on the western intertidal salt marsh ("Randy's Flats") at La Pérouse Bay. Site choice, and thus replication, was limited to homogeneous, intact swards of *Puccinellia phryganodes*. Only three sites met this requirement, and three exclosures at each site were erected. Site is the level of replication (n=3) since the sites were separated by at least 25 m to avoid pseudoreplication. Exclosures were 1.25 m x 1.25 m and made of chicken wire, and each was staked and covered with raspberry netting to prevent goose entry.

The main treatments were nitrogen and phosphorus salt additions, which were added in a full-factorial, randomized block design (refer to section 2.5.1). The concentrations of salt added are based on fertilizer additions to agriculture land and follow Cargill and Jefferies (1984a) and Ngai and Jefferies (2004). Four subplots, 50 cm x 50 cm each, were marked and randomly assigned to one of four treatments, including control plots (0N0P) that received no nutrient amendment. Subplots were displaced 2 - 5 cm from the exclosure's edge to prevent edge effects and corridors of 10 -15 cm were left between subplots within each exclosure to prevent cross-contamination of nutrient salts. Nitrogen was added as NH_4Cl salt (8.5 g N m^{-2}) to nitrogen (1N0P) and nitrogen x phosphorus (1N1P) treatment plots. Phosphorus was added to phosphorus (0N1P) and 1N1P treatment plots as $Na_2HPO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 2H_2O$ salts (6 g P m^{-2}), in equal amounts of the two salts with respect to phosphorus. Nitrogen and phosphorus additions on all plots were made at the beginning of the growing season, 3 June 2003, and mid-growing season, 10 July 2003. In addition, all four subplots of one exclosure from each site were treated with a carbon supplement in the form of glucose (9.6 g C m^{-2}) in July 2003. Photographs of a typical exclosure are shown in Figure 2.3. For clarification of the experimental design of the fertilization experimental refer to Figure 2.4.

In order to look at seasonal changes in nutrient cycling, phosphatase activities and microbial C, N and P were measured in soils from exclosure subplots not only in the summer of 2003 but also after freeze-up in 2003, before thaw in 2004 and throughout the following growing season of 2004. In addition, as a comparison to goose-exclosed sites in the fertilization experiment, on some occasions microbial nutrient immobilization and soil phosphatase activities (Pases) were measured in soil beneath areas with intact, goose-grazed vegetation ("intact"), paired within 1 m of each NP-fertilized exclosure.

Phosphatase activities at La Pérouse Bay had not been measured previously. Accordingly, factors affecting the soil phosphatase assay, including maximal activity in relation to pH, reaction linearity, and dilution stoichiometry, as well as the relative release of acid and

alkaline Pases by plants and soil microbes, and enzyme kinetics were examined. In addition to the experimental plots on the intertidal salt marsh, a baseline study of phosphatase activities across the intertidal, supratidal and freshwater marshes was conducted.

a)

b)

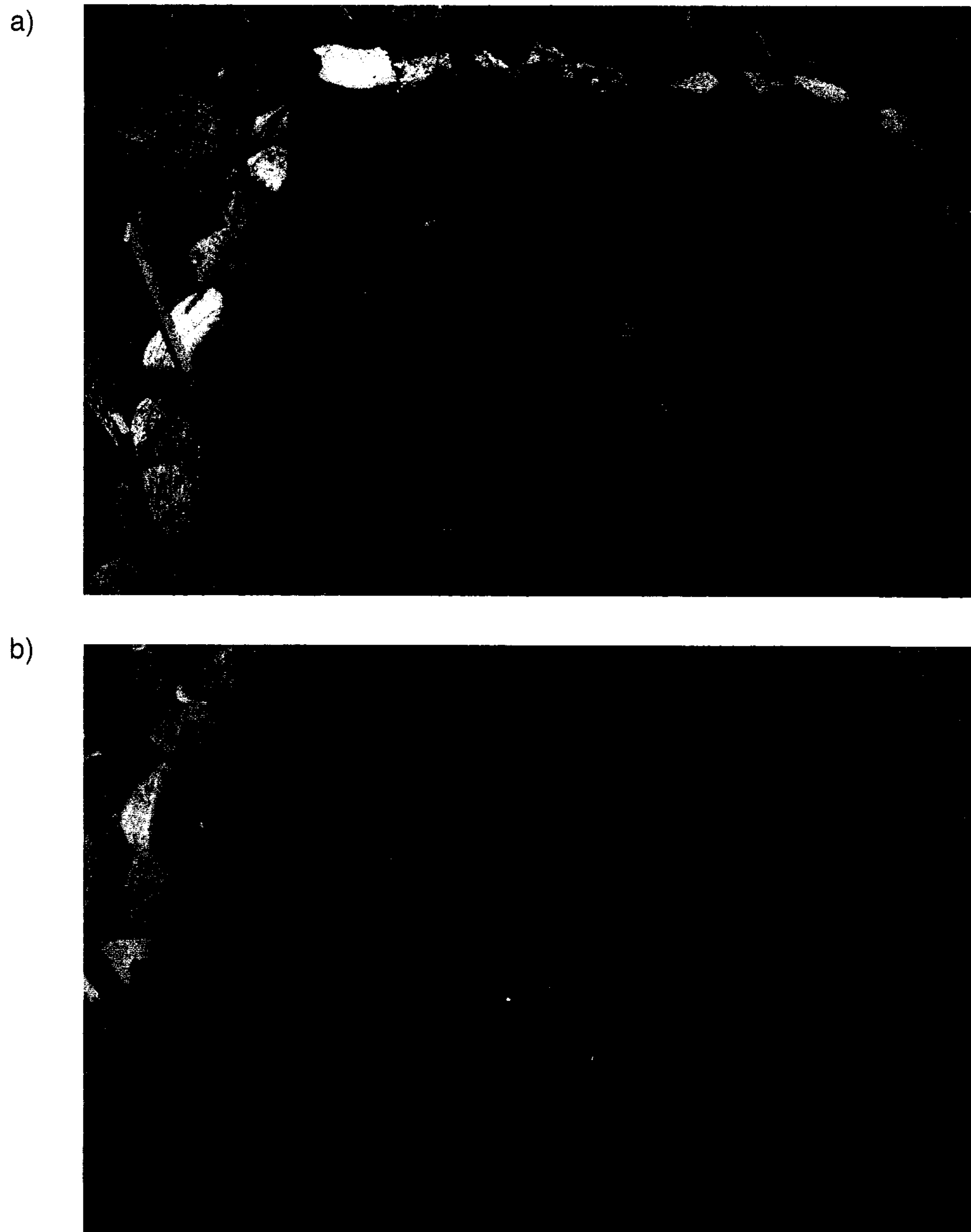


Figure 2.3 Photographs of a typical exclosure on the western intertidal salt marsh at La Pérouse Bay, Manitoba, (a) on the day of nutrient addition, 3 June 2003, and (b) in late July 2003. Treatments clockwise from the top-left subplot: (0N1P), (0N0P), (1N1P), (1N0P). The presence of the rocks, which were put in place to help prevent goose entry, results in the growth response along the edges of the exclosure ("edge effects") by insulating soil temperatures and by acting as protection from the wind. Photographs were taken by the author.

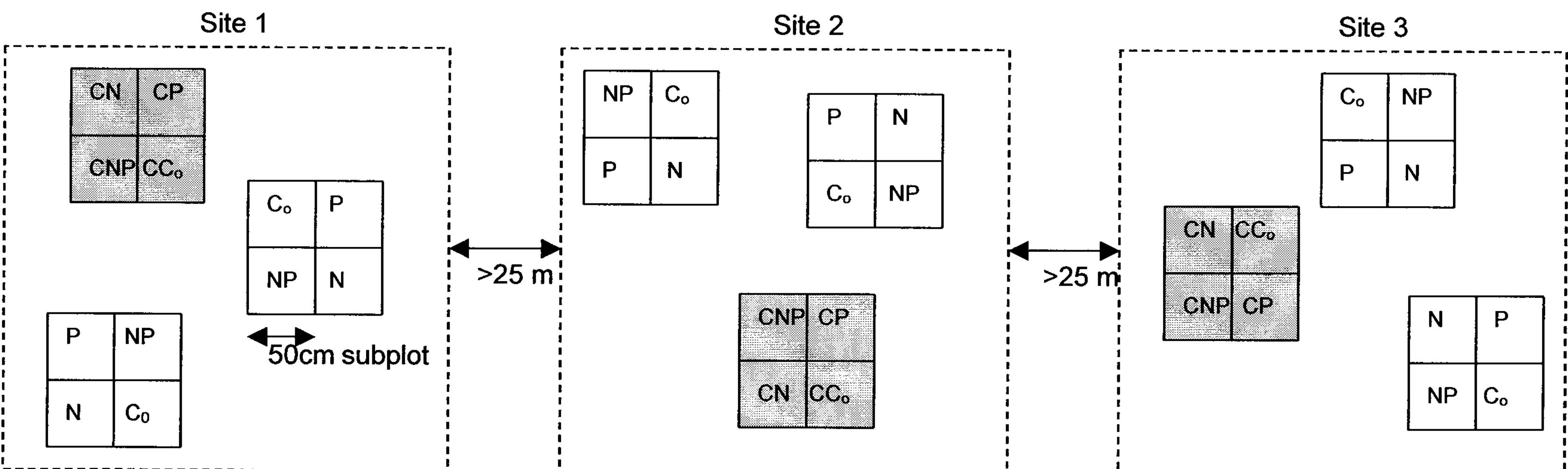


Figure 2.4 Diagram of the design of the fertilization experiment on the western intertidal salt marsh at La Pérouse Bay, Manitoba. Three sites were established (n=3) and three exclosures were erected on each site on 2 June 2003. Nutrients were added as salts on 3 June 2003. The four subplots in all nine exclosures were randomly assigned one of four treatments, including nitrogen (N) addition, phosphorus (P) addition, N + P addition, and control subplots (C_o) that received no nutrient supplement (see section 2.2 for further details). Two exclosures per site (white plots, or NP-fertilized plots) were sampled throughout the summer of 2003, once in October 2003, and in June and July 2004 (refer to Table 2). One exclosure per site (grey plots, or CNP-fertilized plots) was left unsampled until 27 July 2003, at which point carbon (C) was added as a glucose solution and the plot was sampled every two days for six days.

2.3 Sample collection

2.3.1 Sample collection from fertilization plots

Approximately every two weeks throughout the summer of 2003, cores were collected from two experimental plots per site for the following analyses. Using a polyethylene tube, one core 7.2 cm x 6 cm (diameter of tube x rooting depth of *P. phryganodes*) from each of the NP-fertilized subplots, including the control plot, was used for the collection of shoots of *P. phryganodes* and to measure soil physical and chemical properties, including pH, redox potential, and pore water quantities for analysis of salinity and dissolved organic P (Figure 2.5, Table 2.2: Core 1). A second core of the same size from one exclosure per site chosen at random was used for soil extractions to measure inorganic N and microbial biomass in the four subplots (Figure 2.5, Table 2.2: Core 2). Two smaller cores, 2.2 cm x 6 cm, were taken with a cork borer from all plots. One smaller core was used to measure bulk density (Figure 2.5, Table 2.2: Core 3). All living roots of *P. phryganodes* were tweezed from the second smaller core to ensure that root biomass corresponded to a consistent area of shoot biomass (Figure 2.5, Table 2.2: Core 4).

On separate dates, two additional cores (2.2 cm x 6 cm) were collected from the four subplots within the six NP-fertilized exclosures. One core was assayed for acid and alkaline phosphatase activities (Figure 2.5, Table 2.2: Core 5); the other was extracted for total extractable phosphorus using Olsen's and Bray's extractants (Figure 2.5, Table 2.2, Core 6).

Phosphatase activities, microbial C, N and P, and a variety of soil physical and chemical properties were measured from soil sampled from the NP-fertilized plots in October 2003 and during June and July 2004. Note that it was not possible to sample from the fertilization plots in April 2004 because of a dense snow pack.

After glucose addition on 27 July 2003, the previously unsampled exclosure on each site was sampled for estimation of microbial biomass every two days for six days from 27 July to 2 August. Phosphatase activities were measured on day 1 and day 6, while soil physical and chemical properties and above-ground biomass were measured only on the first day of sampling. Sample collection of data used to explore microbial carbon limitation was relatively short because carbon functions as a structural molecule, not a mineral nutrient, and thus once the C added to the soil is taken up, it will be locked up in plant tissue and microbial biomass. Furthermore, since the turnover of microbial populations is so fast, any changes in microbial immobilization of C, N and P in response to the addition of a carbon source will be seen within the six-day sampling period.

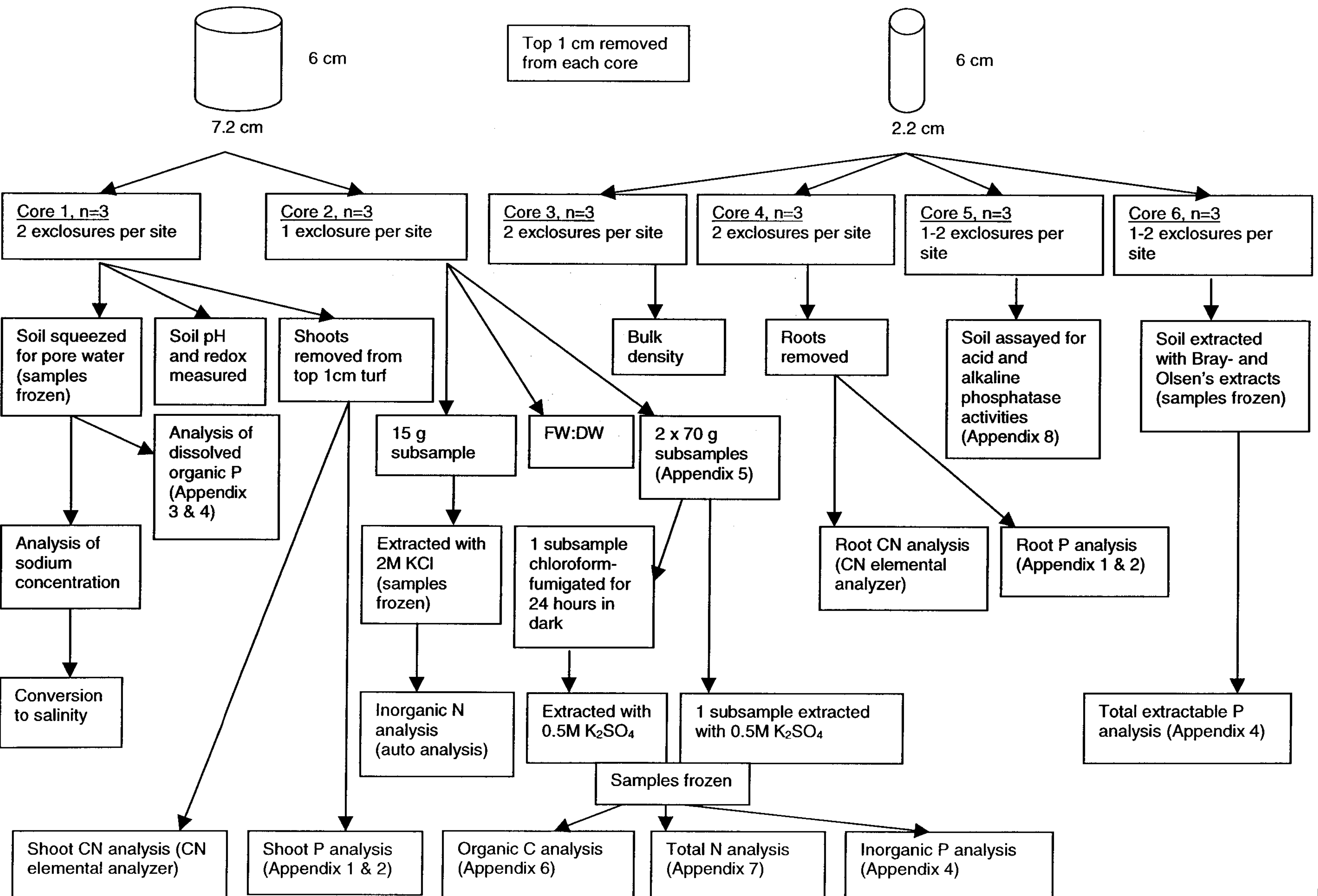

6 cm
7.2 cm
Top 1 cm removed from each core
6 cm
2.2 cm
Core 1, n=3
2 exclosures per site
Core 2, n=3
1 exclosure per site
Core 3, n=3
2 exclosures per site
Core 4, n=3
2 exclosures per site
Core 5, n=3
1-2 exclosures per site
Core 6, n=3
1-2 exclosures per site
Soil squeezed for pore water (samples frozen)
Soil pH and redox measured
Shoots removed from top 1cm turf
Bulk density
Roots removed
Soil assayed for acid and alkaline phosphatase activities (Appendix 8)
Soil extracted with Bray- and Olsen's extracts (samples frozen)
Analysis of dissolved organic P (Appendix 3 & 4)
15 g subsample
FW:DW
2 x 70 g subsamples (Appendix 5)
Analysis of sodium concentration
Extracted with 2M KCl (samples frozen)
1 subsample chloroform-fumigated for 24 hours in dark
Root CN analysis (CN elemental analyzer)
Root P analysis (Appendix 1 & 2)
Conversion to salinity
Inorganic N analysis (auto analysis)
Extracted with 0.5M K_2SO_4
1 subsample extracted with 0.5M K_2SO_4
Total extractable P analysis (Appendix 4)
Samples frozen
Shoot CN analysis (CN elemental analyzer)
Shoot P analysis (Appendix 1 & 2)
Organic C analysis (Appendix 6)
Total N analysis (Appendix 7)
Inorganic P analysis (Appendix 4)

Figure 2.5 Flow diagram of sampling procedures and laboratory analysis for soil collected in June - August 2003, October 2003, April 2004, and June - July 2004, on the western intertidal salt marsh at La Pérouse Bay, Manitoba.

Table 2.2 Timetable of all soil samples collected on the intertidal, supratidal and freshwater marshes at La Pérouse Bay, Manitoba in June - August 2003, October 2003, April 2004, and June - July 2004. Cores 1 - 6 refer to soil cores collected and sampled following the procedures outlined in Figure 2.5. See text for site descriptions (section 2.1).

Date	Core 1	Core 2	Core 3	Core 4	Core 5	Core 6	Site	Notes
2 June 2003					x		Intertidal	Pases from intact and degraded sites
3 June 2003	x	x	x		x	x	NP plots,	No inorganic N analysis
12 June 2003					x		Intertidal intact	Factors affecting Pase assay
16 June 2003					x	x	NP plots	
18 June 2003	x	x	x	x			NP plots	
28 June 2003					x		Intertidal intact	Factors affecting Pase assay: maximal pH
1 July 2003					x	x	NP plots	
3 July 2003	x	x	x	x			NP plots	
14 July 2003		x			x		NP plots & Intertidal intact	Microbial CNP from intertidal, Pases from plots
17 July 2003	x		x	x		x	NP plots	
25 July 2003					x		Intertidal & Supratidal intact	Factors affecting Pase assay: dilution stoichiometry
26 July 2003					x		Freshwater intact	
29 July 2003	x	x	x	x		x	NP plots	
30 July 2003					X		Supratidal intact	Root phosphatase activities
27 July – August 1 2003		x			x		CNP plots	Pase on 27 July and 1 August only
5 August 2003	x	x		x	x	x	NP plots	No inorganic N analysis or soil physical properties
25 October 2003		x	x		x		NP plots & Intertidal intact	No inorganic N analysis
25 April, 2004		x	x		x		Intertidal intact	No inorganic N analysis
19 June, 2004		x			x		NP plots	No inorganic N analysis
29 June, 2004		x			x		NP plots	No inorganic N analysis
9 July, 2004		x			x		NP plots	No inorganic N analysis
28 July, 2004		x			x		Intertidal intact	No inorganic N analysis

2.3.2 Sample collection from intact and degraded sites in the intertidal, supratidal and freshwater marshes

Soil collections from intact sites on the intertidal marsh were made several times throughout the summer of 2003, in October 2003 and April, June and July 2004 in order to measure microbial immobilization of C, N and P and phosphatase activities (refer to Table 2.2). In a comparative study, soils from intact sites on the intertidal, supratidal and freshwater marshes were assayed for phosphatase activities from 25 July to 31 July. Phosphatase activities from soil beneath degraded sites on the intertidal marsh were also measured at the beginning of June 2003 (Table 2.2). Assumptions of the phosphatase assay were tested using soil removed from sites with intact vegetation on the intertidal salt marsh on 12 June and 28 June 2003.

2.4 Sample processing and analysis

Throughout the summer of 2003 samples were brought to the La Pérouse Bay field laboratory, approximately 3 km from the study site, and processed within two days of collection. Samples collected throughout June and July 2004 were processed in the laboratory at the Churchill Northern Studies Centre within two days of sampling. All soils were stored in the dark at ground temperature. Soils sampled in October 2003 and April 2004 were kept at ground temperature (frozen) in coolers, air freighted to Toronto and processed in the University of Toronto laboratory within 4 days of sampling. All solutions were prepared with deionized water. "Analar" (British Drug House) chemicals were used whenever available in this study.

2.4.1 Plant processing and analysis

From intact cores (7.2 cm x 6 cm) above-ground biomass was clipped and rinsed with deionized water, oven-dried at about 50°C for 48 – 72 hours and weighed (Figure 2.5, Table 2.2: Core 1). All biomass was re-dried at 50°C for one week and re-weighed to the nearest milligram in Toronto. Live, fine white roots were tweezed from the small soil cores (2.2 cm x 6 cm), and rinsed in river water, oven-dried at about 50°C for 48 – 72 hours and shipped to the University of Toronto for nutrient analysis (Figure 2.5, Table 2.2: Core 4).

Before nutrient analysis, shoots of *P. phryganodes* were ground at a mesh size of 40 (0.420 mm sieve opening) in a Wiley mill (Model A: Thomas Scientific, U.S.A). There was a limited amount of root biomass, so roots sampled from the six NP-fertilized plots were combined within each site according to treatment and then ground using a mortal and pestle. Amounts of carbon and nitrogen in plant tissue were measured with the use of a CHN elemental analyzer

(Costech International S.I.r. Italy). Plant tissue was digested in a modified Kjeldahl digestion (Appendix 1) before colorimetric analysis of the inorganic phosphorus (orthophosphate) concentration was estimated using the molybdate-blue method modified from Ames (1966) (Appendix 2). In this method, orthophosphate present in the sample complexes with acidified molybdate. This phosphomolybdate complex is then reduced in the presence of ascorbic acid to yield the characteristic molybdenum blue colour (Grimshaw et al. 1989). Because colour development is acid-dependent, acidity of the reagents and sample solutions must be controlled, so both acid and alkaline solutions (samples and standards) were neutralized before analyses. All C:N:P ratios in plant tissues were calculated on an atomic basis.

2.4.2. Analysis of soil physical properties

Soil physical properties were measured from within the fertilization plots on the intertidal salt marsh and from beneath intact sites on the supratidal marsh. Soil physical and chemical properties were not measured in the freshwater marsh, but values from (Ngai 2003) can be used to compare and contrast differences in phosphatase activities between the two marshes.

2.4.2.1 Redox potential, pH and bulk density

Redox potential (E_h) was measured with the use a platinum electrode attached to a Fisher Accumet Portable pH Meter (Model 156) and read on the millivolt scale (Figure 2.5, Table 2.2: Core 1). The instrument was calibrated with ZoBell's solution (ZoBell 1946) to read +430 mV at 25°C. Redox readings were taken when the electrode probe was inserted in a freshly cut soil surface at depths of 2 cm and 5 cm (n=5) in order to measure the redox potential at different rooting depths. Soil pH was measured with a pH probe on a Fisher Accumet Portable pH Meter (Model 156), after the soil was mixed in a 3:1 (v:v) soil to deionized water slurry and equilibrated for 15 minutes (Figure 2.5, Table 2.2: Core 1). The top 1 cm of one of the small cores (2.2 cm x 6 cm) was removed to calculate soil bulk density, using a known volume, and its fresh weight and dry weight determined (Figure 2.5, Table 2.2: Core 3). Soils were dried over the field stove for 48-72 hours at approximately 50 - 60°C.

2.4.2.2 Salinity and dissolved organic P

From the same soil core that was used for pH, redox and above-ground biomass, pore water was hand-squeezed using gloves through a pre-leached (2M KCl and deionized water-rinsed) Whatman No. 1 filter (Figure 2.5, Table 2.2: Core 1). Samples were frozen until they were analyzed for soil salinity and dissolved organic P. Sodium concentration was measured with the use of a Perkin-Elmer atomic absorption spectrophotometer (Model 3110, Norwalk, CT)

in the flame-emission mode at wavelength 589 nm. The concentration of sodium was then converted to salinity using the regression equation: salinity (g dissolved solids L^{-1}) = 3.59 [Na^+] g L^{-1} + 3.85 (r = 0.96) (Srivastava and Jefferies 1995). Soluble organic phosphate concentrations were measured using the molybdate-blue method (Ames 1966)(Appendix 4) before and after conversion of organic P to orthophosphate oxidation via acid potassium persulfate. Dissolved organic phosphorus was calculated as total dissolved phosphorus in soil solution less inorganic phosphorus in the solution (Lajitha et al. 1999)(Appendix 3).

2.4.2.3 Total soil C, N and P

From a selection of soil used for fresh weight to dry weight ratios, dried soil from the top 5 cm of the soil profile was ground in Toronto. Total phosphorus was measured by Laboratory Services at Guelph University (Guelph, Canada) using the molybdate-blue method, after digestion with hydrofluoric acid. Total N and total C were measured using a CHN elemental analyzer (Costech International S.l.r., Italy). Soil C:N:P ratios were calculated based on atomic ratios.

2.4.3 Analysis of soil chemical properties

2.4.3.1 Extractable P

In addition to soil physical properties, soil from each subplot was extracted for chemical properties including amounts of nitrate and ammonium ions and total extractable phosphorus. Both Olsen's- and Bray's extracts (Kuo 1996) were used to extract phosphorus (Figure 2.5, Table 2.2: Core 6). Olsen's extracting solution, 0.5M $NaHCO_3$ buffered at pH8.5, is useful in calcareous soils because the solubility of calcium phosphate is increased by the precipitation of $CaCO_3$. In acid or neutral soils, where phosphates are bound to aluminum and iron, increased OH^- concentrations from the bicarbonate extract decreases the concentration of Al^{3+} and of Fe^{3+}. In contrast, Bray's extracting solution (0.03M NH_4F and 0.025 HCl) for acid soils increases P solubility by decreasing Al activity due to the formation of aluminum and fluoride complexes (Kuo 1996).

Olsen's total extractable phosphorus was extracted using 2 grams fresh weight soil crumbled into 40 mL 0.5M $NaHCO_3$ buffered at pH 8.5 with 1M NaOH. The suspension was hand shaken frequently for one hour and then filtered through a Whatman No. 40 filter paper. For Bray's total extractable phosphorus, one gram fresh weight soil was suspended in 35 mL 0.03 M NH_4F and 0.025 M HCl extracting solution. The volume of extracting solution was determined in order to maintain a pH level below 2.9 for better extraction (Kuo 1996). Soil

suspensions were hand shaken frequently during 30 minutes and then filtered through Whatman No. 40 filter papers. For both Olsen and Bray's extractions, a representative soil sample from the entire depth of the core (5 cm) was used. All filtrates were frozen until phosphate concentrations in the extraction solution were analyzed in Toronto using the molybdate-blue method (Ames 1966) (Appendix 4).

2.4.3.2 Exchangeable N

For extraction of exchangeable nitrate and ammonium ions and microbial C, N and P (refer to section 2.4.4), the soil was mixed in a clean plastic bag and separated into four representative portions (Figure 2.5, Table 2.2: Core 2). One portion of approximately 10 g fresh weight was weighed, dried and reweighed in order to determine a fresh weight to dry weight ratio. For measurement of soil exchangeable nitrate and ammonium ions, a 15 g fresh weight sub-sample was extracted in 75 mL of 2M KCl and hand shaken frequently. After 2 hours the soil suspension was filtered through a Whatman No 40 filter that was pre-leached with 2M KCl and deionized water (Mulvaney 1996). All extracts were frozen until analysis in Toronto, where the inorganic N in the 2M KCl soil extracts was measured colorimetrically with the use of an auto analyzer (Technicon AAII, Tarrytown, NY). Ammonium concentrations were determined using the indophenol blue method (Keeny 1982) that is based on formation of a blue complex when phenol and NH_3 react in the presence of an oxidizing agent under alkaline pH conditions. Nitrate concentrations were determined as nitrite after the extracts were reduced by cadmium and treated with a diazotizing agent (Keeny 1982).

2.4.4 Analysis of microbial C, N and P

From the mixed soil samples (refer to section 2.4.3.2), two sub-samples each of 70 g fresh weight were weighed for the estimation of microbial biomass using a modified chloroform-fumigation-extraction (CFE) method (Witt et al. 2000) (Figure 2.5, Table 2.2: Core 2; Appendix 5). The first 70 g fresh weight sample was immediately extracted with 140 mL 0.5M K_2SO_4, hand shaken frequently over a period of one hour, and filtered through a Whatman GF/A filter paper. The second sub-sample was placed in a tightly sealed, 250 mL Schott bottle with 2 mL of ethanol-free chloroform and incubated in the dark at room temperature for 24 hours. After the incubation, the Schott bottle was left opened in a well-ventilated area for 30 minutes to evaporate excess chloroform. The fumigated sample was then extracted and filtered in the same manner as the non-fumigated sample. Because of limited supply of potassium sulfate at the end of the field season, microbial biomass from the three carbon-enriched plots

(CNP-fertilized plots) were extracted with a 0.5M $NaHCO_3$ solution buffered at pH 8.5 for all four sampling days (40 g fresh weight soil to 200 mL extracting solution). Extracts were frozen until analysis at the University of Toronto.

Microbial C, N and P were each calculated as the difference between amounts of C, N and P in the fumigated and non-fumigated samples. The non-fumigated, or control sample, provides a measure of soil extractable C, N and P. Since it is assumed that chloroform vapour induces lysis of a known percentage of microbial cell membranes, the fumigated sample is a measure of soil C, N and P plus a known percentage of microbial C, N and P (Witt et al. 2000). The total soil microbial C, N and P content of the sample is corrected for by extractability factors (k_{EC}, k_{EN}, k_{EP}, respectively) specific to each nutrient (Jonasson et al. 1996).

Soil extractions typically depend on mechanical shaking to ensure complete exchange of ions from soil colloids to extract solution. At the La Pérouse Bay field laboratory, this procedure was replaced with frequent, but not continuous, manual shaking. The assumption that less vigorous shaking did not significantly affect extraction of microbial biomass was tested by in a previous study by comparing estimates of microbial C, N from intact intertidal soil samples that were either mechanically or manually shaken, and then extracted with 0.5M K_2SO_4 (Buckeridge 2004). The factors affecting chloroform-fumigation-extraction are discussed in section 4.4.1.

An additional consideration is that the chloroform application and the duration of fumigation affect the efficiency of CFE. The application of chloroform may be made by the direct addition of chloroform to Schott bottles (Witt et al. 2000), which was the procedure used in this study. Alternatively, the application of chloroform vapour may be made under vacuum, so that the chloroform quickly vaporizes (Brookes et al. 1985b). This may increase cell lysis and the release of nutrients. For each study site, it is also important to determine the chloroform-fumigation period that maximizes nutrient extraction, which is typically 24 hours (Brookes et al. 1982, Brookes et al. 1985a, Brookes et al. 1985b, Witt et al. 2000).

2.4.4.1 Microbial carbon

Microbial carbon (MBC) extracted by K_2SO_4 and $NaHCO_3$ was estimated by measuring the amount organic C using a dichromatic volumetric method (Nelson and Sommers 1996) (Appendix 6). In this method, organic C is oxidized by $K_2Cr_2O_7$ at high temperatures (between 137 and 170°C). The excess dichromate not utilized in the oxidation is then titrated with ferric ammonium sulfate and *N* – phenylanthranilic acid indicator, such that the carbon-enriched samples require less titrate to reach the end point. Glucose standards are digested to calibrate digestion efficiency, while extractant blanks and undigested dichromate are used to correct for

the thermal decomposition of dichromate and to standardize the titrate. It is also important to correct for interference method by the ferrous iron (Fe^{2+}) when using the dichromate, which results in an overestimation of organic C. However interference at this site has been shown to be insignificant (Henry 2003). Microbial C is calculated by difference after accounting for field blanks and overestimation of C caused by chloroform-C (refer to Appendix 5, step 8). An extractability factor of $k_{EC} = 0.35$ was used to correct for the incomplete recovery (35%) of organic carbon in the sample (Sparling et al. 1990, Jonasson et al. 1996). This value is highly correlated ($r = 0.831$) with estimates of microbial C of Arctic soils measured by substrate-induced respiration (Cheng and Virginia 1993), but is slightly lower than the values used in temperate, aerobic soils ($k_{EC} = 0.38$, Vance 1987; $k_{EC} = 0.45$, Jenkinson 2004).

2.4.4.2 Microbial nitrogen

Microbial N (MBN) was measured as the difference of total nitrogen in K_2SO_4 and $NaHCO_3$ extracts between fumigated and non-fumigated samples. Total nitrogen as nitrite was measured colorimetrically with an auto analyzer (refer to section 2.4.3.2) after conversion to nitrate following alkaline persulfate oxidation (Cabrera and Beare 1993) (Appendix 7). The estimate was corrected on the basis that 40% of the total soil microbial nitrogen was released ($k_{EN} = 0.40$) after cells were killed by chloroform (Sparling et al. 1990, Jonasson et al. 1996). This value has been used in Arctic soils, but is lower than the value recommended generally ($k_{EN} = 0.45$, Jenkinson 2004) and is in the middle of the range of values ($k_{EN} = 0.30$ to 0. 54) that have been reported previously using K_2SO_4 (Jonasson et al. 1996).

2.4.4.3 Microbial phosphorus

Microbial P (MBP) was determined by the difference between orthophosphate (P_i) concentrations in fumigated and non-fumigated samples. After neutralizing the soil extracts when necessary, P_i was measured using the molybdate-blue method (Ames 1966) (Appendix 4). Final estimations of soil microbial P assumed that 40% ($k_{EP} = 0.40$) of the P_i locked up in microbial biomass was mineralized. This value follows the extractability of P_i by K_2SO_4 reported in both Arctic soils (Jonasson et al. 1996) and temperate soils (Jenkinson et al. 2004) and is in between the k_{EP} developed originally ($k_{EP} = 0.47$, Brookes et al. 1982; $k_{EP} = 0.37$, Hedley and Stewart 1982) using a 0.5 M $NaHCO_3$ extract buffered at pH 8.5. Estimates of microbial P using the CFE method are commonly based on P_i concentrations, because organic P usually represents a negligible portion (c. <10%) of the chloroform-released P, which is presumed to be due to the continuation of phosphatase activities throughout fumigation (Brookes et al. 1982). In

addition to the correct estimations of microbial P using an extractability factor, it is also necessary to correct for the amount of inorganic P released from microbes during fumigation that is fixed on soil colloids, or the 'P fixation factor' (Brookes et al. 1982), which is specific to each soil type. In order to do this, orthophosphate spikes equivalent to 1.3 μg P g^{-1} were added to soil extractions of non-fumigated samples in April 2004. The fixation factor (k_{fix}) was then calculated according to as follows:
μg P in spiked sample / (μg P in non-fumigated sample + 1.3 μg P).

2.4.5 Assay of soil phosphatase activity

Acid phosphatase activities (AcidPases) and alkaline phosphatase activities (AlkPases) were measured following Tabatabai (1994) (Figure 2.5, Table 2.2: Core 5; Appendix 8). The assay measures the breakdown of phosphomonoesters (E.C. 3.1.3) on the soil surface by plant and microbial phosphatases. One gram fresh weight of soil was combined with toluene, to suppress microbial growth and uptake of phosphate, and a modified universal buffer was added to buffer enzymatic activity. Last to be added to the incubation suspension was the artificial substrate, p-Nitrophenyl phosphate (*p*-NPP), on which the phosphatase enzymes act and from which the release of *p*-nitrophenol (*p*-NP) was measured. After the soil suspension was incubated for 1 hour, the phosphatase reaction was stopped and a yellow colour was developed by the addition of 0.5 M $CaCl_2$ and 0.5 m NaOH. The solution was then measured colorimetrically at an absorption of 410 nm. The amount of *p*-NP cleaved by phosphatase activities was determined after subtracting the soil control, which accounts both for the background level of organic phosphorus and for trace amounts of coloured soil material that could contaminate sample solutions and result in an overestimation. Phosphatase activities were calculated as the ratio of AcidPases to AlkPases, and by weight and volume of dry soil. Pases were also calculated by volume of soil solution, in order to compare them to the concentration of dissolved organic phosphorus in the soil solution.

2.4.5.1 Factors affecting the soil phosphatase assay

Factors that affect the soil phosphatase assay, including maximal pH, reaction linearity, and dilution stoichiometry, were examined as follows. The maximal pH for the assay of AcidPases and AlkPases specific to the intertidal salt marsh soil was determined using the modified universal buffer in the range of pH 5 – 7 and pH 9 – 11, respectively, in half unit increments. From thereon the assays were buffered at the established pH at which maximum activity was detected.

Next, the procedure was repeated at 30-minute intervals for 90 minutes in order to check that the enzyme reaction was linear. Standards ranged from 0 – 50 ug *p*-NP, and corresponded to an optical density of 0 - 0.584, so it was necessary to dilute sample solutions to within this range before they were measured on the spectrophotometer. Therefore, trials were run to ensure the dilution stoichiometry was linear. As such, samples were diluted and calculated concentrations of *p*-NP were compared.

2.4.5.2 Enzyme kinetics

The tendency for phosphatases to combine with and hydrolyze their substrates is given by the K_m value (Michaelis-Menten constant). K_m is defined as the substrate concentration at which the reaction proceeds at half its maximum rate, such that low K_m values correspond to high substrate affinity (Nobel 1999). Problems with measuring K_m arise when dealing with a complex of enzymes, as with phosphatases, since the Michaelis-Menten value will reflect the additive effect of all enzymes, but not the absolute affinity of each enzyme. However, it is still practical to characterize the phosphatase complex using Michaelis-Menten constant (Jansson et al. 1988). The Lineweaver-Burk plot is a linear transformation of the Michaelis-Menten equation and can be used to calculate values of K_m and V_{max}. Accordingly, activities of acid and alkaline phosphatases were measured at varying concentrations of substrate *p*-NPP (2.5 µmol to 150 µmol), and the reciprocal of enzymes activity ($1/v$) was plotted against the reciprocal of substrate concentration (1/[S]). The y-intercept is equal to ($1/V_{max}$), the x-intercept is equal to (-$1/K_m$) and the slope of the line is (K_m / V_{max}) (Baker 1975).

2.4.5.3 Root and microbial phosphatase exudation

In order to measure the relative release of acid and alkaline Pases by plant roots and soil microorganisms, living roots of *Puccinellia phryganodes* were tweezed carefully from soil sampled from the intertidal salt marsh, and rinsed with river water. Rinsed roots were then buffered at maximal acid and alkaline pH's and incubated following the procedure outlined for soils (Appendix 8). River water was used as the rinse because it contains ions, such as calcium, and thus maintains membrane integrity, which would be lost if the roots were rinsed with pure, deionized water (Epstein 1972). Membrane integrity is essential for root nutrient retention. In order to measure the relative release of acid and alkaline Pases by soil microbes, degraded soil, which had been devoid of vegetation for at least 5 years, was assayed.

2.5 Statistical analyses

Before statistical analyses were performed, all data were tested to ensure they met assumptions of normality (Kolmogorov-Smirnov goodness of fit test, $\alpha > 0.05$) and homogeneity of variances (Bartlett's test, $\alpha > 0.05$). If data were non-normal, they were transformed as needed. If data from the fertilization experiment were both normal and log-normal, log-normal transformations were used to ensure the data were additive, not multiplicative, among treatments (Zar 1999).

Multiple comparisons on parametric data were conducted using the Tukey, or "honestly significant difference" (HSD), test and the Tukey-Kramer test for data of equal and unequal sample sizes, respectively. All analyses, except multivariate analyses of variance (MANOVAs), were performed using the Analyst application in SAS version 8 (SAS Institute Inc. 1999 - 2001). MANOVAs were performed using JMP 4.0.2 (SAS Institute Inc. 1989 - 2000).

2.5.1 Univariate analyses of plant, microbial and soil data from the fertilization experiment

A true repeated measures, or split-plot, experimental design is one in which the same block is applied to some, but not all, of the combinations of factors, and a multiple measurement, or repeated-measure, comprises the replicate data (Zar 1999). However, all treatment combinations were given to every block of the NP-fertilization experiment, so the multiple measurement (time) *and* block (site) comprise the replicate data. Thus, in order to examine the effect of N and P fertilization on plant, microbial and soil data over time, mixed-model, full-factorial multi-way analyses of variance (ANOVAs) were run with nitrogen and phosphorus additions and date as fixed-effects factors, blocked by site (random-effect factor) (Zar 1999). Since the experimental design is not a "true" repeated-measures design, it was not necessary to meet assumptions of sphericity (Zar 1999).

2.5.2 Univariate analyses of microbial C, N and P data from grazed and exclosed sites.

In order to examine the effect of grazing on microbial nutrient immobilization of C, N and P over time, full-factorial three-way ANOVAs were run with grazing treatment (intact sites versus exclosed control subplots) and time as fixed-effect factors, blocked by site. It was necessary to block by site because controls (0N0P subplots) were randomly assigned to each subplot and were paired with an intact site adjacent to the exclosure. Results of microbial C, N and P were compared between sites and among dates using Tukey-Kramer tests.

2.5.3 Univariate analyses of acid and alkaline phosphatase data

In addition to phosphatase data from the fertilization experiment (section 2.5.1), univariate tests were run on other phosphatase data. The effect of grazing on AcidPases and AlkPases was tested separately using the statistical analyses outlined for microbial data (section 2.5.2). In order to determine the maximal pH's, one-way ANOVAs were run for each pH range from soil collected on 12 June and 28 June 2003, for a total of four independent analyses. Tukey's HSD test was used to examine differences within each pH range for each date. Note that, although n=3 at each half-unit increment, the soil used for the assays was taken from a single intact site on the intertidal salt marsh. Thus, the data are pseudoreplicated but, for the purpose of this exercise, results can be generalized regardless of sampling error. The relative release of AcidPases and AlkPases by plant roots was tested (refer to section 2.4.5.3) by performing a paired Student's *t*-test to compare of AcidPases to AlkPases exuded by plant roots.

2.5.4 Multivariate analyses of acid and alkaline phosphatase data

Because AcidPases and AlkPases can help distinguish between plant and microbial enzyme exudation, respectively, it was sometimes important to analyze differences between the release of the two enzyme complexes and not simply test for differences in overall activity among treatments or sites. Accordingly, multivariate analyses of variance (MANOVAs) were run, with AcidPases and AlkPases as the two dependent variables, to examine potential changes in the differential P limitation between plants and microbes. A MANOVA was chosen, as opposed to two ANOVAs, because the multivariate analysis maintains the chosen magnitude of Type I error ($\alpha = 0.05$), while controlling for correlation between the phosphatase complexes (Tabachnick and Fidell 2001). However, problems arise when examining the main effects or interactions of a MANOVA using univariate, multiple comparisons. These problems are especially apparent when the dependent variables are correlated, because the pooled within-in group correlations among them are not zero (unless they were formed by principle components analysis) (Tabachnick and Fidell 2001). Although Bonferroni-type adjustments of error on uncorrelated dependent variables can be made, no straightforward adjustments of error can be made for ANOVAs of each of the correlated dependent variables. Nonetheless, univariate tests, with adjustment of α for multiple tests, are the most common method of interpreting the results of a MANOVA with correlated dependent variables (Tabachnick and Fidell 2001). Therefore, when whole model effects of a MANOVA were significant ($P < 0.05$), the overall Type I error rate (α) was calculated as follows: $\alpha = 1 - (1 - \alpha_{acid})(1 - \alpha_{alkaline})$, such that $\alpha = 0.049$ when the

power of the univariate tests of AcidPases (α_{acid}) equals the power of the univariate tests of AlkPases ($\alpha_{alkaline}$), which both equal 0.025. When whole model effects of a MANOVA were insignificant (P>0.05), effects on AcidPases and AlkPases were compared independently using univariate multiple comparison ($\alpha = 0.05$).

Accordingly, a full-factorial MANOVA was run to look at the effect of grazing (intact sites compared to exclosed 0N0P subplots) on AcidPases and AlkPases simultaneously. Because of the limitation of the statistical program used (JMP 4.0.2 SAS Institute Inc. 1989 - 2000), it was not possible to block by exclosure. A one-way MANOVA was run in order to examine the effect of presence (intact sites) or absence (degraded sites) of plants on AcidPases and AlkPases from soil from the intertidal marsh. A difference between AcidPases and AlkPases in the degraded sites will clarify the relative soil microbial release of the two enzyme complexes, since the degraded sites are devoid of plant roots. A one-way MANOVA was run to look for differences in AcidPases and AlkPases among the intertidal, supratidal and freshwater marshes.

In addition, multi-way MANOVAs were run, with AcidPases and AlkPases as two dependent variables, to examine changes in the relative release of the two enzymes complexes in response to NP-fertilization and CNP-fertilization during the summer 2003 and from June 2003 to July 2004. In these cases, when the overall model was significant ($P < 0.05$) trends in the AcidPase to AlkPase ratio were investigated graphically and the appropriate univariate multicomparisons were run.

2.5.5 Simple linear regression

Simple linear regression was used to examine reaction linearity, dilution stoichiometry, and enzyme kinetics of the phosphatase assay. The ANOVA procedure was used to test the slope of lines of phosphatase release against time, phosphatase release against dilution, and phosphatase release against substrate concentration, where H_0: $\beta = 0$, H_A: $\beta \neq 0$ (Zar 1999). The coefficient of determination, r^2, was used as a measure of the strength of the relationship. A relationship between the dependent and independent variables was considered very strong if $r^2 > 0.90$.

2.5.6. Correlations

In order to examine the relationships between different pools of phosphorus, results of Bray- and Olsen's extractable P, microbial phosphorus, dissolved organic phosphorus, and phosphatase from the summer of 2003 were correlated in pairs. Acid and alkaline phosphatase

activities and concentrations of ammonium and nitrate ions in soil solution were also correlated in pairs. Pearson's correlation coefficient was used to test the strength of the relationships. A correlation between two pools was considered strong if Pearson's correlation coefficient was greater than 0.70 and significant ($P < 0.05$). Since Bray- and Olsen's extractable P measure the same nutrient pool, they are expected to be positively correlated with each other, while dissolved organic phosphorus should be negatively correlated with both Bray- and Olsen's extractable P. If soil microbes are P-limited, MBP and extractable P should be positively correlated in subplots not fertilized with P, and this relationship should weaken as microbes are released from P-limitation with the addition of P fertilizer in 0N1P and 1N1P subplots (Colvan et al. 2001). If more energy is allocated to the acquisition of phosphorus via Pase activities once plants and/or soil microbes are released from N-limitation, then AcidPases and AlkPases should be positively correlated with concentrations of inorganic N in soil solution.

Chapter 3: Results

3.1 Soil phosphatase assay

3.1.1 Factors affecting the soil phosphatase assay

From soil sampled on 12 June 2003, acid phosphatase (AcidPase) activities were highest in soil buffered at pH 6.5 ($F_{4,7}$ = 7.20, P = 0.0126) and alkaline phosphatase (AlkPase) activities were highest in soil buffered at pH 10.5 ($F_{4,7}$ = 12.39, P=0.0011) (Figure 3.1, Table 3.1). Therefore, pH 6.5 and pH 10.5 were considered the optimal pH values for the assay of AcidPases and AlkPases, respectively, in soil from La Pérouse Bay, and from thereon all assay mixtures were buffered at pH 6.5 for AcidPases and pH 10.5 for AlkPases. Similar results were obtained mid-summer from soil sampled on 28 June 2003, as AcidPases peaked at pH 6.5 and AlkPases peaked at pH 10.5, but activities were not statistically different across the soil solutions buffered at different pH values (AcidPases: $F_{4,7}$ = 2.18 P = 0.1732; AlkPases: $F_{4,7}$ = 3.39 P=0.0763).

Figure 3.2 shows the affect of incubation time on the amount of *p*-nitrophenol released per gram dry weight of soil. First-order linear regressions of incubation time on AcidPase activities ($F_{1,10}$ = 285.03, P<0.0001) and AlkPase activities ($F_{1,10}$ = 109.92,P<0.0001) indicated that the slope of both regressions lines are significantly different from zero and that both lines have coefficients of determination greater than 0.90 ($r^2_{AcidPase}$ = 0.9667; $r^2_{AlkPase}$ = 0.9166). Therefore, it was concluded that the accumulation of *p*-nitrophenol increases linearly with respect to incubation time for at least 90 minutes. Soils were incubated throughout the year for 60 minutes.

All samples were diluted to within the absorption range of standards before they were read spectrophotomically. It is apparent that dilutions do not affect final estimates of AcidPase activities (Figure 3.3a), since the slope of line was not significantly different from zero ($F_{1,2}$ = 2.16; P=0.2793). In a second dilution method, a single sample was diluted serially so that the regression line of absorption values decreased linearly with increasing dilution, and the linear fit of the line was excellent ($F_{1,2}$ = 6007.76; P<0.0002; r^2 = 0.9997) (Figure 3.3b).

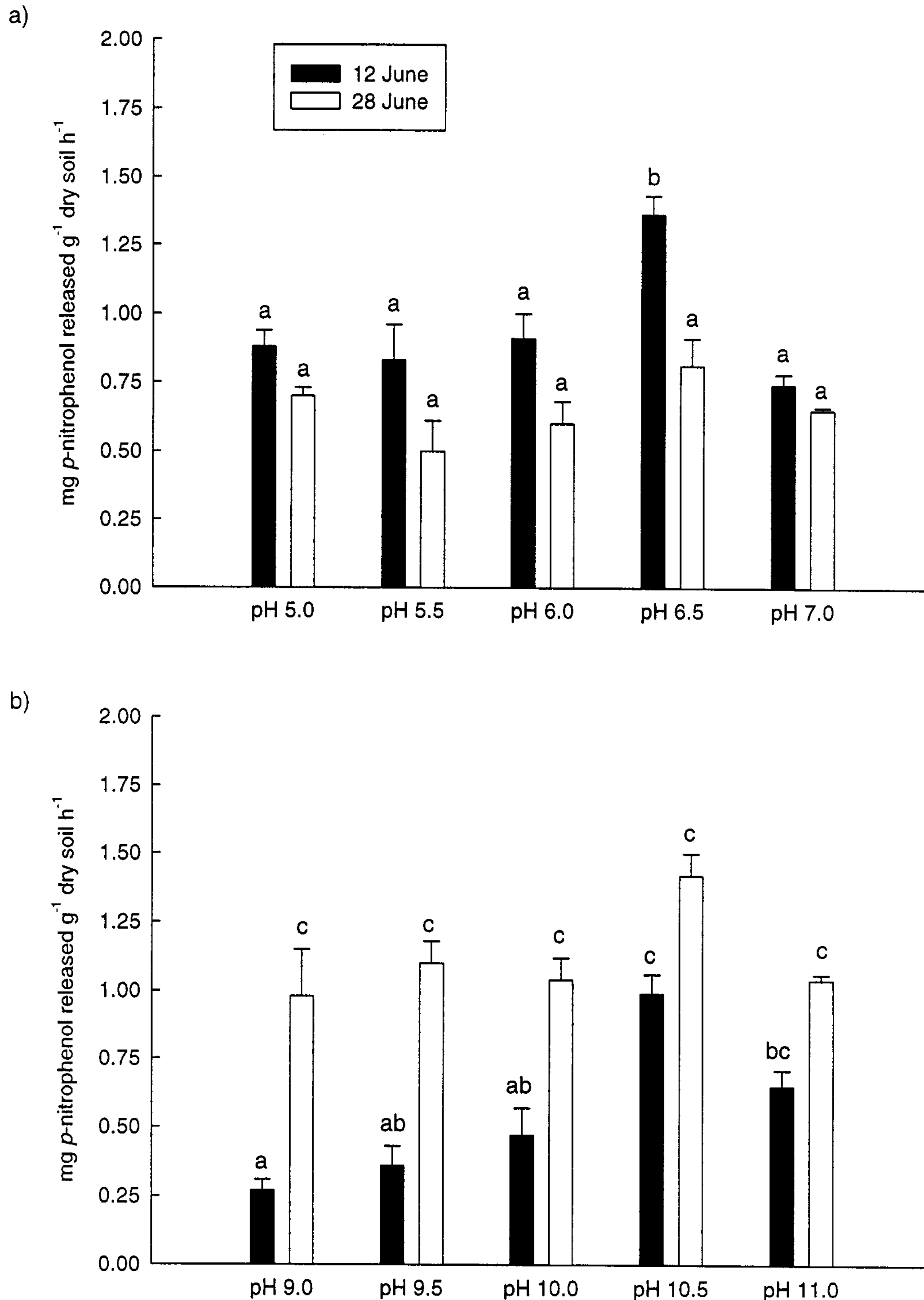


Figure 3.1 (a) Acid and (b) alkaline phosphatase activities in soil collected from the western intertidal marsh at La Pérouse Bay, Manitoba and buffered between pH 5 - pH 7 and pH 9 - pH 11, respectively, on 12 June and 28 June 2003. Soils were collected beneath intact swards to a depth of 5 cm (rooting depth) and incubated for 60 minutes. Means and standard errors (+/- SEM) are shown (n=3). Common lower case letters denote a lack of significant difference within each sampling date (Tukey's HSD test).

Table 3.1 Results of one-way ANOVAs of phosphatase activities within acid (pH 5.0 - pH 7.0) and alkaline (pH 9.0 - pH 11.0) ranges on 12 June and 28 June 2003 (n=3). Soils were collected from the top 5 cm of the soil profile (rooting depth) and incubated for 60 minutes. * P<0.05; ** P<0.01.

Range	Date	df	F	P
pH 5.0 - 7.0	12 June 2003	4,7	7.20	0.0126 *
pH 5.0 - 7.0	28 June 2003	4,7	2.18	0.1732
pH 9.0 - 11.0	12 June 2003	4,7	12.39	0.0011 **
pH 9.0 - 11.0	28 June 2003	4,7	3.39	0.0763

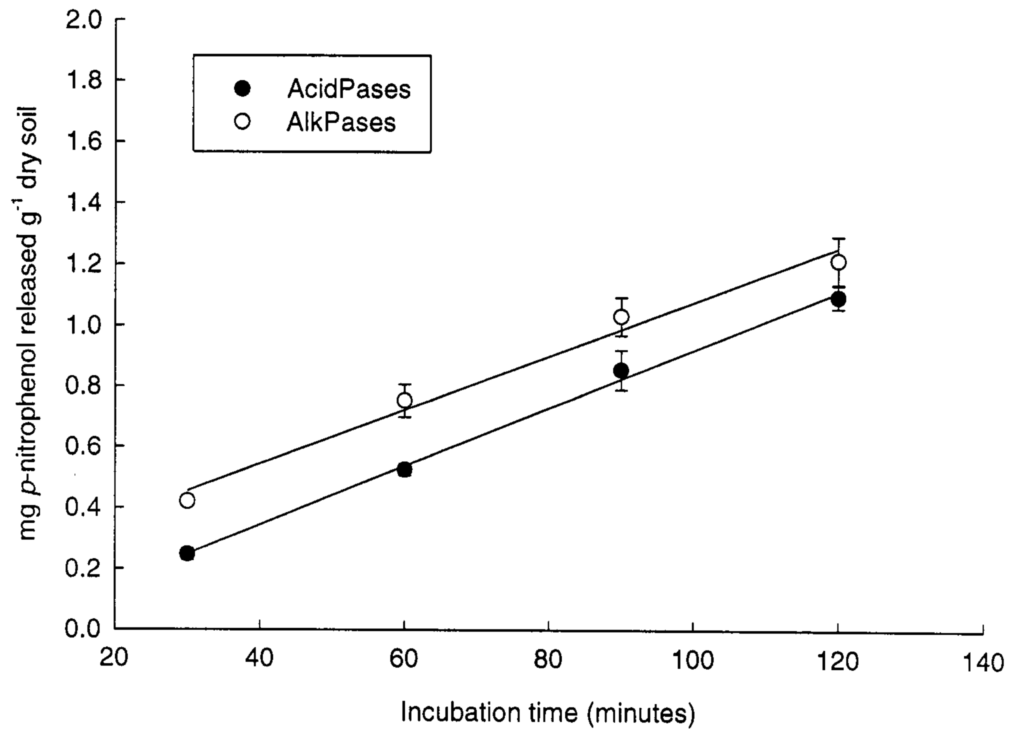


Figure 3.2 Acid and alkaline phosphatase activities (AcidPases and AlkPases, respectively) in soils from intact sites on the western intertidal marsh at La Pérouse Bay, Manitoba after 30, 60, 90 and 120 minute incubation. Means and standard errors (+/-SEM) are shown (n=3). First-order linear regressions of incubation time on phosphatase activities: AcidPases $F_{1,10}$=285.03, P<0.0001, r^2=0.9667; AlkPases: $F_{1,10}$=109.92, P<0.0001, r^2=0.9166.

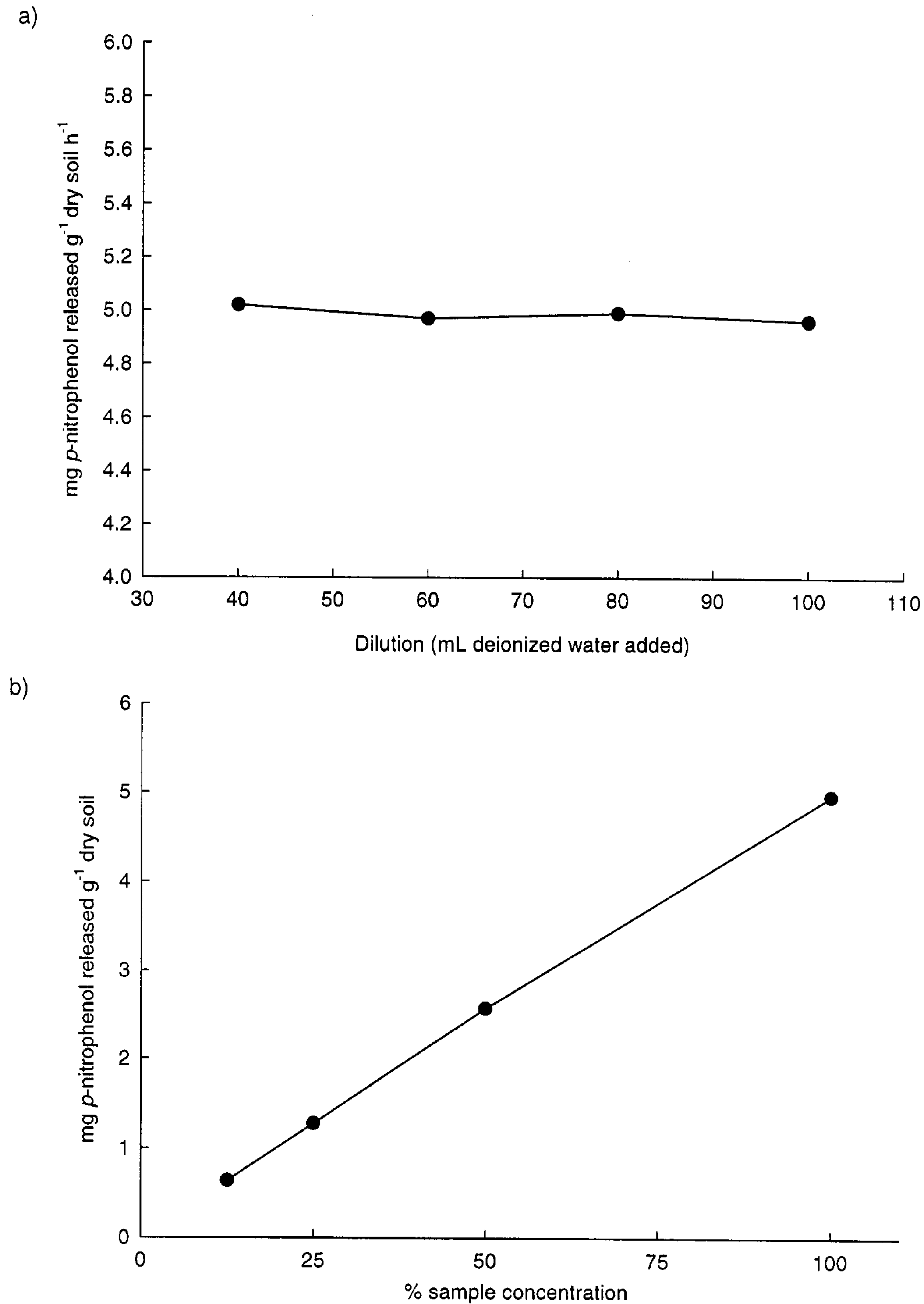


Figure 3.3 The effect of dilution on estimates of acid phosphatase activities. (a) Estimates of *p*-nitrophenol released after the sample was read spectrophotomically at different dilutions. $F_{1,2}$=2.16, P=0.2793, r^2 = 0.5194. (b) Estimates of *p*-nitrophenol released at serial dilutions of a single sample. $F_{1,2}$=6007.76, P<0.0002, r^2 = 0.9997. Soils were collected from the supratidal marsh on 25 July 2003 at rooting depth (5 cm), buffered at pH 6.5 and incubated for 60 minutes.

3.1.2 Determination of kinetic parameters

From plots of the reciprocal of phosphatase activities (1/ *v*) versus the reciprocal of enzyme substrate (1 / [S]), $y_{AcidPases}$ = 5.412x + 1.2739, R^2= 0.8696 and $y_{AlkPases}$ = 4.1842x + 0.8474, R^2 = 0.8951. Table 3.2 gives values taken from the Lineweaver-Burk plots of AcidPases and AlkPases which were used to calculate K_m and V_{max}. The substrate affinity of AcidPases (K_m = 4.59 mM *p*-Nitrophenyl phosphate) was slightly higher than that of AlkPases (K_m = 4.93 mM *p*-Nitrophenyl phosphate). The V_{max} of AcidPases was 0.785 mg *p*-nitrophenol released per gram dry weight soil per hour, while the V_{max} of AlkPases was higher and was 1.18 mg *p*-nitrophenol released per gram dry weight soil per hour.

Table 3.2 Values taken from the Lineweaver-Burk linear transformations of the Michaelis-Menten equation. V_{max} is expressed as mg p-nitrophenol released g^{-1} dry soil h^{-1}; K_m is expressed as mM *p*-Nitrophenyl phosphate.

	Equation	$1/V_{max}$ (y-intercept)	$-1/K_m$ (x-intercept)	V_{max}	K_m
AcidPases	y = 5.8412x+ 1.2739	1.2739	-0.218	0.785	4.59
AlkPases	y = 4.1842x+ 0.8474	0.8474	-0.203	1.18	4.93

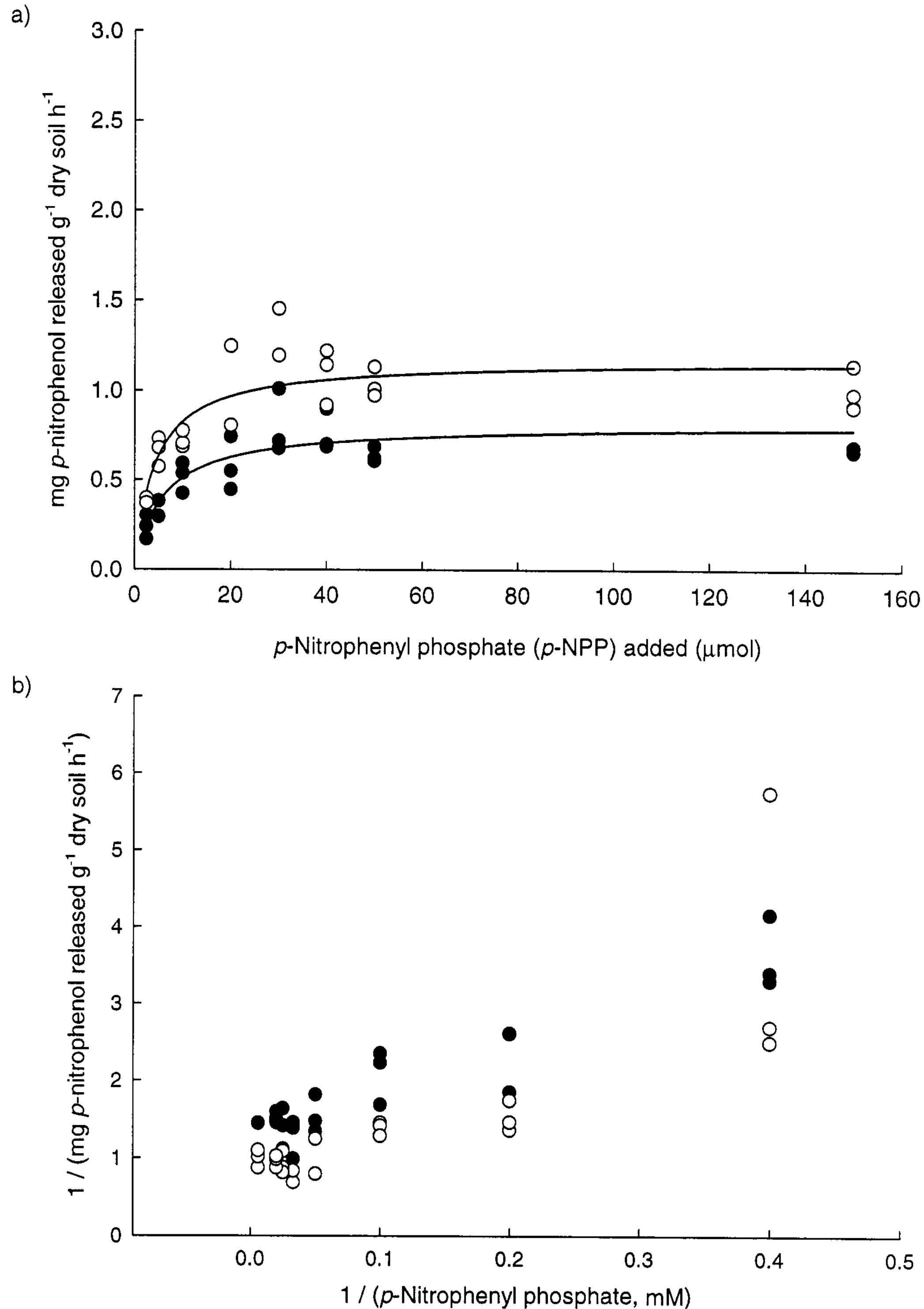


Figure 3.4 (a) Acid and alkaline phosphatase activities (AcidPases and AlkPases, respectively) as mg g^{-1} at varying concentrations of the enzyme substrate *p*-Nitrophenyl phosphate. (b) A plot of the reciprocals of substrate concentration (1/S) and enzyme activities (1/ V) for calculation of K_m and V_{max}. $y_{AcidPases} = 5.8412x + 1.273$, $R^2 = 0.8698$; $y_{AlkPases} = 4.1842x + 0.8474$, $R^2 = 0.8951$. Soils were incubated for 60 minutes.

3.1.3 Root and microbial phosphatase release

Roots of *Puccinellia phryganodes* released significantly more AcidPases than AlkPases (t_2 = 8.537, P=0.0134) (Figure 3.5). In contrast, multivariate analyses of variance (MANOVAs) of the effect of soil site (intact and degraded) on AcidPases and AlkPases were insignificant when expressed on a unit mass basis ($F_{1,3}$ = 0.0112, P=0.9223) or on a unit volume basis ($F_{1,3}$ = 0.4459, P=0.5408) (Figure 3.6, Table 3.3). This indicates that there was neither a difference in the activities between AcidPases and AlkPases from soil beneath intact versus degraded sites, nor was there an effect of site on the relative activities of AcidPases and AlkPases in June of 2003 (i.e. the ratio of activities of AcidPases to AlkPases did not change between intact and degraded soils) (Table 3.3). Thus, soil microorganisms excreted both AcidPases and AlkPases in the different soil types in similar amounts expressed on a unit mass basis and a unit volume basis. All Pase data from intact and degraded sites were log-transformed. Because MANOVAs were insignificant, no Bonferroni-type adjustments of Type I error were made for the univariate analyses of variance (ANOVAs) (α = 0.05). In general, activities of AcidPases ($F_{1,4}$ = 12.15, P=0.0399) and AlkPases ($F_{1,4}$ = 13.48, P=0.0214) were significantly higher in intact sites than in degraded sites (Figure 3.6). However, when results are expressed per unit volume based on dry soil (Figure 3.6b), the difference in activities between sites is reduced since the bulk density of degraded soils (0.76 +/- 0.04 g cm^{-3} SEM at time of sampling) is greater than that of intact soils (0.69 +/- 0.02 g cm^{-3} SEM at time of sampling) due to compaction of soil in the absence of vegetation (McLaren and Jefferies 2004). On a volume basis, only AlkPases were significantly different between intact and degraded sites ($F_{1,4}$ = 11.44, P=0.0277)(Table 3.3).

3.1.4 Soil phosphatase activities in soil from the coastal marshes

Activities of AcidPases and AlkPases varied in soils from the intertidal, supratidal and freshwater marshes (Figure 3.7). A MANOVA of the effect of marsh site on activities of AcidPases and AlkPases expressed on a unit area basis was significant ($F_{1,9}$ = 14.3317, P=0.0043, Table 3.4), indicating that the relative activities, or ratio of activities, of AcidPases and AlkPases changed at the different sites in the marshes. Accordingly, Bonferroni-type adjustments (α = 0.025) were made to the results of AcidPases and AlkPases expressed on a unit area basis only. AcidPase activities were lower, but statistically similar, to AlkPase activities in the intertidal marsh. The activities of the two enzyme complexes were similar in the supratidal marsh, but AcidPase activities were significantly higher than AlkPase activities in the freshwater marsh. When expressed per unit mass, activities of AcidPases were highest in the P-limited freshwater marsh (means between 4.0 and 6.5 mg *p*-nitrophenol released g^{-1} of dried

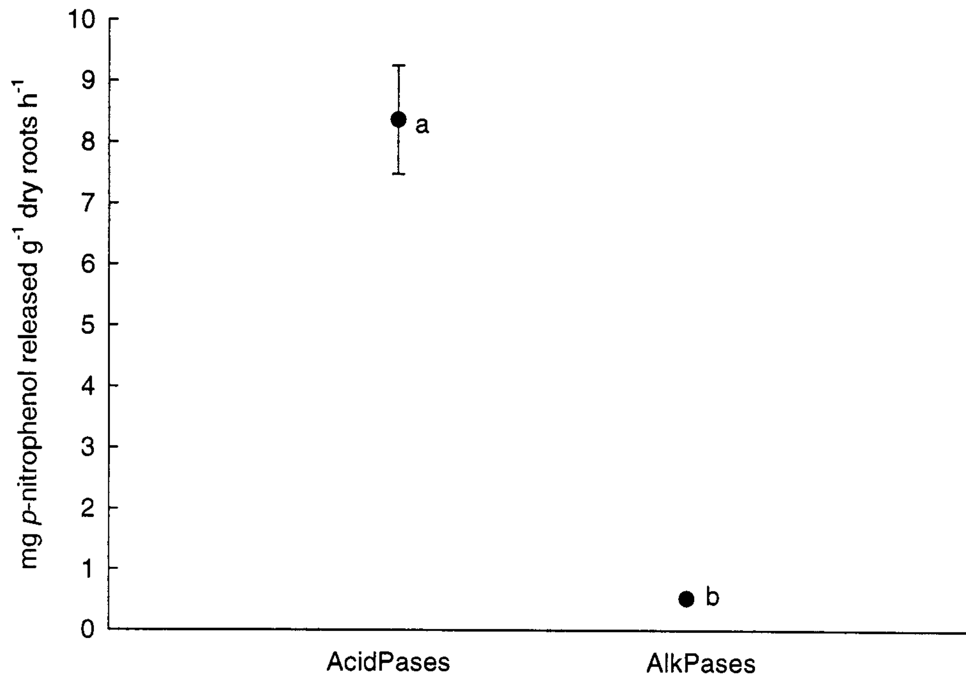


Figure 3.5 Acid phosphatase activities (AcidPases) and alkaline phosphatase activites (AlkPases) exuded by roots of *Puccinellia phryganodes*. Living roots were sampled on 30 July 2003 from intact sites on the supratidal marsh. For the assay, roots were rinsed with river water, buffered at pH 6.5 (AcidPases) and pH 10.5 to (AlkPases), and incubated for 60 minutes. Means and standard errors (+/- SEM) are shown (n=3). Common lower case letters denote a lack of significance ($P<0.05$; paired *t*-test).

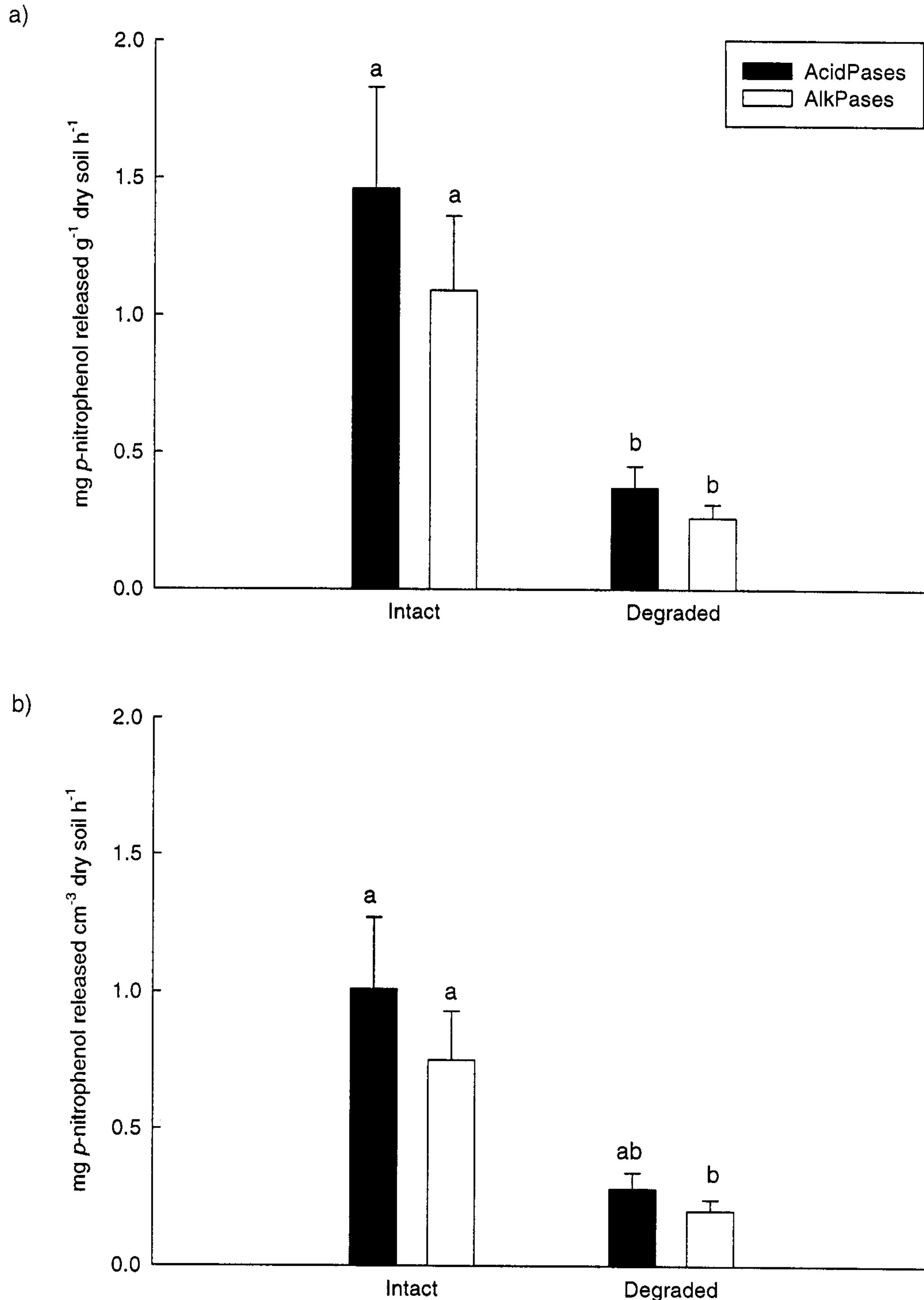


Figure 3.6 Acid phosphatase activities (AcidPases) and alkaline phosphatase activities (AlkPases) (a) per gram dry soil and (b) per unit volume of soil taken beneath intact and degraded sites on the western intertidal marsh at La Pérouse Bay, Manitoba in June 2003. Means and standard errors (+/-SEM) are shown (n=3). Common lower case letters denote a lack of significance between intact and degraded sites for AcidPases and AlkPases separately (Tukey's HSD test).

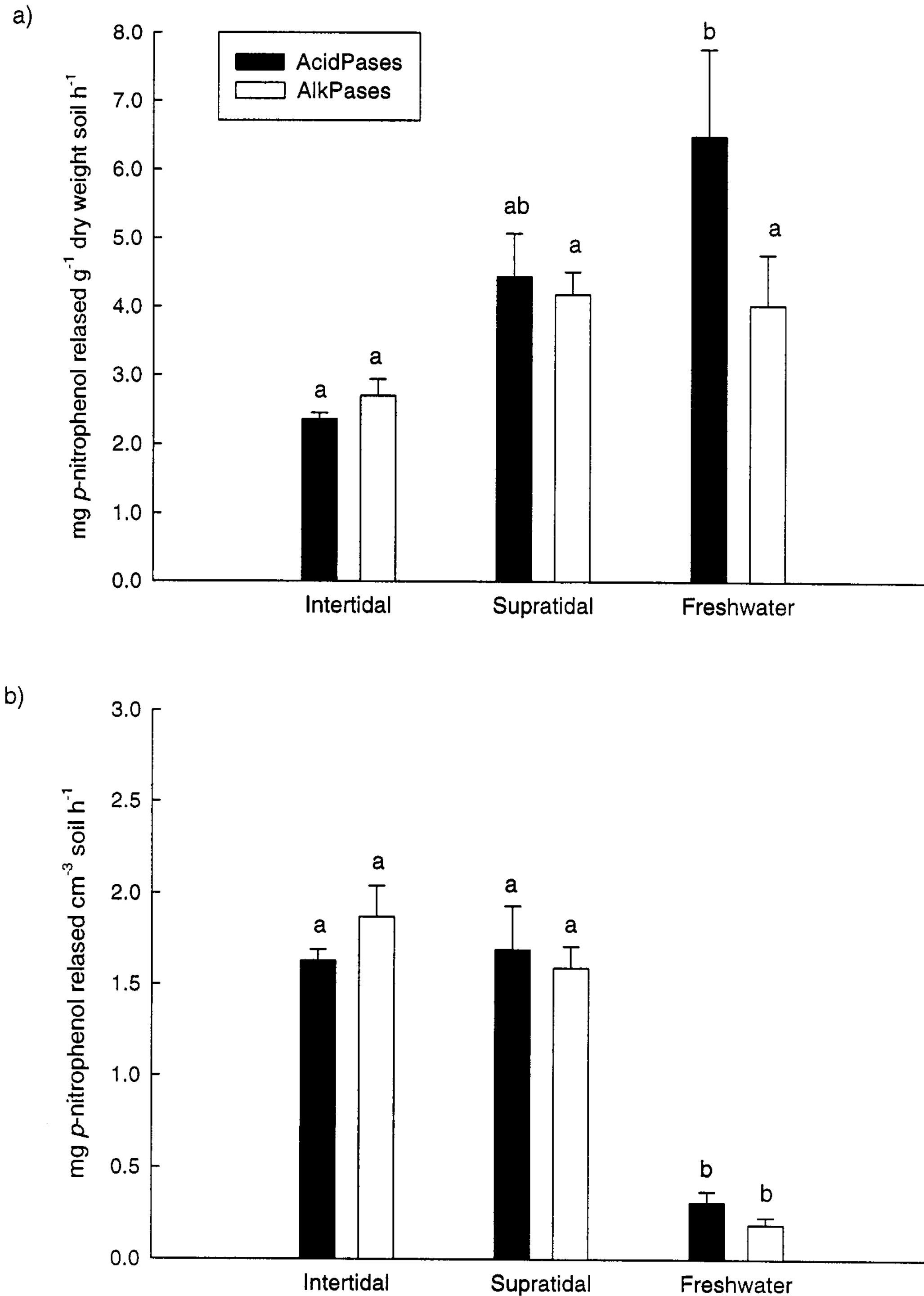


Figure 3.7 Acid phosphatase activities (AcidPases) and alkaline phosphatase activities (AlkPases) (a) per gram dry soil and (b) per cm^3 of soil at sites with intact vegetation in the intertidal (I), supratidal (S) and freshwater (F) marshes at La Pérouse Bay, Manitoba in July 2003. Soil was incubated for 60 minutes. Means and standard errors (+/-SEM) are shown. $n_{I,S}$ =4; n_F =3. Common lower case letters denote a lack of significance between marshes (Tukey-Kramer test).

Table 3.3 One-way MANOVAs and ANOVAs of acid and alkaline phosphatase activities (AcidPases and AlkPases, respectively) from soil beneath intact and degraded sites (Site) on the western intertidal marsh at La Pérouse Bay, Manitoba, in June 2003. * P<0.05.

Analysis	Data	Effects	α	Den df	Num df	F	P	
MANOVA	log (g^{-1})	Model	0.05	1	3	0.0112	0.9223	
	log (cm^{-3})	Model	0.05	1	3	0.4459	0.5408	
ANOVA	log(AcidPases) (g^{-1})	Site	0.05	1	4	12.15	0.0399	*
	log(AlkPases) (g^{-1})	Site	0.05	1	4	13.48	0.0214	*
	log(AcidPases) (cm^{-3})	Site	0.05	1	4	1.98	0.2323	
	log(AlkPases) (cm^{-3})	Site	0.05	1	4	11.44	0.0277	*

Table 3.4 One-way MANOVAs and ANOVAs of acid and alkaline phosphatase activities (AcidPases and AlkPases, respectively) from soil beneath intact swards in the intertidal, supratidal and freshwater marshes ("Marsh") at La Pérouse Bay, Manitoba, in July 2003. * P<0.05; ** P<0.01; *** P<0.0001.

Analysis	Data	Effect	α	Den df	Num df	F	P	
MANOVA	(g^{-1})	Model	0.05	1	9	14.3317	0.0043	**
	(cm^{-3})	Model	0.05	1	9	1.6538	0.2305	
ANOVA	AcidPases (g^{-1})	Marsh	0.025	2	8	8.01	0.0123	*
	AlkPases (g^{-1})	Marsh	0.025	2	8	1.98	0.2007	
	AcidPases (cm^{-3})	Marsh	0.05	2	8	20.72	0.0007	**
	AlkPases (cm^{-3})	Marsh	0.05	2	8	40.54	<0.0001	***

Table 3.5. Results of microbial phosphorus used to determine the phosphorus fixation factor (k_{fix}) for determination of soil microbial P (MBP). Spike and MBP content in the non-fumigated sample, and spiked sample are all given as μg P g^{-1} dry soil.

Replicate	$MBP_{non-fumigated}$	MBP_{spike}	k_{fix}
1	5.1	4.9	0.77
2	3.8	3.9	0.76
3	5.2	5.3	0.82
4	3.6	3.1	0.63
Average			0.77

soil), at intermediate levels in the organic-rich supratidal marsh (means between 4.2 and 4.4 mg *p*-nitrophenol released g^{-1} of dried soil), and lowest in the mineral-rich intertidal marsh (means between 2.4 and 2.7 mg *p*-nitrophenol released g^{-1} of dried soil) ($F_{2, 8} = 8.01$, P=0.0123) (Table 3.4). Activities of AlkPases expressed on a unit area basis were statistically indistinguishable between soils from the three marshes ($F_{2, 8} = 1.98$, P=0.2007, Table 3.4).

If the ANOVA results are placed on a unit volume basis, activities of AcidPases ($F_{2, 8} = 20.72$, P=0.0007) and AlkPases ($F_{2, 8} = 40.54$, P<0.0001, Table 3.4) were lowest in the freshwater marsh (Figure 3.7). These results, which are the reverse of the above, are due to the large differences in bulk density between the more densely packed intertidal (mean of 0.69 g cm^{-3} dry soil at the time of sampling) and supratidal (mean of 0.38 g cm^{-3} dry soil at the time of sampling) soils, and the organic-rich freshwater substrate (mean of 0.048 g cm^{-3} dry soil; from Ngai 2004). Furthermore, when expressed on a unit volume basis, the relative activities of AcidPase and AlkPase did not change across the three marshes (MANOVA: $F_{1, 9} = 1.6538$, P=0.2305) (Table 3.4).

3.2 Phosphate fixation for estimation of microbial phosphorus

Results from the determination of P fixation during microbial P extraction are given in Table 3.5. From the equation modified from Brookes et al. (1982), where the fixation factor, K_{fix} = µg P in spiked sample / (µg P in non-fumigated sample + 1.3 µg P), the average fixation factor for soils taken from the intertidal salt marsh at La Pérouse Bay was 0.77. This value was used in all calculations of microbial P (refer to Appendix 5).

3.3 Results from the fertilization experiment on the western intertidal marsh during the summer of 2003

3.3.1 Soil physical properties

A summary of soil physical and chemical properties is given in Table 3.6. Means are of data collected over the summer of 2003. Total amounts of carbon (C), nitrogen (N) and phosphorus (P) in the soil are expressed as means because they were relatively static during the summer of 2003. Total soil nutrient content refers to both biologically available and biologically unavailable nutrients. As expected, carbon comprises most of the total soil nutrient content. On a dry weight basis, there was around 30 times more total carbon than total nitrogen and around 180 times more total carbon than total phosphorus. Expressed in atomic ratios, these differences in total soil nutrient content give a mean soil C:N ratio of 37.2 +/- 0.468 SEM, a mean C:P ratio of 520.2 +/- 24 SEM, and a mean N:P ratio of 14.1 +/- 2.0 SEM.

The mean soil pH, calculated as the concentration of hydrogen ions, was near neutral (pH 7.4), and varied little throughout the season. Bulk density did increase throughout the summer 2003, as the soil dried and shrunk, and the seasonal changes can be seen indirectly when the results are used to convert soil chemical data, soil phosphatase data and soil microbial data from a unit area basis to a unit volume basis. Soils from the exclosures on the intertidal marsh had a mean value of 6.4 g of dissolved solutes per litre in the hyposaline soil solution, whereas seawater has c.32 g of dissolved solutes per litre.

3.3.2 Soil chemical properties in response to NP-fertilization during the summer of 2003

Dissolved organic phosphorus (DOP), measured for the first time at La Pérouse Bay, showed a negative, yet inconsistent, response when inorganic nitrogen and phosphorus were added together (Figure 3.8, Table 3.7). DOP in control (0N0P) subplots remained statistically similar from June to October 2003 (Figure 3.8). There is an N by P interaction when data are expressed per unit of soil solution ($F_{1, 51.5} = 16.68$, $P = 0.0002$), and the average DOP in these subplots was significantly lower than corresponding values in the 0N1P and 1N0P subplots on three sampling dates. There was also an effect of N addition over time on DOP ($F_{4, 51.7} = 3.51$, $P = 0.0131$), as average DOP in N-alone fertilized subplots (1N0P) increased throughout the summer, peaking in late summer on the fourth sampling date. Although DOP tended to increase in N-alone and P-alone fertilized subplots after the second nutrient addition on 10 July

2003, the results are variable and results of DOP in 1N1P subplots are inconsistent so the second nutrient addition did not appear to significantly affect the overall results of DOP.

Results from the summer of 2003 show that Bray's extract was a more efficient extractant of P, and extracted around 1 to 2 times more phosphorus per unit mass and per unit volume than Olsen's extract (Figures 3.9 and 3.10). Since bulk density on the intertidal marsh was always less than 1 g dry weight per cm^{-3} (average of 0.709 +/- 0.006), Bray's and Olsen's extractable P is lower when expressed by volume than by mass. Nonetheless, trends in Bray's extractable P and Olsen's extractable P during the summer of 2003 are similar. In general, total extractable P changed significantly over time ($P < 0.01$, Table 3.8), and was lowest at the start of the growing season and peaked slightly in mid- to late summer on the fourth sampling date. There were no significant response to N or P, with the exception of a P by time interaction for Bray's extractable P based on volume ($F_{3, 80} = 3.17$, $P = 0.00289$), which corresponded to the overall rise and fall of available P during the summer.

Results of soil exchangeable (2M KCl-extracted) ammonium and nitrate concentrations are less clear and this may be because samples were taken from only one exclosure per site, and only on three days throughout the summer of 2003 (Figure 3.11 and 3.12, Table 3.9). On the first and third sampling day, average exchangeable nitrate was higher by unit mass ($F_{1, 26} = 4.48$, $P = 0.0439$) and by unit volume ($F_{1, 26} = 4.75$, $P = 0.0385$), but generally soil nitrate concentrations remained unaffected by N and P addition and by time, except in the 1N1P treatment where nitrate concentrations were higher on two of the three sampling occasions. All soil chemical properties (DOP, extractable P and exchangeable N) were log-transformed before statistical analyses were performed.

Correlations between soil microbial, soil phosphatases, Bray- and Olsen's extractable inorganic phosphate, and DOP reveal only two significant relationships (Table 3.10). Bray- and Olsen's extractable P were strongly positively correlated (Pearson's correlation coefficient = 0.7981, $P < 0.0001$) and soil exchangeable ammonium and nitrate were moderately positively correlated (Pearson's correlation coefficient = 0.5281, $P = 0.0019$).

Table 3.6 Physical and chemical properties of soil from the NP-fertilization experiment on the western intertidal marsh at La Pérouse Bay, Manitoba. Means are of data collected from 3 June to 5 August 2003. DW = dry weight of soil.

Property	Unit	n	Mean	SEM
Bulk density	g DW cm^{-3}	102	0.709	0.006
pH	$-log_{10} [H^+]$	96	7.4	0.02
Redox (2cm depth)	mV	96	369	4.5
Redox (5cm depth)	mV	96	352	4.9
Total C	mg C g^{-1} DW	114	92.9	0.522
Total N	mg N g^{-1} DW	114	2.98	0.052
Total P	mg P g^{-1} DW	5	0.456	0.019
Soil C:N	Atomic ratio	114	37.2	0.5
Soil C:P	Atomic ratio	5	520.2	24
Soil N:P	Atomic ratio	5	14.1	2.0
Salinity	g dissolved solids L^{-1}	98	6.4	0.1
Dissolved organic P	µg P mL^{-1} soil solution	70	0.347	0.024
	µg P g^{-1} DW	70	0.222	0.016
	µg P cm^{-3} DW	70	0.157	0.011
Bray's extractable P	µg P g^{-1} DW	102	28.4	1.6
	µg P cm^{-3} DW	102	20.4	1.1
Olsen's extractable P	µg P g^{-1} DW	102	11.0	0.8
	µg P cm^{-3} DW	102	7.6	1.1
Total exchangeable NH_4^+ - N	µg N g^{-1} DW	32	1.59	0.18
	µg N cm^{-3} DW	32	1.11	0.13
Total exchangeable NO_3^- - N	µg N g^{-1} DW	35	1.09	0.10
	µg N cm^{-3} DW	35	0.79	0.07

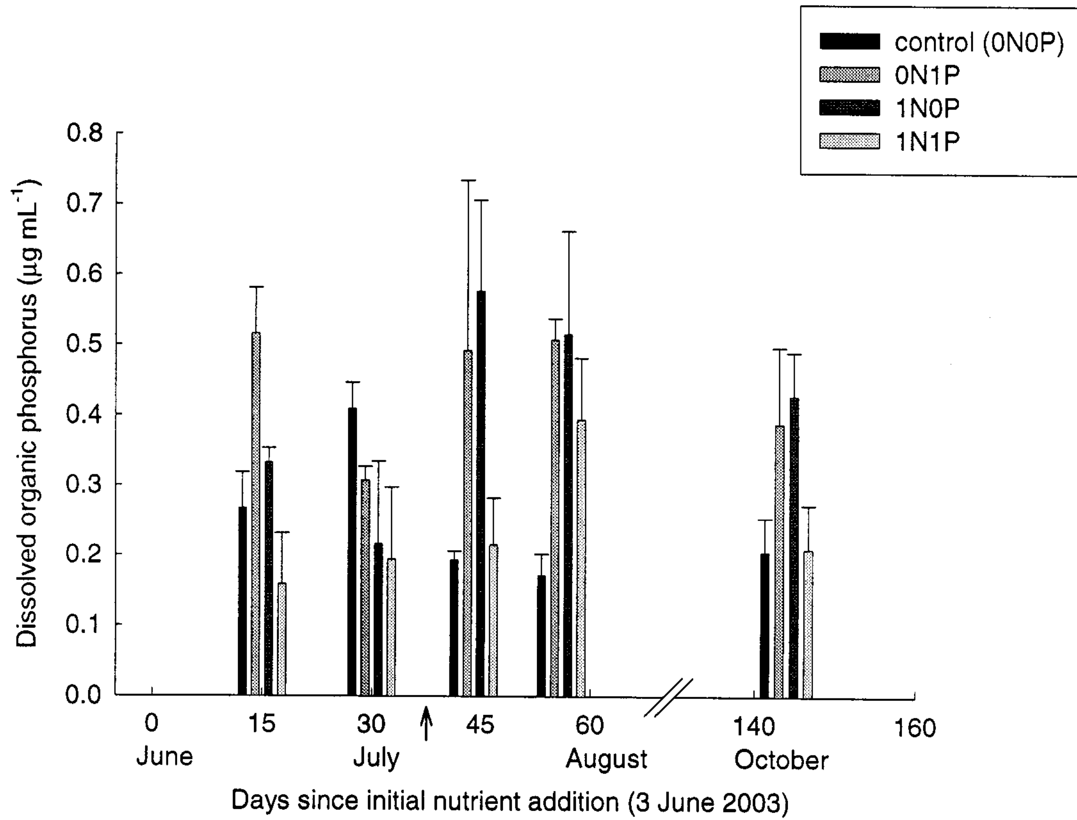


Figure 3.8 Dissolved organic phosphorus (DOP) results per mL soil solution from soil solution sampled from the control and fertilization subplots on the western intertidal marsh at La Pérouse Bay, Manitoba from June to October 2003. The arrow at day 37 indicates the second nutrient addition on 10 July 2003. Means and standard errors (+/-SEM) are shown (n=3).

Table 3.7 Multi-way ANOVAs of dissolved organic phosphorus (DOP) in response to NP-fertilization on the western intertidal marsh at La Pérouse Bay, Manitoba from June to October 2003. * P<0.05; ** P<0.01.

Data	Fixed effects	Num df	Den df	F	P	
log(DOP) (mL soil solution^{-1})	Time	4	52.3	1.78	0.1460	
	Nitrogen	1	51.6	1.66	0.2033	
	Phosphorus	1	51.4	0.11	0.7361	
	Nitrogen x Time	4	51.7	3.51	0.0131	*
	Phosphorus x Time	4	51.7	1.02	0.4054	
	Nitrogen x Phosphorus	1	51.5	16.68	0.0002	**

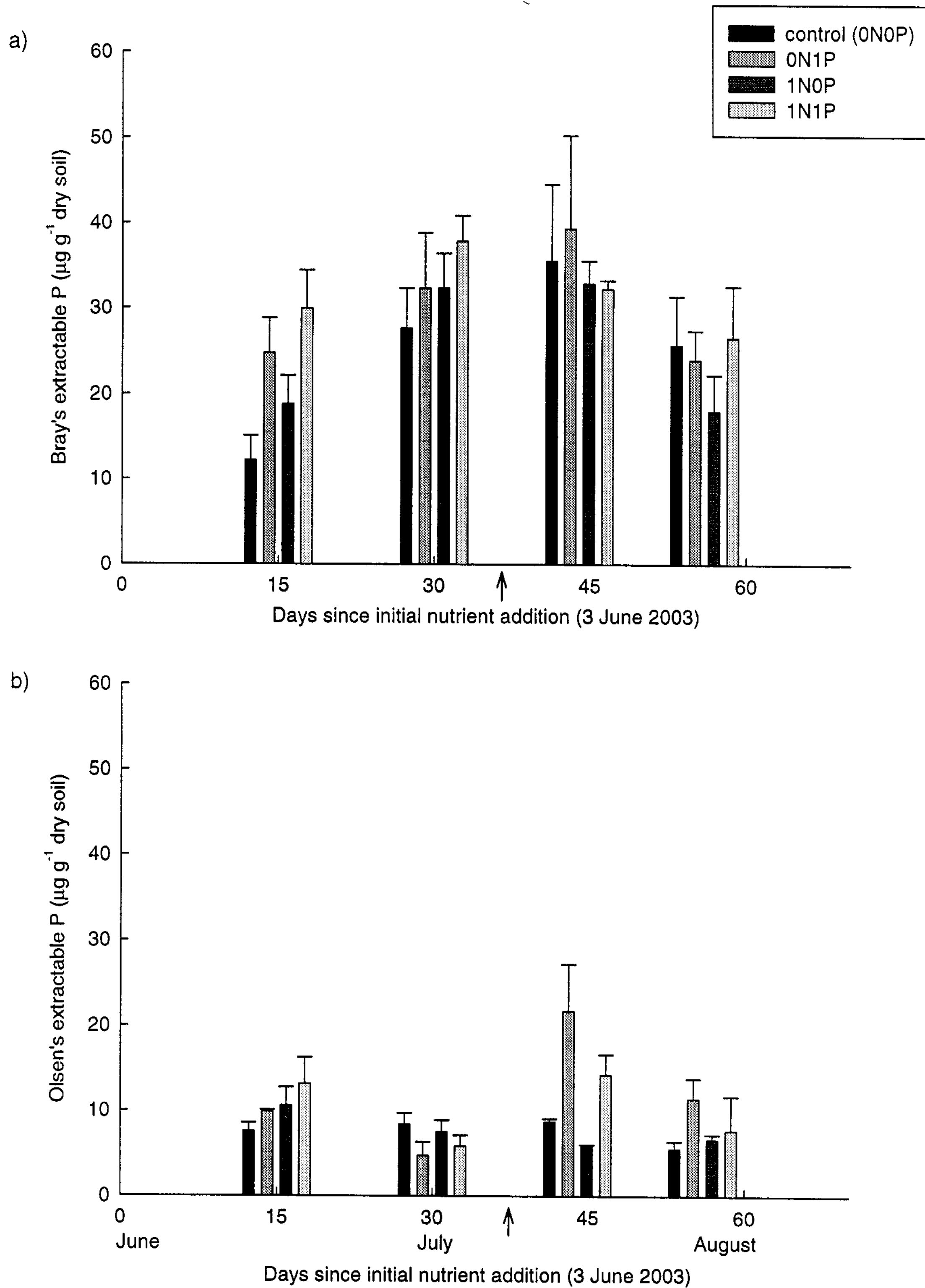


Figure 3.9 (a) Bray's and (b) Olsen's total extractable P (μg g^{-1} dry soil) from control and fertilized subplots on the intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at rooting depth (5 cm). The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/-SEM) are shown.

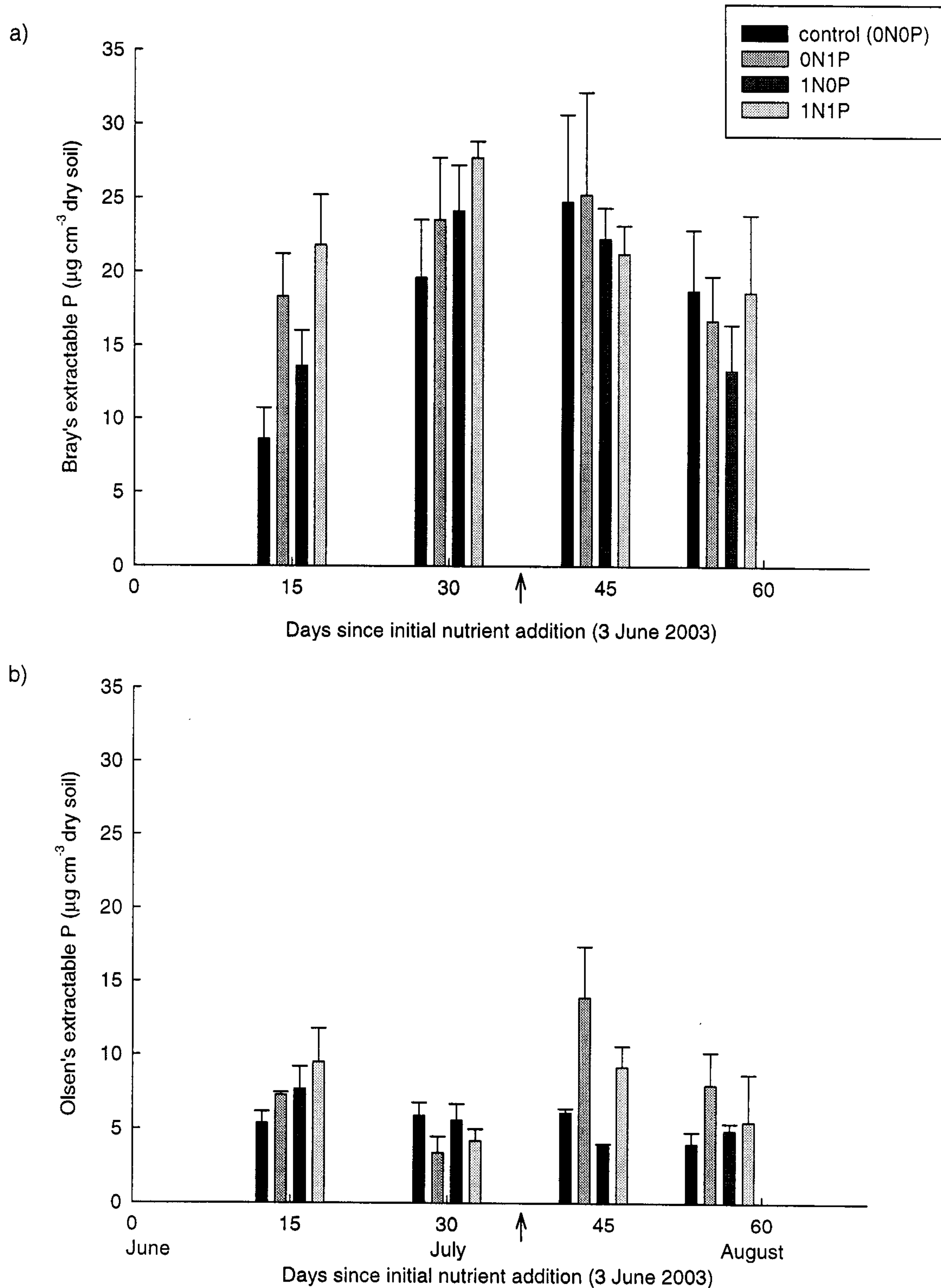


Figure 3.10 (a) Bray's and (b) Olsen's total extractable P (µg cm^{-3} dry soil) from control and fertilized subplots on the intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at rooting depth (5 cm). The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/-SEM) are shown.

Table 3.8 Multi-way ANOVAs of Bray's and Olsen's exchangeable phosphorus (P) in response to NP-fertilization on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. * P<0.05; ** P<0.01.

Data	Fixed effects	Num df	Den df	F	P	
log(Bray's P g^{-1})	Time	3	80	7.49	0.0002	**
	Nitrogen	1	80	0.62	0.4352	
	Phosphorus	1	80	2.48	0.1194	
	Nitrogen x Time	3	80	1.05	0.3759	
	Phosphorus x Time	3	80	2.51	0.0642	
	Nitrogen x Phosphorus	1	80	0.04	0.8483	
log(Olsen's P g^{-1})	Time	3	80.1	5.92	0.0011	**
	Nitrogen	1	80.1	0.23	0.6316	
	Phosphorus	1	80.1	1.27	0.2623	
	Nitrogen x Time	3	80.1	0.39	0.7579	
	Phosphorus x Time	3	80.1	2.54	0.0623	
	Nitrogen x Phosphorus	1	80.1	2.83	0.0964	
log(Bray's P cm^{-3})	Time	3	80	6.62	0.0005	**
	Nitrogen	1	80	0.71	0.4009	
	Phosphorus	1	80	1.79	0.1853	
	Nitrogen x Time	3	80	1.11	0.3517	
	Phosphorus x Time	3	80	3.17	0.0289	*
	Nitrogen x Phosphorus	1	80	0.01	0.9196	
log(Olsen's P cm^{-3})	Time	3	80	5.00	0.0031	**
	Nitrogen	1	80	0.23	0.5978	
	Phosphorus	1	80	0.95	0.3327	
	Nitrogen x Time	3	80	0.43	0.7292	
	Phosphorus x Time	3	80	2.43	0.0715	
	Nitrogen x Phosphorus	1	80	3.03	0.0857	

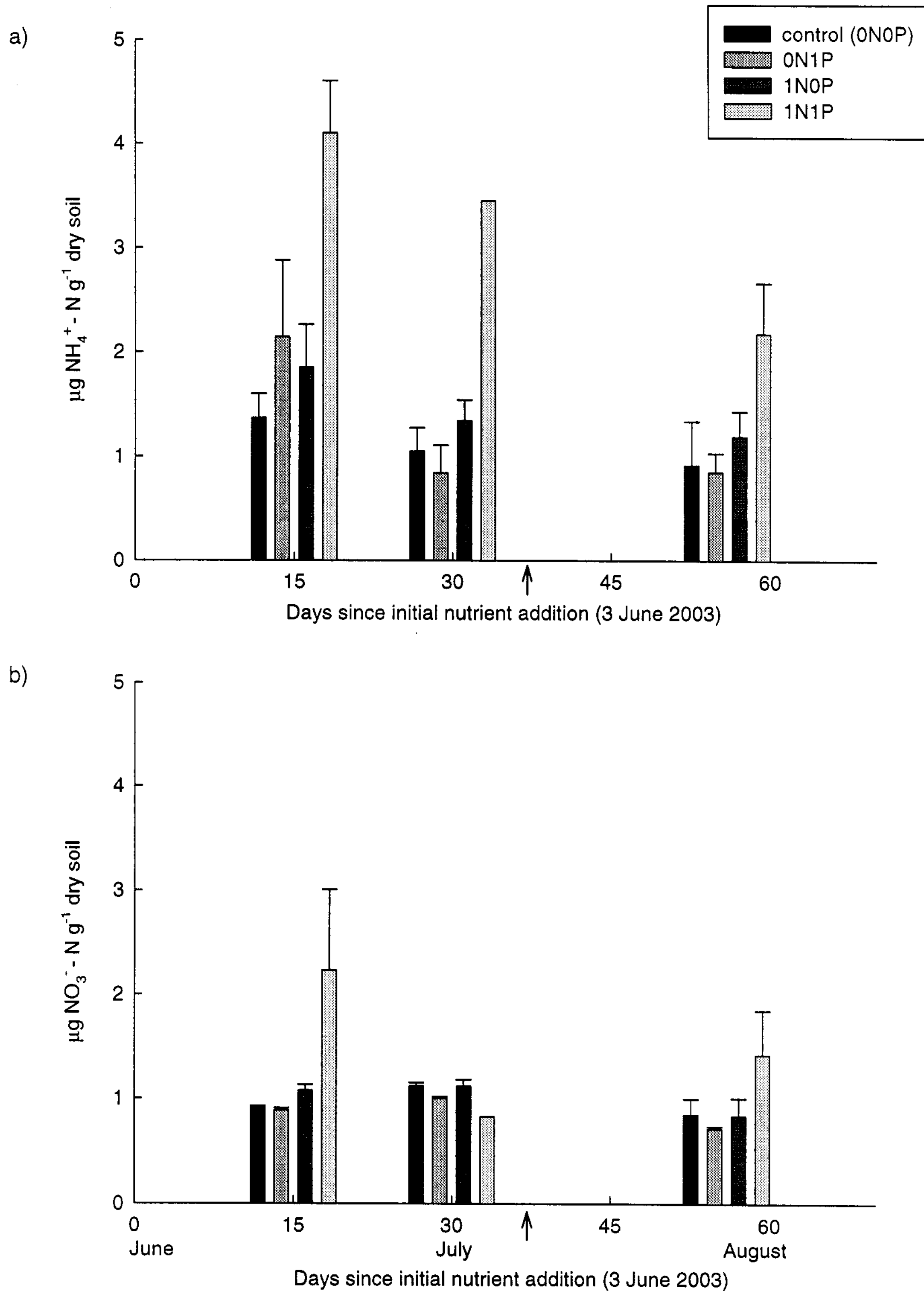


Figure 3.11 Soil exchangeable and soluble (2M KCl) (a) ammonium and (b) nitrate concentrations per gram dry soil from control and fertilized subplots on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at a depth of 5 cm (rooting depth). The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/-SEM) are shown (n=3).

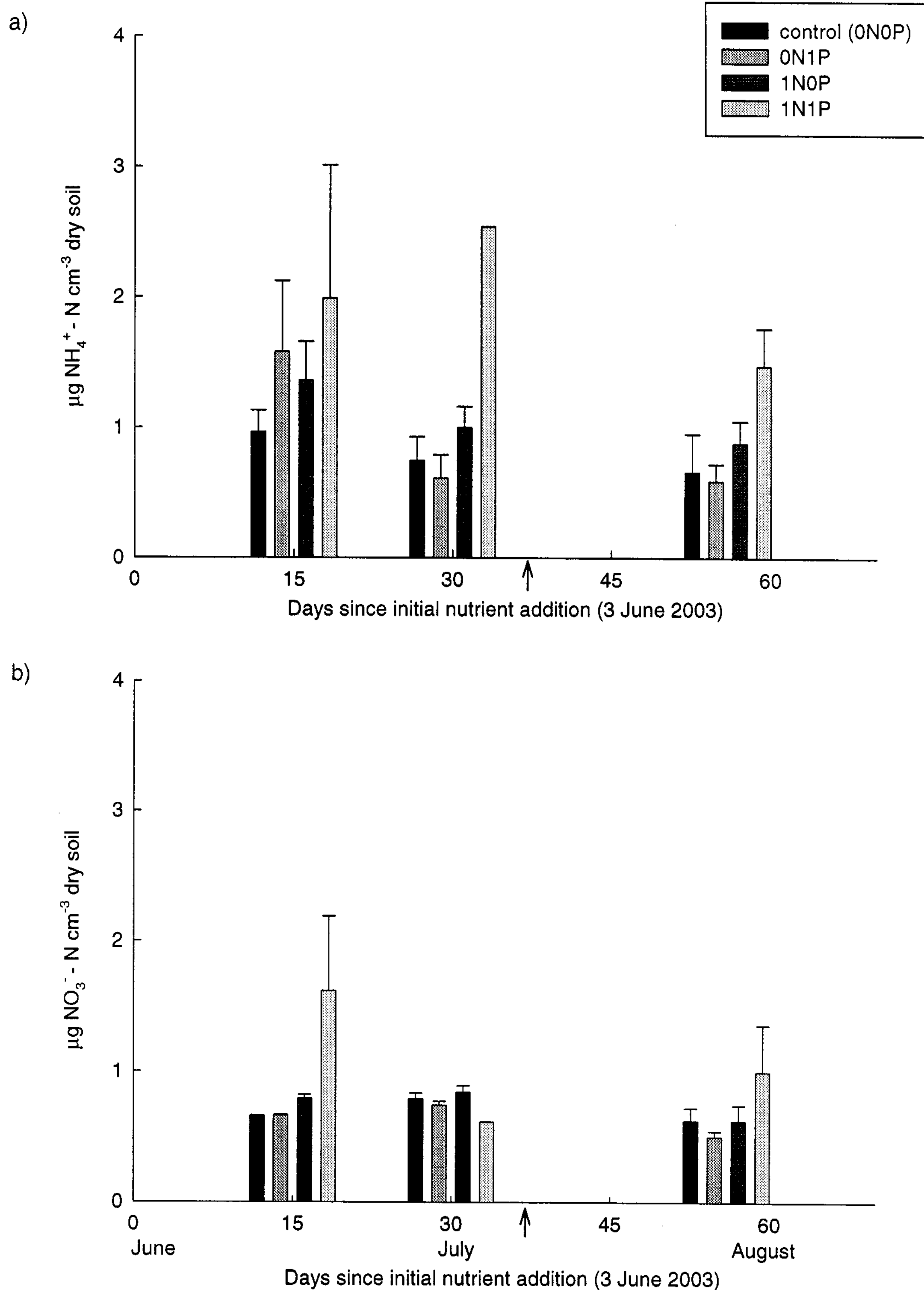


Figure 3.12 Soil exchangeable and soluble (2M KCl) (a) ammonium and (b) nitrate concentrations per cm^3 dry soil from control and fertilized subplots on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected from the top 5 cm of the soil profile (rooting depth). The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/-SEM) are shown (n=3).

Table 3.9 Multi-way ANOVAs of soil exchangeable ammonium and nitrate in response to the NP-fertilization experiment on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Nitrate data by volume are non-parametric. * P<0.05; ** P<0.01.

Data	Fixed effects	Num df	Den df	F	P	
$\log(NH_4^+)$ (g^{-1})	Time	2	24	7.62	0.0027	**
	Nitrogen	1	24	17.40	0.0003	**
	Phosphorus	1	24	3.72	0.0657	
	Nitrogen x Time	2	24	0.01	0.9887	
	Phosphorus x Time	2	24	0.15	0.8576	
	Nitrogen x Phosphorus	1	24	2.20	0.1509	
$\log(NO_3^-)$ (g^{-1})	Time	2	26	2.96	0.0696	
	Nitrogen	1	26	4.48	0.0439	*
	Phosphorus	1	26	0.07	0.7972	
	Nitrogen x Time	2	26	3.36	0.0502	
	Phosphorus x Time	2	26	0.64	0.5334	
	Nitrogen x Phosphorus	1	26	0.14	0.7145	
$\log(NH_4^+)$ (cm^{-3})	Time	2	24	8.00	0.0022	**
	Nitrogen	1	24	18.25	0.0003	**
	Phosphorus	1	24	3.58	0.0705	
	Nitrogen x Time	2	24	0.02	0.9787	
	Phosphorus x Time	2	24	0.18	0.8344	
	Nitrogen x Phosphorus	1	24	1.94	0.1768	
$\log(NO_3^-)$ (cm^{-3})	Time	2	26	3.12	0.0611	
	Nitrogen	1	26	4.75	0.0385	*
	Phosphorus	1	26	0.11	0.7452	
	Nitrogen x Time	2	26	3.17	0.0587	
	Phosphorus x Time	2	26	0.41	0.6670	
	Nitrogen x Phosphorus	1	26	0.05	0.8184	

Table 3.10 Pearson's correlation coefficients for correlations between acid phosphatase activities (AcidPases), alkaline phosphatase activities (AlkPases), dissolved organic phosphorus (DOP), microbial phosphorus (MBP), and Bray- and Olsen's extractable phosphorus. ** P < 0.01, *** P < 0.0001.

Correlation	Pearson Coefficient	P	n
AcidPases, AlkPases	0.7981	<0.0001***	43
DOP, AcidPases	-0.0581	0.7329	37
DOP, AlkPases	0.7764	0.6385	39
DOP, Bray	-0.2606	0.1090	39
DOP, Olsen	-0.0293	0.8596	39
Bray, Olsen	0.3414	0.1796	47
Bray, AcidPases	-0.0436	0.8094	33
Bray, AlkPases	0.3344	0.0574	33
Olsen, AcidPases	0.1893	0.2913	33
Olsen, AlkPases	0.3259	0.0642	33
Ammonium, Nitrate	0.5281	0.0019**	32
Ammonium, AcidPases	-0.1913	0.3201	29
Ammonium, AlkPases	0.0265	0.8856	32
Nitrate, AcidPases	-0.1831	0.3159	32
Nitrate, AlkPases	-0.2146	0.2157	35
MBP, Bray	-0.0191	0.9118	36
MBP, Bray (P-treated subplots)	-0.1985	0.4298	18
MBP, Bray (non P-treated subplots)	-0.0841	0.6962	24
MBP, Olsen	0.7010	0.6846	36
MBP, Olsen P-treated subplots)	0.1085	0.6681	18
MBP, Olsen (non P-treated subplots)	0.0385	0.8582	24

3.3.3 Plant response to NP-fertilization during the summer of 2003

Results from the NP-fertilization experiment during the summer of 2003 show that above-ground biomass is clearly co-limited by both nitrogen and phosphorus (Figure 3.13a, Table 3.11). During the summer of 2003, above-ground biomass in control and phosphorus-alone treatment subplots increased slightly and remained statistically similar ($F_{4, 102} = 1.05$, $P = 0.3845$). Above-ground biomass in nitrogen-alone subplots responded with a significant increase in production beyond that in control subplots as of mid-July 2003 ($F_{1,102} = 304.82$, $P < 0.0001$; $F_{4,102} = 14.67$, $P < 0.0001$). Average above-ground biomass in these subplots continued to increase to a maximum on day 56. The strongest and most immediate response of above-ground biomass was in response to the interaction of nitrogen and phosphorus addition (1N1P) ($F_{1,102} = 24.12$, $P < 0.0001$). Average above-ground biomass production in 1N1P subplots was significantly higher than the average in 0N0P, 1N0P and 0N1P subplots after just 30 days. On the final harvest, at the end of the growing season on 5 August 2003, average above-ground biomass production was equally low in control and 0N1P subplots (means of 79.4 and 74.3 g m^{-2}, respectively), significantly higher in 1N0P subplots (mean of 192.8 g m^{-2}), and significantly highest in the 1N1P subplots (mean of 266.2 g m^{-2}) (Figure 3.13b).

The allocation of C, N and P to shoots of *Puccinellia phryganodes* varied in response to N and P fertilization (Figure 3.14, Table 3.11). Shoot C content fluctuated with no clear trend because most carbon is structural, and not acting as a mineral nutrient. Average allocation of N to shoot tissues showed a significant and positive response to N addition (1N0P and 1N1P subplots) ($F_{1, 106} = 642.38$, $P < 0.0001$). During the summer of 2003, there was also a significant seasonal change in shoot N allocation ($F_{1, 106} = 100.82$, $P < 0.0001$), as well as a significant N by time interaction ($F_{4, 106} = 7.55$, $P < 0.0001$). Average shoot N content was generally higher in 1N0P and 1N1P subplots and shoot N in these subplots increased and then decreased following each of the two nutrient additions on 3 June and 10 July 2003. Average allocation of phosphorus to shoots was generally higher in 1N1P subplots than in 0N1P subplots ($F_{1, 105} = 18.75$, $P < 0.0001$) (Figure 3.15). Shoot P content in P-fertilized subplots was significantly higher than average P shoot allocation in control (0N0P) and 1N0P subplots ($F_{1, 105} = 319.67$, $P < 0.0001$). Although there was a significant effect of time ($F_{5, 105} = 19.16$, $P < 0.0001$) and N fertilization ($F_{1, 105} = 8.04$, $P < 0.0055$), which corresponded to a slight decrease in the average P content, shoot P content remained relatively constant throughout the growing season despite the second nutrient addition on 10 July 2003.

Similar to results of shoot C allocation, allocation of carbon to roots of *Puccinellia phryganodes* changed significantly over time ($F_{4, 44} = 21.49$, $P < 0.0001$) and with nitrogen

addition ($F_{4, 44}$ = 5.99, P = 0.0184) but no strong patterns in C allocation emerged between roots collected from the different subplots because carbon is largely a structural element (Figure 3.16, Table 3.11). Average nitrogen content in control and 0N1P subplots dropped in mid-summer (third sampling day) ($F_{4, 43}$ = 32.65, P <0.0001), and remained low ($F_{1, 43}$ = 0.04, P = 0.8376) over time ($F_{4, 43}$ = 0.07, P = 0.9911). Average N content in roots was equal and highest in 1N0P and 1N1P subplots (N-effect: $F_{1, 43}$ = 146.24, P <0.0001; NxP: $F_{1, 43}$ = 0.01, P = 0.9287). On the final harvest (5 August 2003), average shoot N content in N-fertilized plots had dropped but was still higher than in that of the control and P-alone fertilized subplots. Significantly more phosphorus was allocated to roots in 0N1P and 1N1P subplots ($F_{1, 34}$ = 79.40, P <0.0001), but the P content in roots decreased over time in these subplots ($F_{4, 34}$ = 3.26, P = 0.0228). Furthermore, in subplots that received no P amendment (0N0P and 1N0P subplots) roots contained equally low amounts of phosphorus ($F_{1, 34}$ = 0.24, P = 0.6274), and the P content did not change during the summer ($F_{4, 34}$ = 1.73, P = 0.1657).

Since C is a structural molecule, only atomic shoot and root N:P ratios were analyzed (Figure 3.16 and 3.17). Average shoot N:P ratio in control (0N0P) and 0N1P subplots was lower than 23 throughout the summer, while average shoot N:P in N-fertilized subplots was higher than 14 with a maximum value of 36.8. Average root atomic N:P ratios ranged from below an average of 17.2 in control, 0N1P and 1N1P subplots, to an average between 20.1 and 31.3 in 1N0P subplots. Generally, average shoot and root atomic N:P ratios decreased throughout the summer ($F_{5, 104}$ = 20.18, P <0.0001; $F_{4, 31.6}$ = 6.54, P = 0.0006, respectively), and were approximately equal in the control and 1N1P subplots, lower in 0N1P subplots, and highest in 1N0P subplots. Refer to Table 3.11 for statistical analyses run on all non-transformed and log-transformed plant data.

Table 3.12 gives the shoot and root N and P contents (pool sizes) expressed per m^2 in comparison to control subplots and to the amount of N and P fertilizer added on 3 June and 10 July 2003. When expressed on an area basis, results incorporate both shoot and root nutrient content and growth. Results indicate that the shoots and roots of *P. phryganodes* accounted for from one third to two thirds of the N fertilizer added and less than one tenth of the P fertilizer. It is also clear that the allocation of N to shoots and roots is enhanced by N-fertilization and when N and P fertilizers are added together. Furthermore, the allocation of P to shoots and roots increased with P-fertilization and NP-fertilization, and the allocation of shoot P increased in N-alone fertilized subplots.

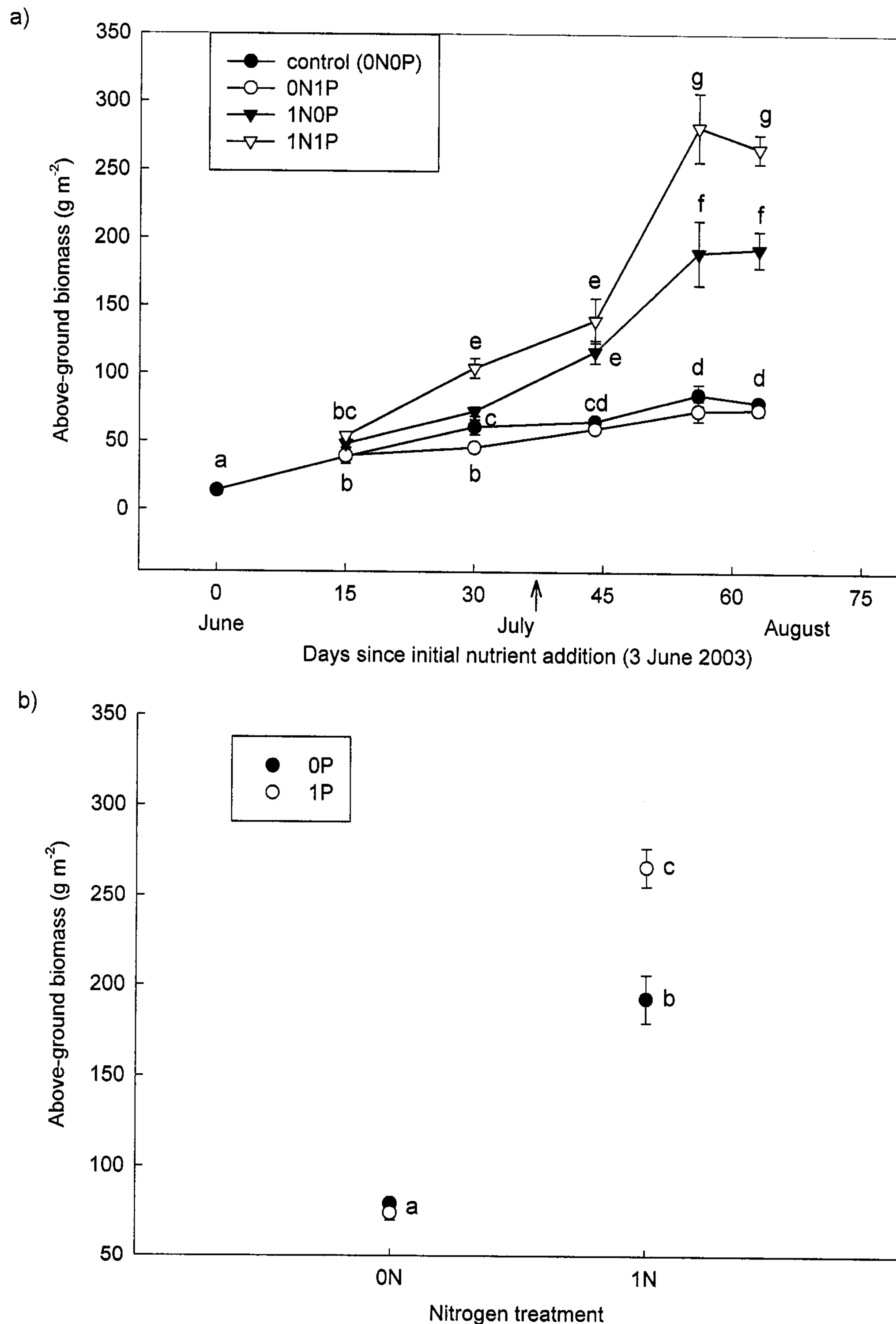
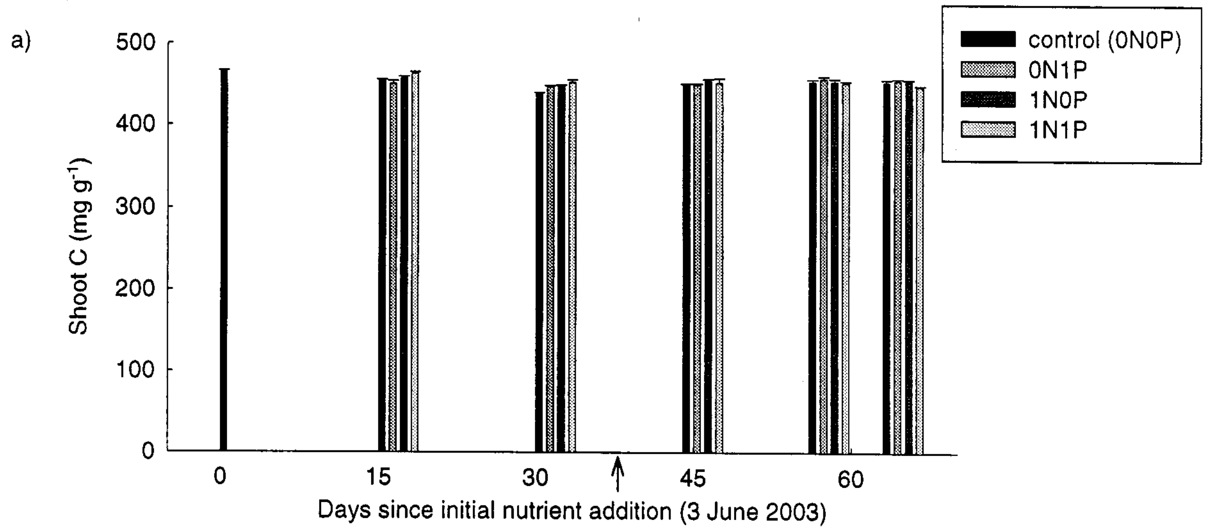
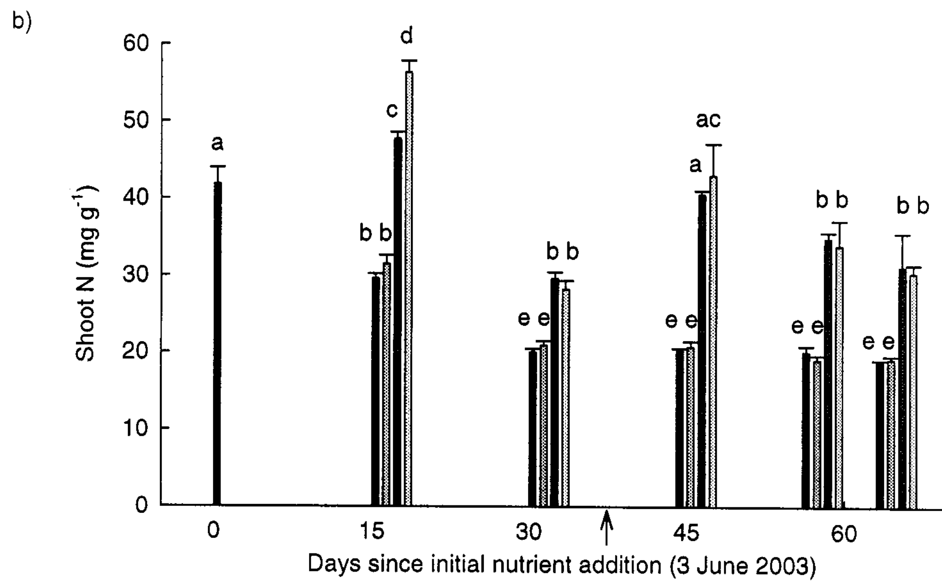


Figure 3.13 (a) Above-ground biomass (g m^{-2}) of results from control and fertilized plots during the summer of 2003 on the western intertidal marsh at La Pérouse Bay, Manitoba. The arrow at day 37 indicates the second nutrient addition on 10 July 2003. (b) the main effects (nitrogen and phosphorus) plotted for the above-ground biomass response on 5 August 2003. Means and standard errors (+/- SEM) are given (n=3). Common lower case letters denote a lack of significance (Tukey's HSD test).

a)



b)



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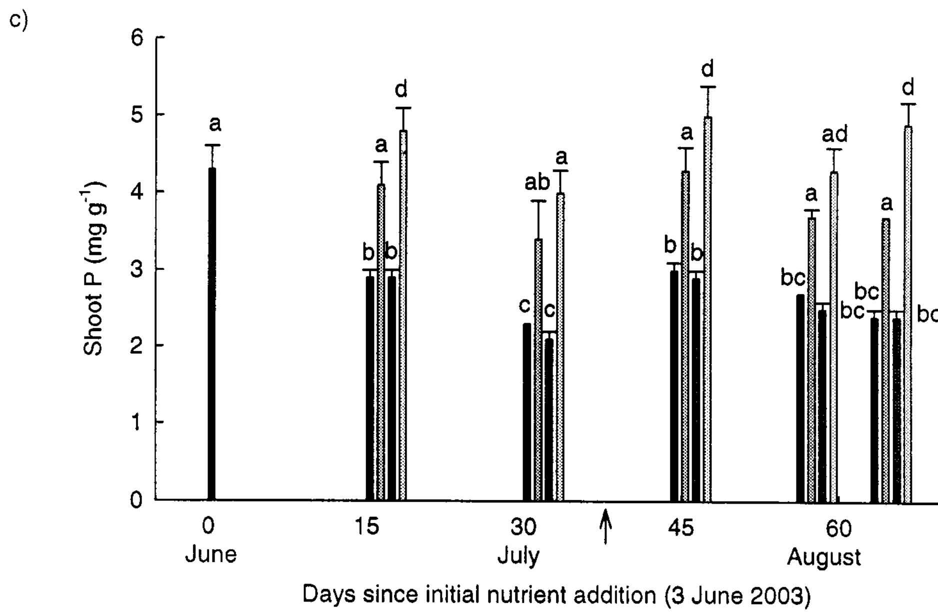
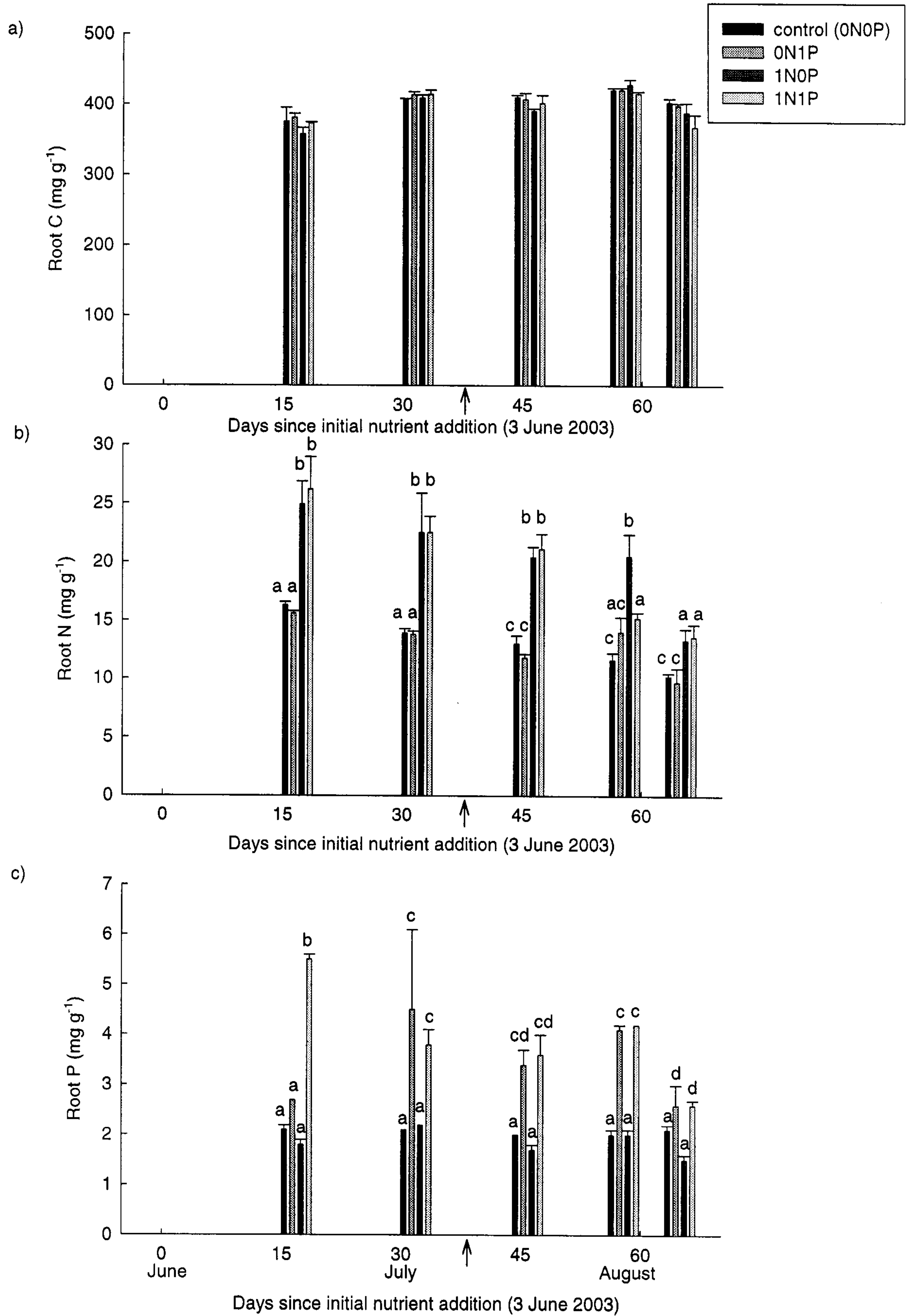


Figure 3.14 Shoot (a) C, (b) N and (c) P as mg g^{-1} dry mass of shoot tissue of *Puccinellia phryganodes* growing in the control and fertilization subplots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/- SEM) are shown (n=3). Common lower case letters denote a lack of significant difference between treatments and across time (Tukey's HSD test). Letters are either above or to the right of and parallel to the corresponding error bars.



a)
Root C (mg g-1)
500
400
300
200
100
0
15
30
45
60
Days since initial nutrient addition (3 June 2003)
control (0N0P)
0N1P
1N0P
1N1P
b)
Root N (mg g-1)
30
25
20
15
10
5
0
15
30
45
60
Days since initial nutrient addition (3 June 2003)
c)
Root P (mg g-1)
7
6
5
4
3
2
1
0
0
June
15
30
July
45
60
August
Days since initial nutrient addition (3 June 2003)

Figure 3.15 Root (a) C, (b) N and (c) P as mg g^{-1} dry mass of root tissue of *Puccinellia phryganodes* growing in the control and fertilization subplots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/- SEM) are shown (n=3). Common lower case letters denote a lack of significant difference between treatments and across time (Tukey's HSD test).

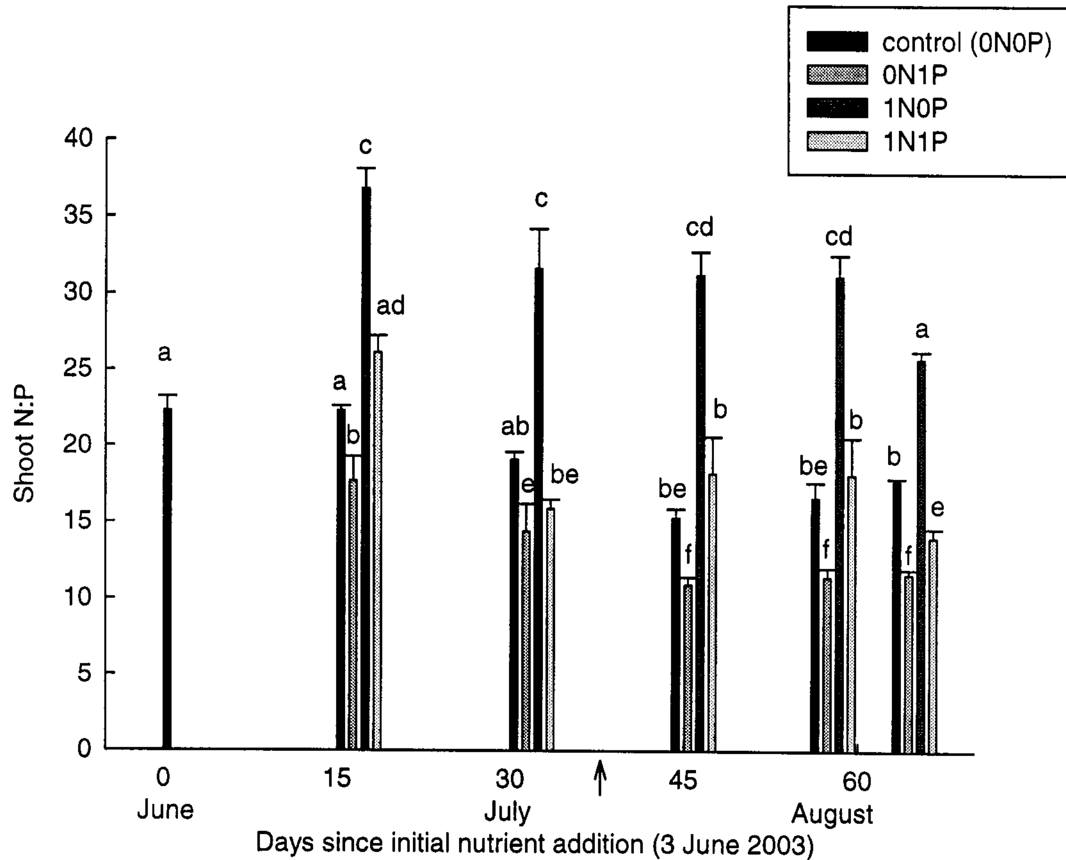


Figure 3.16 Atomic ratio of N:P in shoot tissue of *Puccinellia phryganodes* growing in control and fertilized plots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Means and standard errors (+/- SEM) are shown (n=3). The arrow at day 37 indicates the second nutrient addition on 10 July 2003. Common lower case letters denote a lack of significance (Tukey's HSD test).

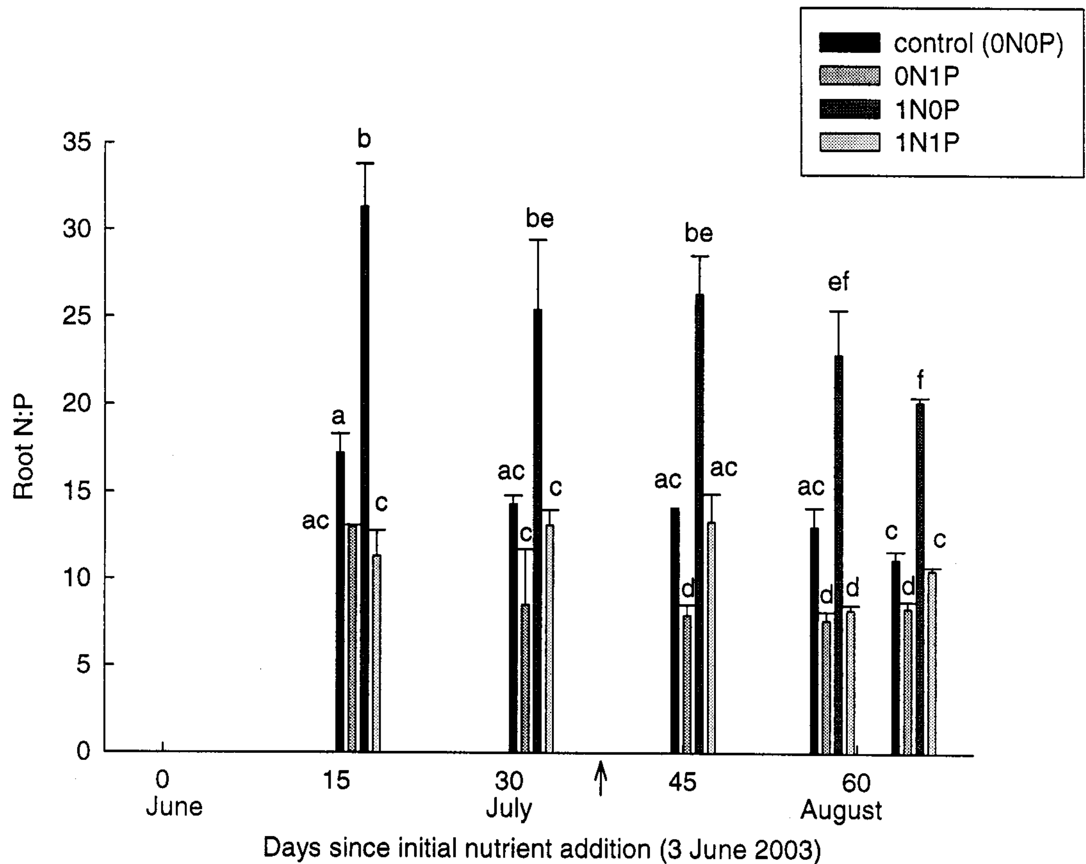


Figure 3.17 Atomic ratio of N:P in root tissue of *Puccinellia phryganodes* growing in control and fertilized plots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. The arrow at day 37 indicates the second nutrient addition on 10 July 2003. Means and standard errors (+/- SEM) are shown (n=3). Common lower case letters denote a lack of significance (Tukey's HSD test). Letters appear either above or to the left of and parallel to the corresponding error bars.

Table 3.11 Multi-way ANOVAs of above-ground biomass and shoot and root nutrients in response to NP-fertilization on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. * P<0.05; ** P<0.01; *** P<0.0001.

Data	Fixed effects	Num df	Den df	F	P	
log(Biomass)	Time	5	102	131.28	<0.0001	***
	Nitrogen	1	102	304.82	<0.0001	***
	Phosphorus	1	102	5.26	0.0238	*
	Nitrogen x Time	4	102	14.67	<0.0001	***
	Phosphorus x Time	4	102	1.05	0.3845	
	Nitrogen x Phosphorus	1	102	24.12	<0.0001	***
log(Shoot C)	Time	5	108	11.92	<0.0001	***
	Nitrogen	1	108	4.93	0.0285	*
	Phosphorus	1	108	0.57	0.4528	
	Nitrogen x Time	4	108	3.48	0.0102	*
	Phosphorus x Time	4	108	1.43	0.2306	
	Nitrogen x Phosphorus	1	108	0.69	0.4070	
log(Shoot N)	Time	5	106	100.82	<0.0001	***
	Nitrogen	1	106	642.38	<0.0001	***
	Phosphorus	1	106	0.83	0.3656	
	Nitrogen x Time	4	106	7.55	<0.0001	***
	Phosphorus x Time	4	106	1.73	0.1485	
	Nitrogen x Phosphorus	1	106	0.00	0.9847	
log(Shoot P)	Time	5	105	19.16	<0.0001	****
	Nitrogen	1	105	8.04	0.0055	**
	Phosphorus	1	105	319.67	<0.0001	***
	Nitrogen x Time	4	105	0.53	0.7163	
	Phosphorus x Time	4	105	0.86	0.4892	
	Nitrogen x Phosphorus	1	105	18.75	<0.0001	***
Root C	Time	4	44	21.49	<0.0001	***
	Nitrogen	1	44	5.99	0.0184	*
	Phosphorus	1	44	0.00	0.9474	
	Nitrogen x Time	4	44	1.56	0.2019	
	Phosphorus x Time	4	44	1.33	0.2752	
	Nitrogen x Phosphorus	1	44	0.02	0.8789	
log(Root N)	Time	4	43	32.65	<0.0001	***
	Nitrogen	1	43	146.24	<0.0001	***
	Phosphorus	1	43	0.04	0.8376	
	Nitrogen x Time	4	43	2.29	0.0756	
	Phosphorus x Time	4	43	0.07	0.9911	
	Nitrogen x Phosphorus	1	43	0.01	0.9287	
Root P	Time	4	34	3.26	0.0228	*
	Nitrogen	1	34	0.24	0.6274	
	Phosphorus	1	34	79.40	<0.0001	***
	Nitrogen x Time	4	34	1.73	0.1657	
	Phosphorus x Time	4	34	1.73	0.1652	
	Nitrogen x Phosphorus	1	34	2.97	0.0937	
log(Shoot N:P)	Time	5	104	20.18	<0.0001	***
	Nitrogen	1	104	217.00	<0.0001	***
	Phosphorus	1	104	225.68	<0.0001	***
	Nitrogen x Time	4	104	4.37	0.0026	**
	Phosphorus x Time	4	104	1.84	0.1274	
	Nitrogen x Phosphorus	1	104	13.08	0.0005	***
log(Root N:P)	Time	4	31.6	6.54	0.0006	
	Nitrogen	1	31.6	59.49	<0.0001	
	Phosphorus	1	32.5	126.47	<0.0001	
	Nitrogen x Time	4	31.7	1.78	0.1581	
	Phosphorus x Time	4	31.5	0.65	0.6315	
	Nitrogen x Phosphorus	1	31.8	8.77	0.0058	

Table 3.12 Nitrogen (N) and phosphorus (P) contents in shoots and roots of *Puccinellia phryganodes* taken from the NP-fertilization experiment on 3 June (Day 0), 3 July (Day 30), 17 July (Day 44) and 5 August (Day 63) 2003. 0N0P, 0N1P, 1N0P and 1N1P refer to nutrient treatments added to exclosure subplots. Means +/- SEM are given. Total fertilizer addition to date refers to the total amount of N fertilizer added to 1N0P and 1N1P subplots and the total amount of P fertilizer added to 0N1P and 1N1P as of each sampling date. Fertilizer differential equals the difference between the total amount of fertilizer added to date and shoot + root nutrient content. Fertilizer was added on 3 June and 10 July 2003. Shoot and root contents, shoot + root, total fertilizer addition to date and fertilizer differential are expressed as g (nutrient) m^{-2}.

Nutrient	Day	Treatment subplot	Shoot content	Root content	Shoot + root	Total fertilizer addition to date	Fertilizer differential	% use
N	0	0N0P	0.57 +/- 0.09		0.57			
	30	0N0P	1.15 +/- 0.03	0.51 +/- 0.05	1.66			
		0N1P	1.24 +/- 0.15	0.38 +/- 0.01	1.62			
		1N0P	2.33 +/- 0.13	0.97 +/- 0.10	3.30	8.5	5.20	38
		1N1P	3.06 +/- 0.18	1.41 +/- 0.14	4.47	8.5	4.03	53
	44	0N0P	1.31 +/- 0.04	0.51 +/- 0.02	1.82			
		0N1P	1.23 +/- 0.09	0.42 +/- 0.03	1.65			
		1N0P	5.35 +/- 0.35	1.60 +/- 0.03	6.95	17	10.05	41
		1N1P	5.91 +/- 0.30	1.79 +/- 0.30	7.70	17	9.30	46
	63	0N0P	1.51 +/- 0.06	0.49 +/- 0.02	2.00			
		0N1P	1.48 +/- 0.16	0.45 +/- 0.05	2.93			
		1N0P	5.19 +/- 0.96	1.32 +/- 0.12	6.51	17	10.49	38
		1N1P	8.16 +/- 0.56	2.18 +/- 0.23	10.34	17	6.66	61
P	0	0N0P	0.07 +/- 0.02		0.07			
	30	0N0P	0.14 +/- 0.01	0.05 +/- 0.02	0.19			
		0N1P	0.15 +/- 0.02	0.08 +/- 0.05	0.23	6	5.77	4
		1N0P	0.16 +/- 0.01	0.09 +/- 0.01	0.25			
		1N1P	0.41 +/- 0.05	0.24 +/- 0.03	0.45	6	5.55	7
	44	0N0P	0.19 +/- 0.01	0.08 +/- 0.00	0.27			
		0N1P	0.25 +/- 0.02	0.12 +/- 0.01	0.37	12	11.63	3
		1N0P	0.39 +/- 0.05	0.14 +/- 0.01	0.53			
		1N1P	0.57 +/- 0.17	0.31 +/- 0.06	0.88	12	11.12	7
	63	0N0P	0.18 +/- 0.01	0.10 +/- 0.01	0.28			
		0N1P	0.28 +/- 0.02	0.12 +/- 0.02	0.40	12	11.60	3
		1N0P	0.42 +/- 0.04	0.15 +/- 0.01	0.57			
		1N1P	1.38 +/- 0.18	0.28 +/- 0.14	1.66	12	10.34	14

3.3.4 Soil microbial C, N and P in response to NP-fertilization and CNP-fertilization

Before statistical analyses were performed, microbial N and P, microbial C:P and N:P ratios from the NP-fertilization were log-transformed. On a unit mass basis, results of soil microbial biomass were variable in response to N and P fertilization during the summer of 2003 (Figure 3.18, Table 3.13). Soil microbial carbon (MBC) also varied over time ($F_{4, 37}$ = 12.83, P <0.0001), as MBC in all subplots increased throughout the summer to about 5 times the baseline amount of a mean of 0.19 mg C g^{-1} on 3 June 2003 to a approximately 1.1 mg C g^{-1} in August 2003. Soil microbial N (MBN) also increased in all subplots approximately 3-fold during the summer of 2003 ($F_{4, 33.1}$ = 7.09, P <0.0003) from approximately 43.5 µg N g^{-1} to about 120 µg N g^{-1} in August 2003. High and less variable MBN in soil from the 1N0P subplot on the last sampling day (5 August 2003) accounted for the significant N effect per unit mass ($F_{4, 33.1}$ = 6.90, P = 0.0130). Soil microbial P (MBP) ranged from a mean of 1.89 to 6.56 µg P g^{-1}. Amounts of MBP in the experimental subplots, expressed per unit area, were unaffected by N and P additions and did not significantly change from June to August 2003. Nevertheless, MBP was lowest in June 2003 and there was a non-significant increase throughout the summer.

Microbial biomass results also were similar when data were analyzed on a volume basis (Figure 3.19, Table 3.13). MBC ($F_{4, 37}$ = 12.68, P <0.0001) and MBN ($F_{4, 35}$ = 3.98, P <0.0091) increased significantly from June to August 2003. There was a P by time interaction ($F_{3, 37}$ = 2.88, P = 0.0486) for MBC and a significant N effect ($F_{4, 35}$ = 9.46, P = 0.0041) for MBN, but these significant effects were a result of differences on a single date and therefore did not contribute to a general treatment effect. Again, MBP did not significantly change in response to N, P or time.

Atomic ratios of microbial C:N, C:P and N:P follow the same overall trends as MBC, MBN and MBP (Figure 3.20, Table 3.13). Microbial C:N increased two-fold from June to August 2003 ($F_{4, 35}$ = 6.57, P = 0.0005), while microbial C:P increased around 3-fold during the summer 2003 ($F_{4, 26}$ = 5.12, P = 0.0035). Microbial N:P only responded significantly to phosphorus addition ($F_{1, 23.5}$ = 10.20, P = 0.0040). This result is because MBP was lowest in 0N1P and 1N1P subplots on the fourth sampling date.

In response to glucose added on 27 July 2003, only MBC showed an overall change over the six-day period (Figure 3.21, Table 3.14). MBC increased to a maximum mean of 0.72 mg C g^{-1} on day 4 and dropped back to baseline values (a mean of about 0.3 mg C g^{-1}) on day 6 ($F_{3, 28.2}$ = 3.80, P = 0.0210). MBP also varied significantly over the six-day period ($F_{3, 32}$ = 4.78, P = 0.0073), but this was because of an unusual low amount of MBP in soil from the 0N1P subplot

on day 0. Bulk density did not change within the six-day period, so data were not expressed per unit volume.

Expressed as atomic ratios, MB C:N and MB C:P increased on day 2 and 4 but dropped again on day 6 (MB C:N: $F_{3,28.2} = 3.78$, P = 0.0213; MB C:P: $F_{3, 28.2} = 4.41$, P = 0.0115) (Figure 3.22, Table 3.14). These responses were mostly driven by changes in MBC in response to glucose addition. There were no significant changes in amounts of average MB N:P in response to glucose addition.

Note that estimates of MBP from soil taken from the CNP-fertilized plots are much higher than estimates in soil taken from the NP-fertilized plots (about 30 μg P g^{-1} compared to about 3 μg P g^{-1}). This is likely due to a difference in the capacity of 0.5M K_2SO_4 and 0.5M $NaHCO_3$ to extract P, which will be addressed later in the Discussion (section 4.4.2). In order to meet assumptions of normality, MBC and all nutrient ratios from the CNP-fertilization experiment were log-transformed.

3.3.5 Soil phosphatase activities in response to NP-fertilization during the summer of 2003

Results of soil acid and alkaline phosphatase activities in response to N and P addition to plots on the western intertidal marsh during the summer 2003 are variable (Figure 3.23, 3.24, 3.25) (Table 3.15a). Generally, AlkPase activities were higher than AcidPases activities. Expressed per unit mass, per unit volume, and per unit soil solution, log-transformed activities of AcidPases and AlkPases did not change significantly in response to N and P during the summer. Although statistically insignificant, activities of AcidPases peaked in early June 2003 (first sampling date) and then decreased and leveled off in mid-June 2003, irrespective of the unit of measure used to express the data. In contrast, when expressed per unit area and per unit volume, activities of AlkPases increased slightly (but insignificantly) in mid-summer. When expressed per unit soil solution, activities of AlkPases increased slightly (but insignificantly) at the end of the summer.

Results of MANOVA of the effect of N and P fertilization during the summer of 2003 on activities of both AcidPases and AlkPases were significant only when data were expressed on a unit mass basis ($F_{8, 58} = 2.8180$, P = 0.0484) (Table 3.15b). For this reason, Bonferroni-type adjustments ($\alpha = 0.025$) were made for the multi-way ANOVAs of AcidPases and AlkPases on a unit mass basis described above. The ratio of AcidPase to AlkPase per unit dry weight of soil revealed no consistent trend because of the marginal significance of the results (Figure 3.26).

Furthermore, results of activities in soils collected on day 0 and day 6 after the application of glucose indicated that there were no significant differences in activities of AcidPases or AlkPases among subplots of CNP-fertilized exclosures (Figure 3.27, Table 3.15a). There was also no significant change in the relative activities of the two enzyme complexes (MANOVA on log-transformed data: $F_{8, 14} = 1.0936$, $P = 0.4217$). All statistical analyses of Pase activities are presented in Table 3.15.

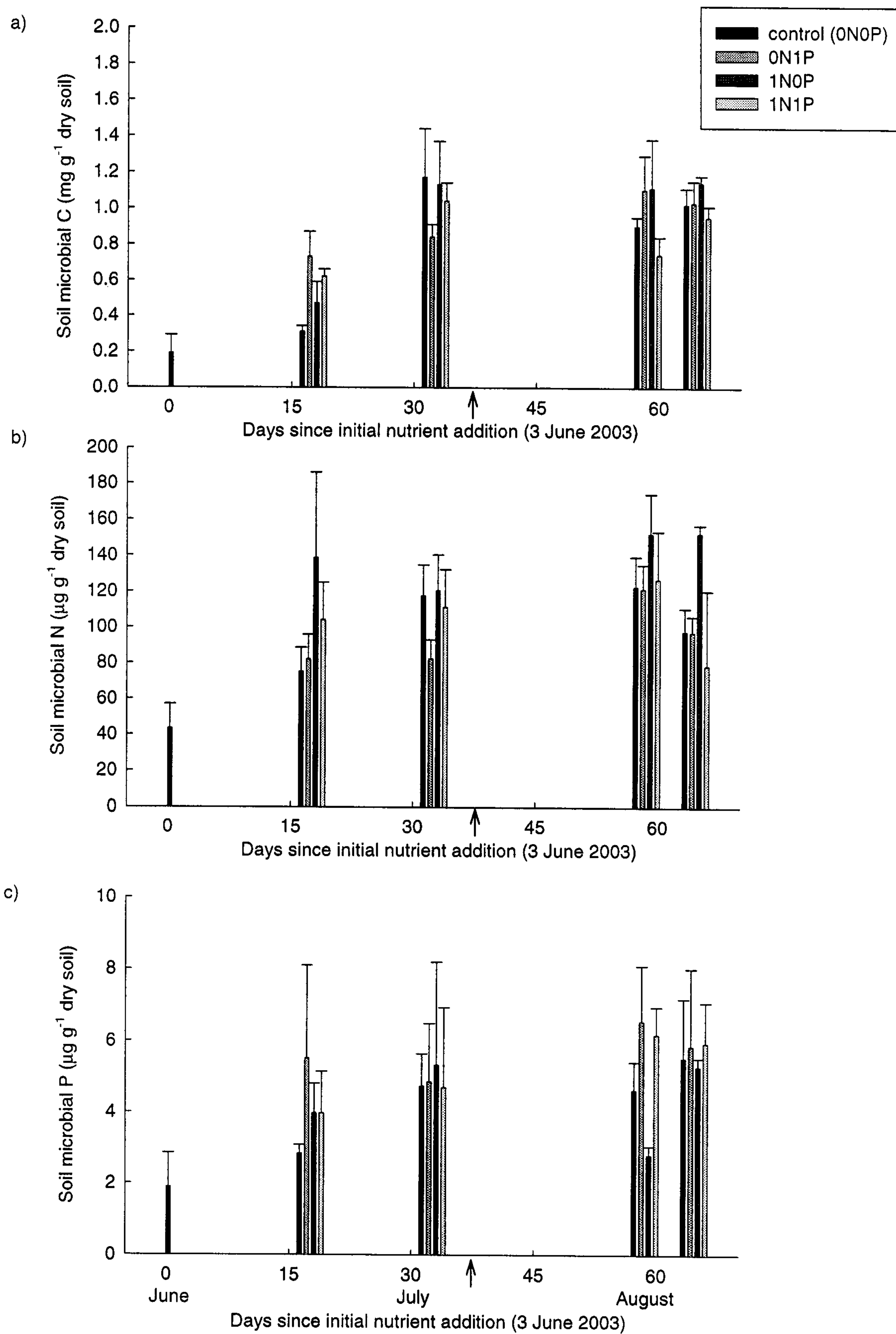

a)
b)
c)
control (0N0P)
0N1P
1N0P
1N1P
Soil microbial C (mg g^{-1} dry soil)
Soil microbial N (µg g^{-1} dry soil)
Soil microbial P (µg g^{-1} dry soil)
0
15
30
45
60
June
July
August
Days since initial nutrient addition (3 June 2003)

Figure 3.18 Soil microbial (a) carbon (MBC), (b) nitrogen (MBN) and (c) phosphorus (MBP) per gram dry weight in control and fertilized subplots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Soil was collected at rooting depth (5cm). Means and standard errors (+/- SEM) are shown (n=3). Final calculations of MBC, MBN and MBP were corrected for using extractability factors $k_{EC} = 0.35$, $k_{EN} = 0.40$ and $k_{EP} = 0.40$, respectively, and a P fixation factor, $k_{fix} = 0.77$.

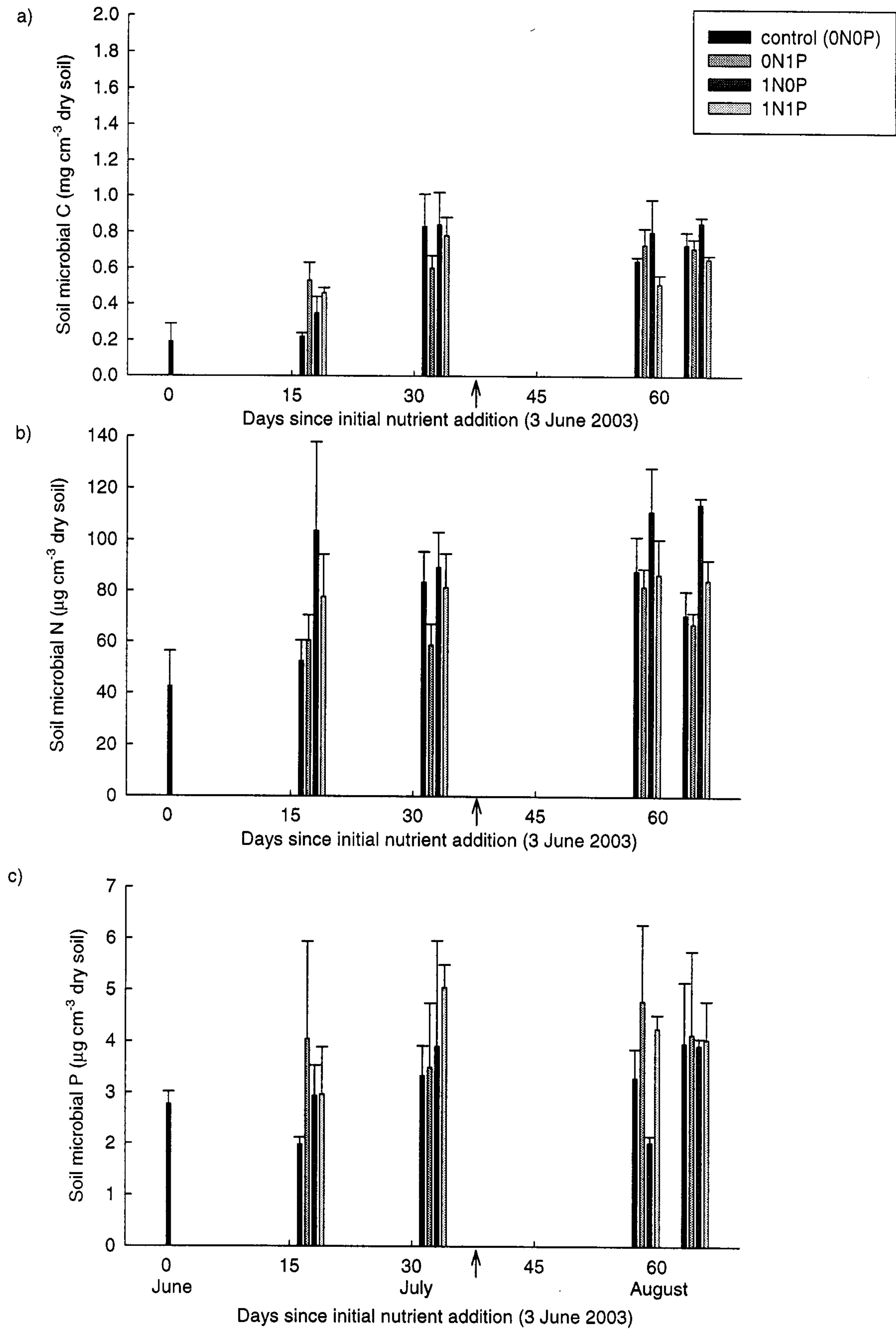

a)
Soil microbial C (mg cm^{-3} dry soil)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control (0N0P)
0N1P
1N0P
1N1P
0
15
30
45
60
Days since initial nutrient addition (3 June 2003)
b)
Soil microbial N (μg cm^{-3} dry soil)
140
120
100
80
60
40
20
0
0
15
30
45
60
Days since initial nutrient addition (3 June 2003)
c)
Soil microbial P (μg cm^{-3} dry soil)
7
6
5
4
3
2
1
0
0
June
15
30
July
45
60
August
Days since initial nutrient addition (3 June 2003)

Figure 3.19 Soil microbial (a) carbon (MBC), (b) nitrogen (MBN) and (c) phosphorus (MBP) per cm^3 dry soil in control and fertilized subplots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Soil was collected at rooting depth (5cm). Means and standard errors (+/- SEM) are shown (n=3). Final calculations of MBC, MBN and MBP were corrected for using extractability factors $k_{EC} = 0.35$, $k_{EN} = 0.40$ and $k_{EP} = 0.40$, respectively, and a P fixation factor, $k_{fix} = 0.77$.

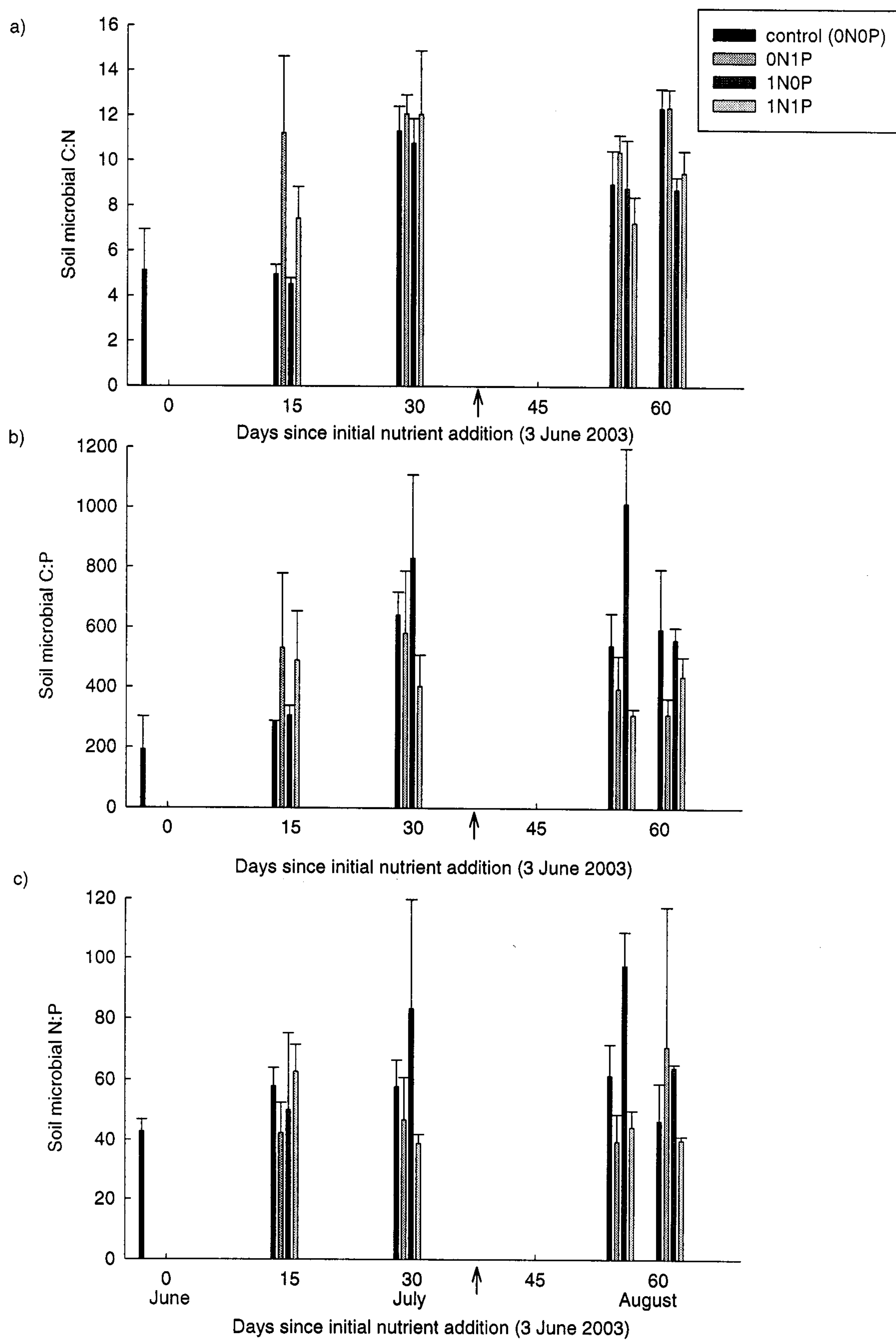

a)
Soil microbial C:N
16
14
12
10
8
6
4
2
0
0
15
30
45
60
Days since initial nutrient addition (3 June 2003)
control (0N0P)
0N1P
1N0P
1N1P
b)
Soil microbial C:P
1200
1000
800
600
400
200
0
0
15
30
45
60
Days since initial nutrient addition (3 June 2003)
c)
Soil microbial N:P
120
100
80
60
40
20
0
0
June
15
30
July
45
60
August
Days since initial nutrient addition (3 June 2003)

Figure 3.20 Soil microbial (a) C:N, (b) C:P and (c) N:P in control and fertilized subplots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Soil was collected at rooting depth (5cm) and ratios were calculated on an atomic basis. Means and standard errors (+/- SEM) are shown (n=3).

Table 3.13 Multi-way ANOVAs soil microbial C, N and P in response to NP-fertilization on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. * $P<0.05$; ** $P<0.01$; *** $P<0.0001$.

Data	Fixed effects	Num df	Den df	F	P	
Microbial C (g^{-1})	Time	4	37	12.83	<0.0001	***
	Nitrogen	1	37	0.04	0.8410	
	Phosphorus	1	37	0.11	0.7426	
	Nitrogen x Time	3	37	0.21	0.8867	
	Phosphorus x Time	3	37	2.16	0.1095	
	Nitrogen x Phosphorus	1	37	1.86	0.1806	
log(Microbial N) (g^{-1})	Time	4	33.1	7.09	0.0003	**
	Nitrogen	1	33.1	6.90	0.0130	*
	Phosphorus	1	33.2	1.89	0.1786	
	Nitrogen x Time	3	33.2	0.52	0.6717	
	Phosphorus x Time	3	33.1	0.15	0.9306	
	Nitrogen x Phosphorus	1	33.2	0.48	0.4944	
log(Microbial P) (g^{-1})	Time	4	36	0.86	0.4957	
	Nitrogen	1	36	0.45	0.5061	
	Phosphorus	1	36	0.87	0.3572	
	Nitrogen x Time	3	36	0.49	0.6911	
	Phosphorus x Time	3	36	1.08	0.3709	
	Nitrogen x Phosphorus	1	36	0.10	0.7556	
Microbial C (cm^{-3})	Time	4	37	12.68	<0.0001	***
	Nitrogen	1	37	0.39	0.5359	
	Phosphorus	1	37	0.56	0.4595	
	Nitrogen x Time	3	37	0.27	0.8444	
	Phosphorus x Time	3	37	2.88	0.0486	*
	Nitrogen x Phosphorus	1	37	2.27	0.1404	
log(Microbial N) (cm^{-3})	Time	4	35	3.98	0.0091	**
	Nitrogen	1	35	9.46	0.0041	**
	Phosphorus	1	35	2.61	0.1152	
	Nitrogen x Time	3	35	0.56	0.6468	
	Phosphorus x Time	3	35	0.19	0.8998	
	Nitrogen x Phosphorus	1	35	0.59	0.4474	
log(Microbial P) (cm^{-3})	Time	4	36	0.59	0.6743	
	Nitrogen	1	36	0.27	0.6043	
	Phosphorus	1	36	0.63	0.4318	
	Nitrogen x Time	3	36	0.45	0.7165	
	Phosphorus x Time	3	36	1.01	0.3999	
	Nitrogen x Phosphorus	1	36	0.14	0.7121	
Microbial C:N	Time	4	35	6.57	0.0005	**
	Nitrogen	1	35	5.80	0.0214	*
	Phosphorus	1	35	3.56	0.0674	
	Nitrogen x Time	3	35	0.67	0.5774	
	Phosphorus x Time	3	35	1.90	0.1471	
	Nitrogen x Phosphorus	1	35	0.65	0.4269	
log(Microbial C:P)	Time	4	26	5.12	0.0035	**
	Nitrogen	1	26	0.17	0.6842	
	Phosphorus	1	26	3.89	0.0592	
	Nitrogen x Time	3	26	1.30	0.2954	
	Phosphorus x Time	3	26	0.76	0.5248	
	Nitrogen x Phosphorus	1	26	0.03	0.8555	
log(Microbial N:P)	Time	4	23.4	1.14	0.3623	
	Nitrogen	1	23.3	3.20	0.0866	
	Phosphorus	1	23.5	10.20	0.0040	**
	Nitrogen x Time	3	23.2	1.66	0.2037	
	Phosphorus x Time	3	23.9	0.49	0.6948	
	Nitrogen x Phosphorus	1	23.7	0.20	0.6602	

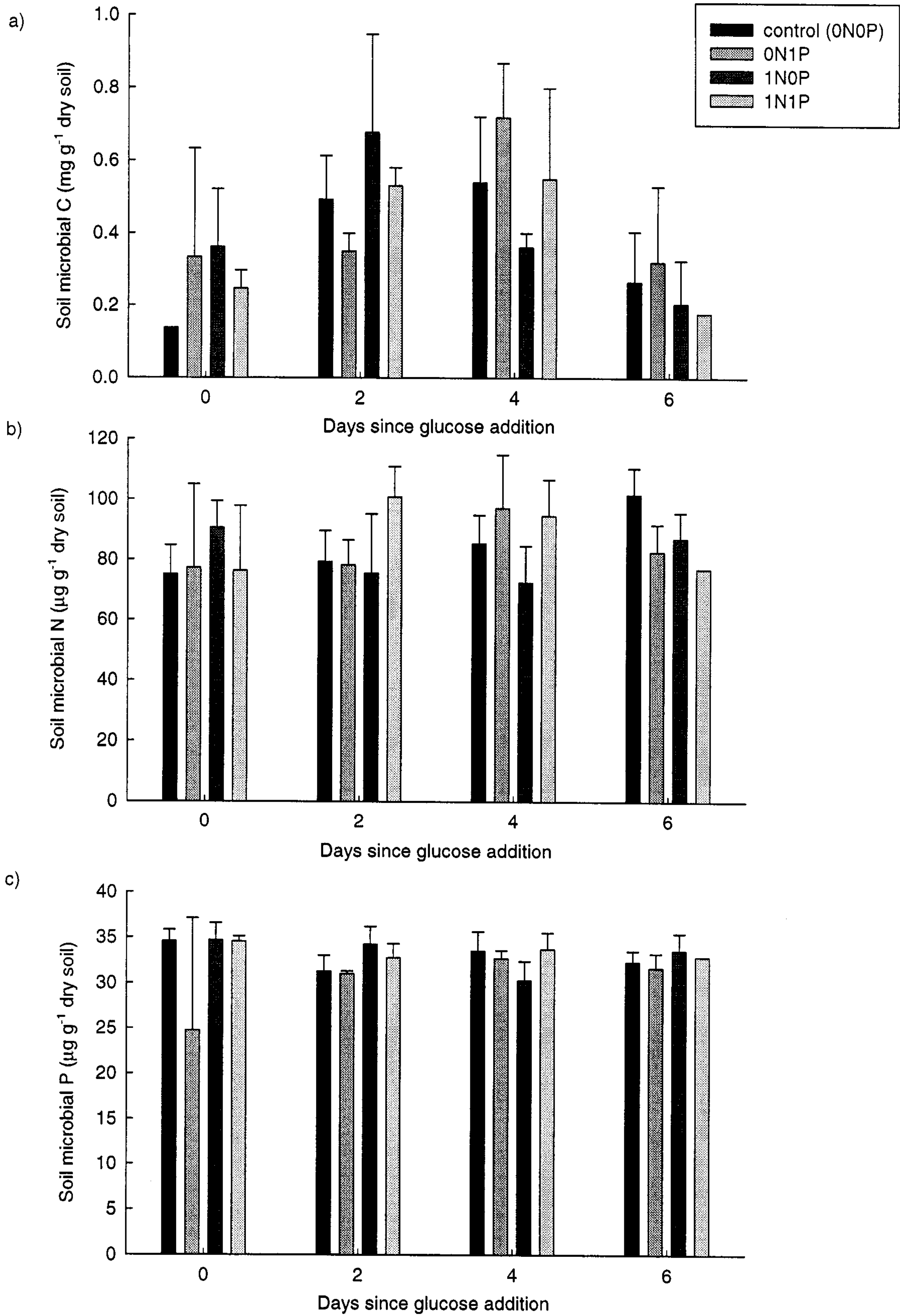

a)
1.0
0.8
0.6
0.4
0.2
0.0
Soil microbial C (mg g^{-1} dry soil)
0
2
4
6
Days since glucose addition
control (0N0P)
0N1P
1N0P
1N1P
b)
120
100
80
60
40
20
0
Soil microbial N (μg g^{-1} dry soil)
0
2
4
6
Days since glucose addition
c)
40
35
30
25
20
15
10
5
0
Soil microbial P (μg g^{-1} dry soil)
0
2
4
6
Days since glucose addition

Figure 3.21 Soil microbial (a) carbon (MBC), (b) nitrogen (MBN) and (c) phosphorus (MBP) per gram dry weight in glucose fertilized (CNP-fertilized) plots on the western intertidal marsh at La Pérouse Bay, Manitoba in July 2003. Soil was collected at rooting depth (5cm). Means and standard errors (+/- SEM) are shown (n=3). Final calculations of MBC, MBN and MBP were corrected for using extractability factors $k_{EC} = 0.35$, $k_{EN} = 0.40$ and $k_{EP} = 0.40$, respectively, and a P fixation factor, $k_{fix} = 0.77$.

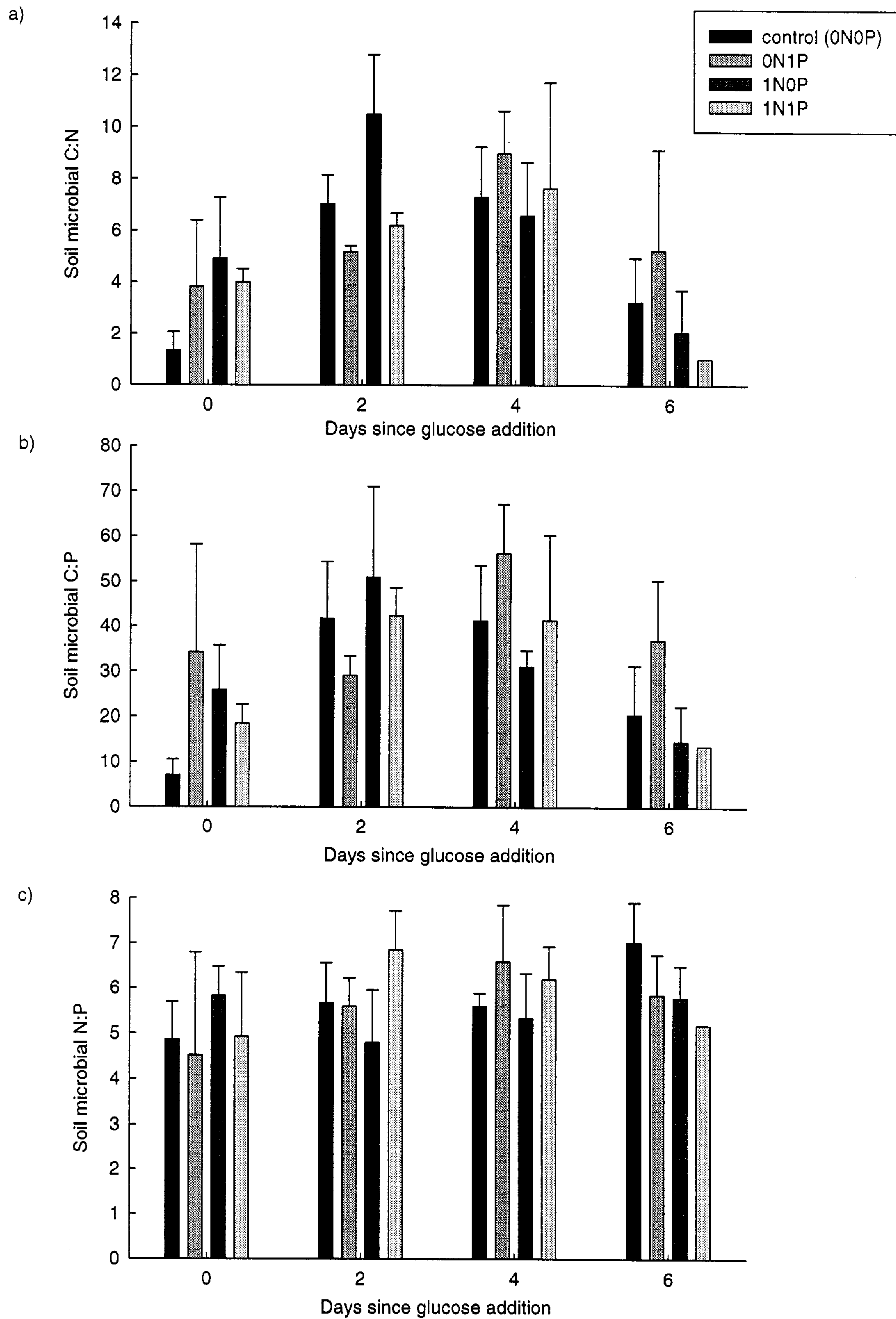

a)
b)
c)
control (0N0P)
0N1P
1N0P
1N1P
Soil microbial C:N
Soil microbial C:P
Soil microbial N:P
Days since glucose addition
Days since glucose addition
Days since glucose addition
0
2
4
6

Figure 3.22 Soil microbial (a) C:N, (b) C:P and (c) N:P in glucose fertilized (CNP-fertilized) plots on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soil was collected at rooting depth (5cm) and ratios were calculated on an atomic basis. Means and standard errors (+/- SEM) are shown (n=3).

Table 3.14 Multi-way ANOVAs of soil microbial C, N and P in response to glucose addition (CNP-fertilization plots) on the western intertidal marsh at La Pérouse Bay, Manitoba, July 2003. * P<0.05; ** P<0.01.

Data	Fixed effects	Num df	Den df	F	P	
log(Microbial C) (g^{-1})	Time	3	28.2	3.80	0.0210	*
	Nitrogen	1	28.6	0.00	0.9891	
	Phosphorus	1	28.1	0.77	0.3888	
	Nitrogen x Time	3	28.5	1.13	0.3540	
	Phosphorus x Time	3	28.1	0.47	0.7078	
	Nitrogen x Phosphorus	1	28.1	0.97	0.3333	
Microbial N (g^{-1})	Time	3	35	0.27	0.8464	
	Nitrogen	1	35	0.00	0.9668	
	Phosphorus	1	35	0.10	0.7595	
	Nitrogen x Time	3	35	0.54	0.6559	
	Phosphorus x Time	3	35	1.20	0.3254	
	Nitrogen x Phosphorus	1	35	0.29	0.5922	
Microbial P (g^{-1})	Time	3	32	4.78	0.0073	**
	Nitrogen	1	32	0.91	0.3481	
	Phosphorus	1	32	0.02	0.8823	
	Nitrogen x Time	3	32	2.33	0.0930	
	Phosphorus x Time	3	32	0.98	0.4146	
	Nitrogen x Phosphorus	1	32	0.018	0.6746	
log(Microbial C:N)	Time	3	28.2	3.78	0.0213	*
	Nitrogen	1	28.5	0.02	0.9002	
	Phosphorus	1	28.1	0.67	0.4194	
	Nitrogen x Time	3	28.4	0.75	0.5319	
	Phosphorus x Time	3	28.1	1.04	0.3887	
	Nitrogen x Phosphorus	1	28.1	2.35	0.1364	
log(Microbial C:P)	Time	3	28.2	4.41	0.0115	*
	Nitrogen	1	28.9	0.00	0.9490	
	Phosphorus	1	28.2	0.77	0.3869	
	Nitrogen x Time	3	28.6	1.08	0.3730	
	Phosphorus x Time	3	28.1	0.47	0.7073	
	Nitrogen x Phosphorus	1	28.1	1.02	0.3204	
log(Microbial N:P)	Time	3	28.2	1.20	0.3272	
	Nitrogen	1	28.7	0.03	0.8717	
	Phosphorus	1	28.2	0.01	0.9291	
	Nitrogen x Time	3	28.5	0.35	0.7870	
	Phosphorus x Time	3	28.1	1.08	0.3748	
	Nitrogen x Phosphorus	1	28.1	1.02	0.3200	

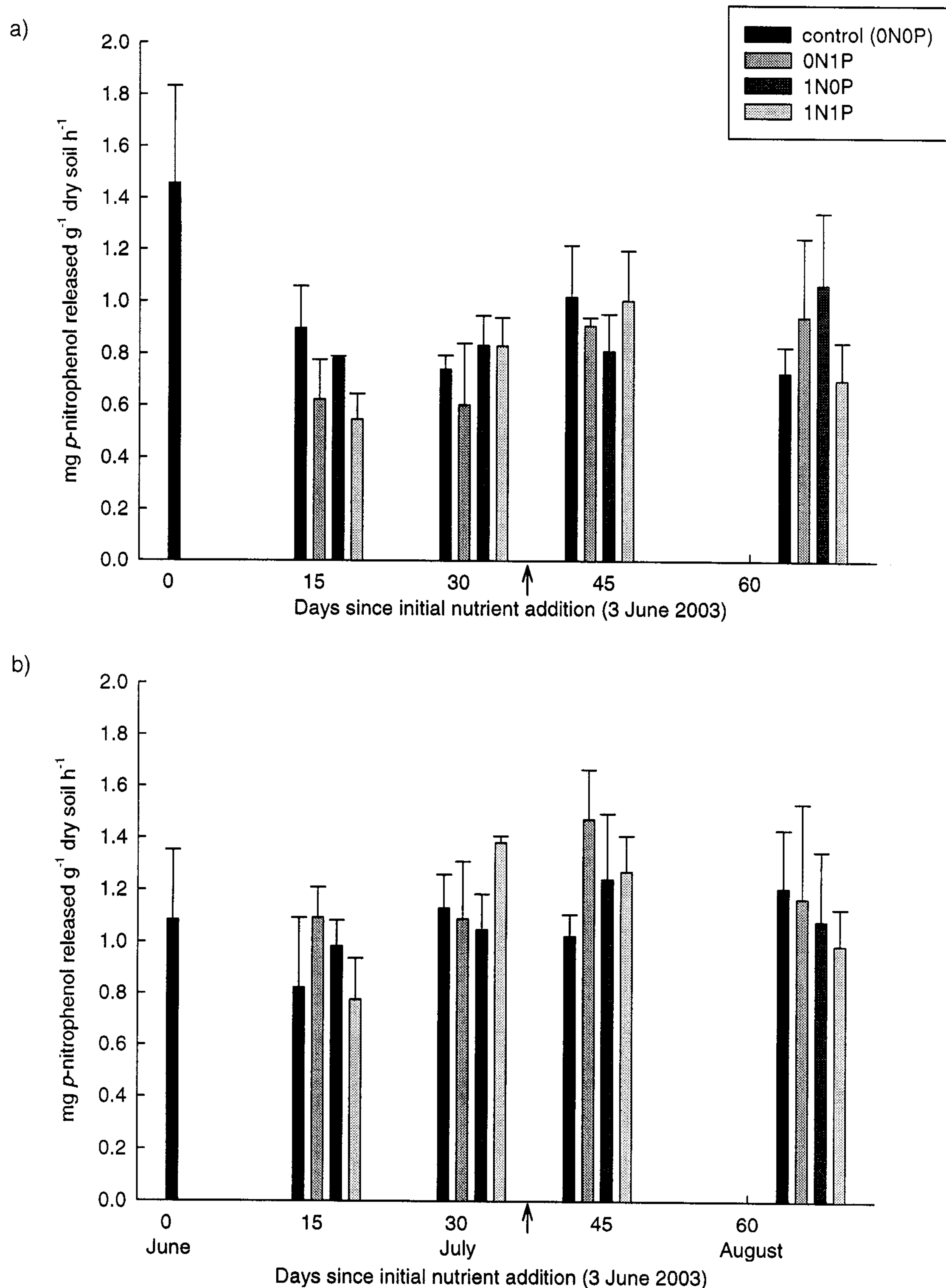


Figure 3.23 (a) Acid and (b) alkaline phosphatase activities per gram dry weight of soil in the control and fertilized plots in the western intertidal salt marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at rooting depth (5 cm) and incubated for 60 minutes. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/- SEM) are shown (n=3). There are no significant differences among treatments and across time (P>0.05).

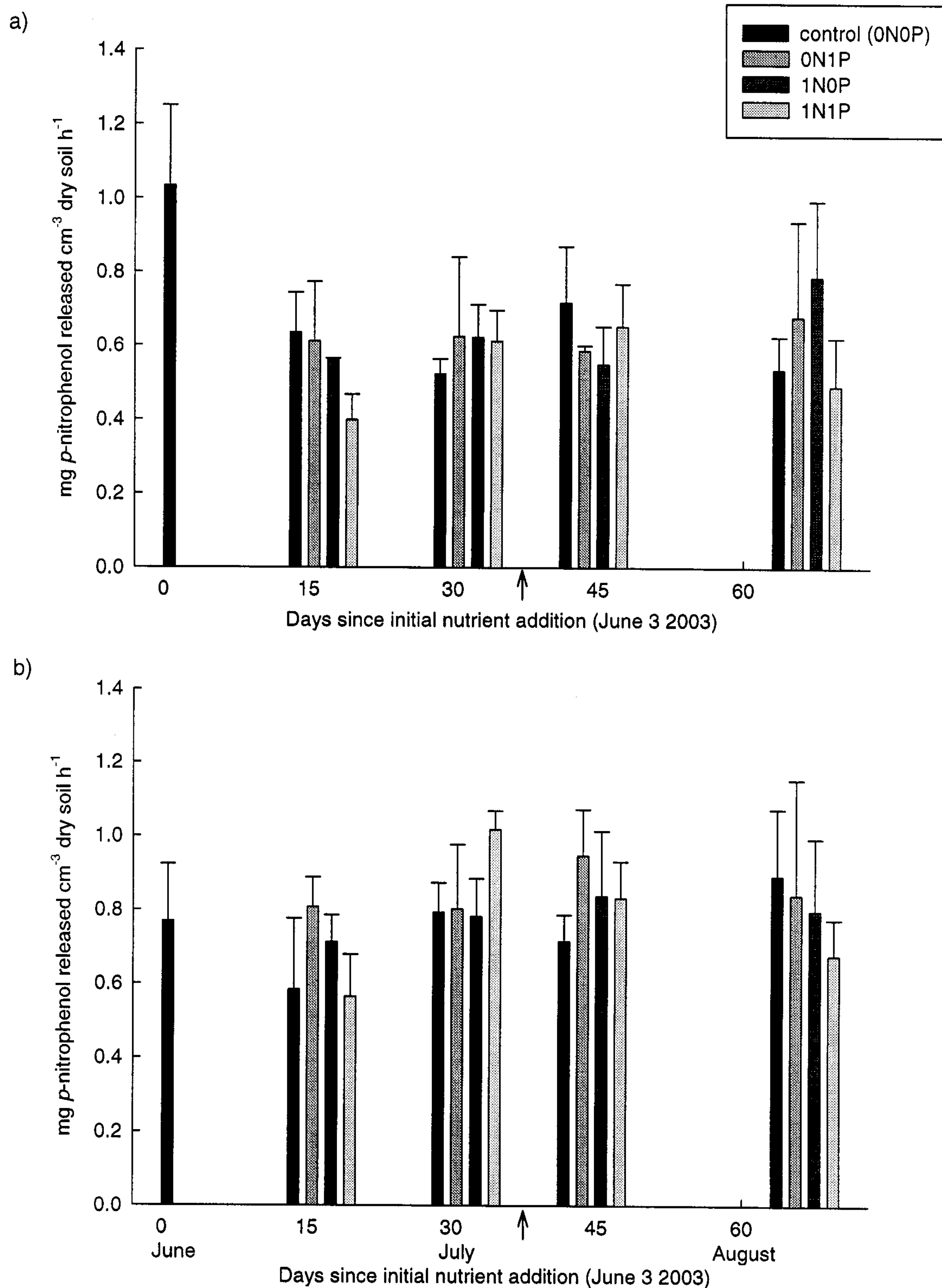


Figure 3.24 (a) Acid and (b) alkaline phosphatase activities per cm^3 of dry soil in the control and fertilized plots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at 5 cm (rooting depth) and incubated for 60 minutes. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Means and standard errors (+/- SEM) are shown (n=3). There are no significant differences among treatments and across time (P>0.05).

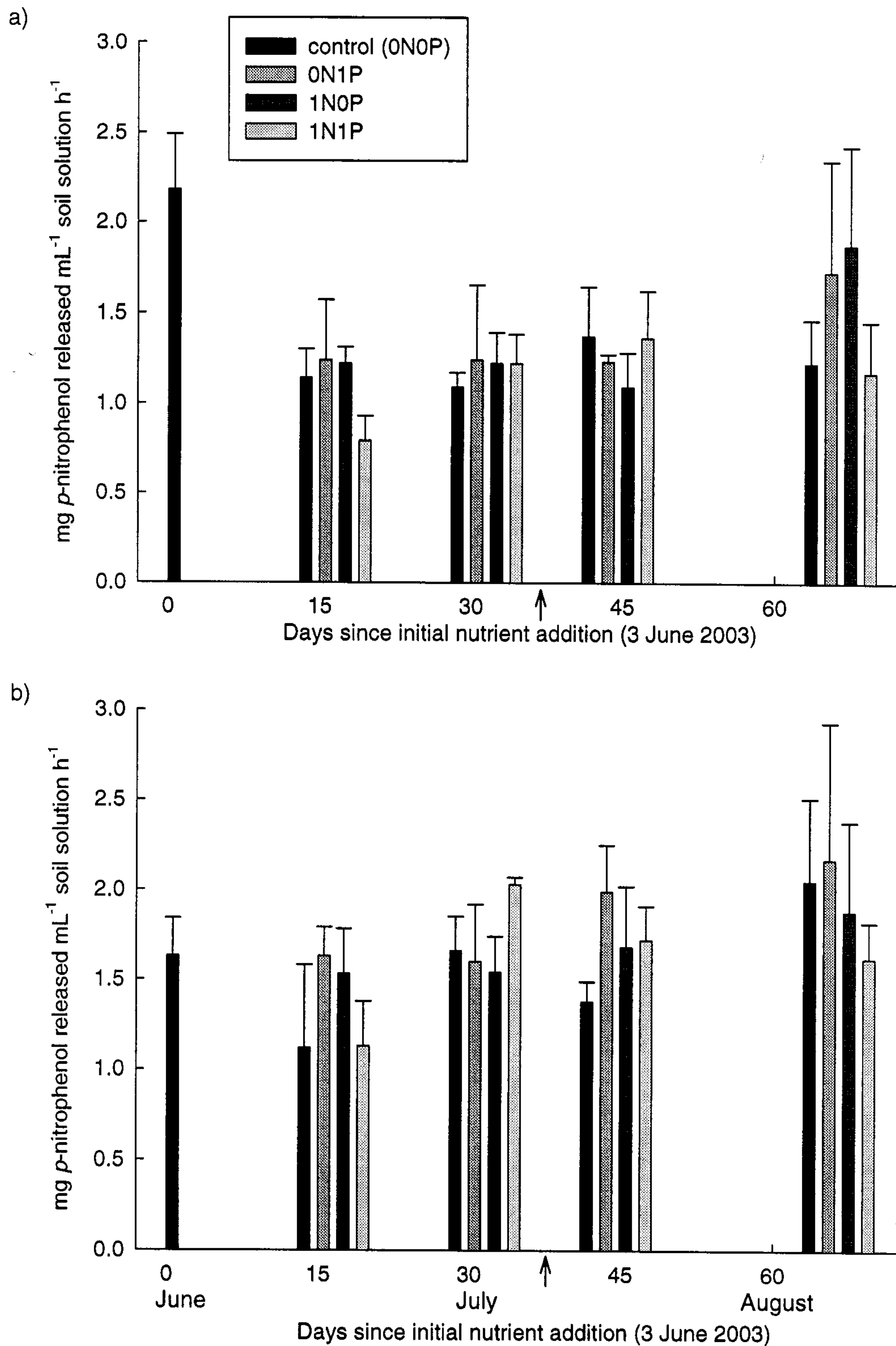


Figure 3.25 (a) Acid and (b) alkaline phosphatase activities per mL of soil solution in the control and fertilized plots in the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at 5 cm (rooting depth) and incubated for 60 minutes. The arrows at day 37 indicate the second nutrient addition on 10 July 2003. Mean and standard errors (+/- SEM) are shown (n=3). There are no significant differences among treatments and across time (P>0.05).

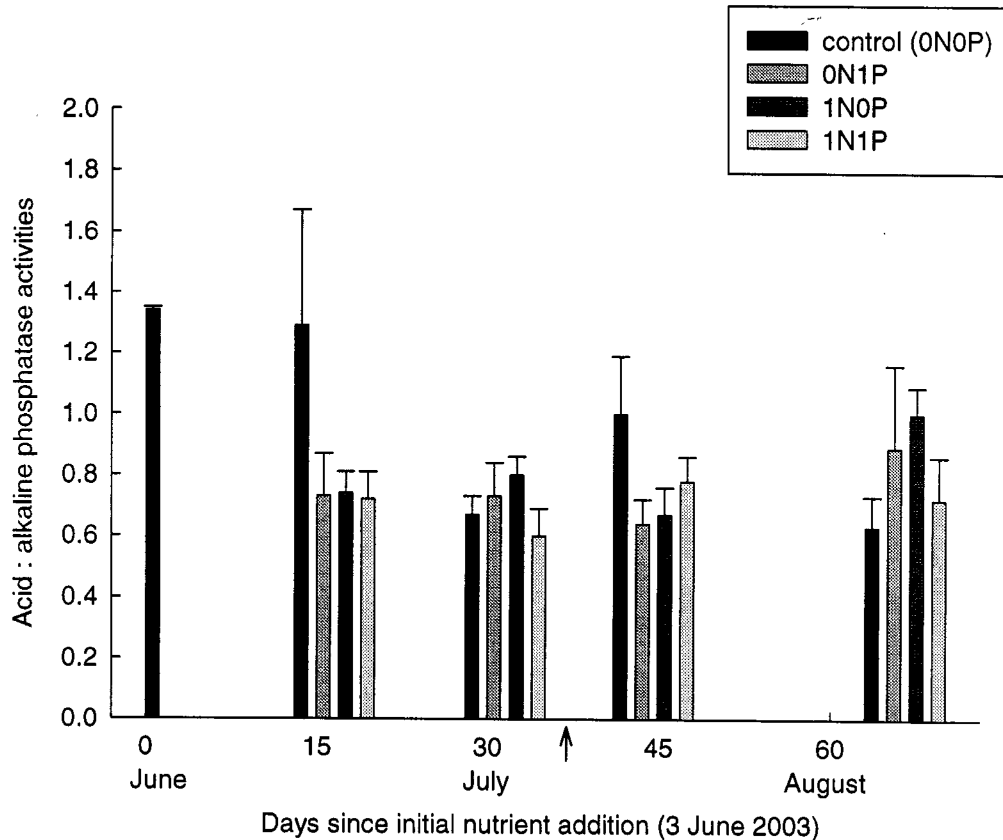


Figure 3.26 The ratio of acid to alkaline phosphatase activities per gram dry weight in control and fertilized plots in the western intertidal salt marsh at La Pérouse Bay, Manitoba during the summer of 2003. Soils were collected at 5 cm (rooting depth) and incubated for 60 mintues. The arrow at day 37 indicates the second nutrient addition on 10 July 2003. Means and standard errors (+/-SEM) are shown (n=3). There are no significant differences among treatments and across time (P>0.05).

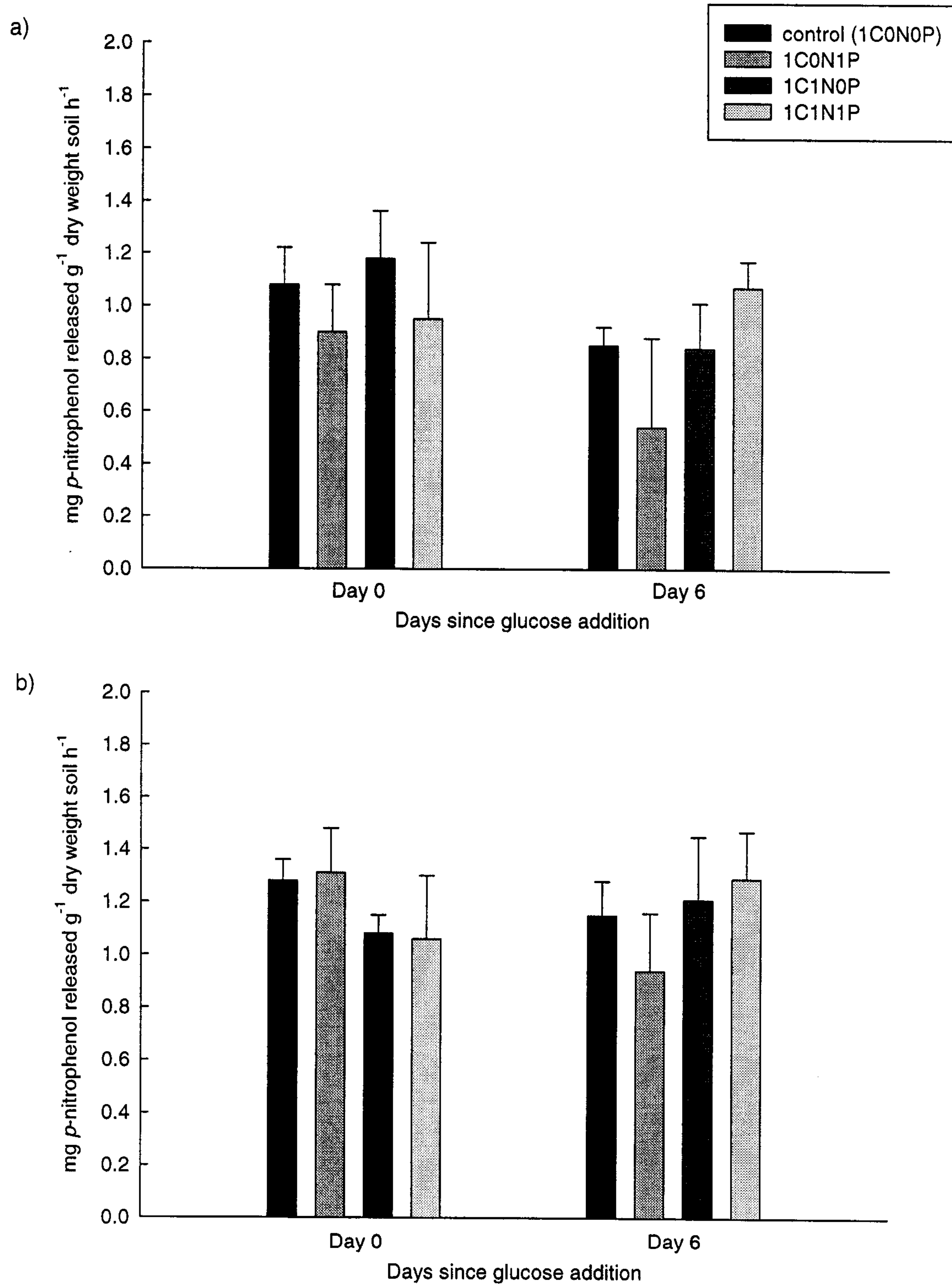


Figure 3.27 (a) Acid and (b) alkaline phosphatase activites in the soil of carbon-enriched fertilization plots on the western intertidal marsh at La Pérouse Bay, Manitoba. Assays were run before glucose addition (day 0; 27 July 2003) and after six days (1 August 2003) and soils were incubated for 60 minutes. Means and standard errors (+/- SEM) are shown (n=3). There are no significant differences among treatments and across time (P>0.05).

Table 3.15 a) Multi-way ANOVAs of acid and alkaline phosphatase activities (AcidPases and AlkPases, respectively) in response to NP-fertilization and glucose addition (CNP-fertilization plots) on the western intertidal marsh at La Pérouse Bay, Manitoba during the summer of 2003.

Data	Fixed effects	Num df	Den df	F	P
log(AcidPases) (g^{-1}); α = 0.025 NP-fertilization	Time	3	58.1	2.02	0.1207
	Nitrogen	1	58.1	0.04	0.8377
	Phosphorus	1	58.1	1.82	0.1824
	Nitrogen x Time	2	58.1	0.10	0.9019
	Phosphorus x Time	2	58.1	1.07	0.3489
	Nitrogen x Phosphorus	1	58	0.00	0.9495
log(AlkPases) (g^{-1}); α = 0.025 NP-fertilization	Time	3	57	2.60	0.0609
	Nitrogen	1	57	0.09	0.7667
	Phosphorus	1	57	0.22	0.6390
	Nitrogen x Time	2	57	0.59	0.5582
	Phosphorus x Time	2	57	0.57	0.5693
	Nitrogen x Phosphorus	1	57	0.08	0.7726
log (AcidPases) (cm^{-3}); α = 0.05 NP-fertilization	Time	3	60	0.10	0.9608
	Nitrogen	1	60	0.37	0.5444
	Phosphorus	1	60	0.99	0.3247
	Nitrogen x Time	2	60	0.43	0.6552
	Phosphorus x Time	2	60	0.29	0.7480
	Nitrogen x Phosphorus	1	60	0.07	0.7889
log(AlkPases) (cm^{-3}); α = 0.05 NP-fertilization	Time	3	58	2.02	0.1208
	Nitrogen	1	58	0.06	0.8095
	Phosphorus	1	58	0.05	0.8221
	Nitrogen x Time	2	58	0.53	0.5941
	Phosphorus x Time	2	58	0.76	0.4728
	Nitrogen x Phosphorus	1	58	0.15	0.7033
log (AcidPases) (mL^{-1} soil solution; α = 0.05 NP-fertilization	Time	3	60	0.95	0.4217
	Nitrogen	1	60	0.42	0.5174
	Phosphorus	1	60	0.69	0.4091
	Nitrogen x Time	2	60	0.20	0.8175
	Phosphorus x Time	2	60	0.12	0.8906
	Nitrogen x Phosphorus	1	60	0.70	0.4076
log (AlkPases) (mL^{-1} soil solution) α = 0.05 NP-fertilization	Time	3	58	2.60	0.0608
	Nitrogen	1	58	0.09	0.7698
	Phosphorus	1	58	0.26	0.6148
	Nitrogen x Time	2	58	0.26	0.7695
	Phosphorus x Time	2	58	0.26	0.7731
	Nitrogen x Phosphorus	1	58	1.46	0.2318
AcidPases: AlkPases (g^{-1}); α = 0.05 NP-fertilization	Time	3	58	0.54	0.6597
	Nitrogen	1	58	0.62	0.4340
	Phosphorus	1	58	1.83	0.1812
	Nitrogen x Time	2	58	0.79	0.4579
	Phosphorus x Time	2	58	0.62	0.5441
	Nitrogen x Phosphorus	1	58	0.65	0.4243
AcidPases (g^{-1}); α = 0.05 CNP-fertilization	Time	1	17	2.06	0.1689
	Nitrogen	1	17	1.42	0.2502
	Phosphorus	1	17	0.73	0.4045
	Nitrogen x Time	1	17	0.41	0.5314
	Phosphorus x Time	1	17	0.32	0.5813
	Nitrogen x Phosphorus	1	17	0.76	0.3951
log(AlkPases) (g^{-1}); α = 0.05 CNP-fertilization	Time	1	15	0.15	0.7005
	Nitrogen	1	15	0.01	0.9180
	Phosphorus	1	15	0.31	0.5850
	Nitrogen x Time	1	15	3.51	0.0807
	Phosphorus x Time	1	15	0.04	0.8364
	Nitrogen x Phosphorus	1	15	0.29	0.5961

Table 3.15 b) Multi-way MANOVAs of acid and alkaline phosphatase activities (Pases) in soil from NP-fertilization and CNP-fertilization plots on the intertidal marsh at La Pérouse Bay, Manitoba, during the summer of 2003. * P<0.05.

Data	Plots	Effect	Den df	Num df	F	P	
log(Pases) (g^{-1})	NP-fertilization	Whole Model	8	58	2.8180	0.0484	*
log(Pases) (cm^{-3})	NP-fertilization	Whole Model	8	60	1.4218	0.2061	
log(Pases) (mL soil solution)	NP-fertilization	Whole Model	8	60	1.4218	0.2061	
log(Pases) (g^{-1})	CNP-fertilization	Whole Model	8	14	1.0936	0.4217	

3.4 Seasonal results from the NP-fertilization experiment on the western intertidal marsh from June 2003 to July 2004

3.4.1 Seasonal changes in soil microbial C, N and P in response to NP-fertilization

In order to examine seasonal variation in soil microbial immobilization of C, N and P in response to NP-fertilization, results from early spring (2 June), mid-summer (14 June), late fall (25 October) 2003, late spring (19 June), early summer (29 June), and early summer (9 July) 2004 were compared (Figure 3.28, Table 3.16). All data were log-transformed, except the microbial C:N ratio, which was normally distributed. Multi-way ANOVAs of MBC, MBN and MBP indicate a significant effect of time (MBC: $F_{6, 53} = 12.19$, P <0.0001; MBN: $F_{6, 55} = 15.50$, P <0.0001; MBP: $F_{6, 51,1} = 18.70$, P <0.0001). Closer examination of the data reveals a peak in MBC (a mean of 4.4 mg C g^{-1}) and MBP (a mean of 45.1 µg P g^{-1}) when the soil is frozen in October 2003. Microbial N peaked at a mean of 410 µg N g^{-1} June 2004. Despite the seasonal changes in MBC and MBN, there were no changes among treatment subplots within a single sampling date. There was a significant effect of P-fertilization on soil microbial immobilization of P ($F_{1, 51} = 11.81$, P = 0.0012), due to high average MBP in mid-summer 2003, but this does not correspond to a general seasonal trend.

Atomic ratios of microbial C:N, C:P and N:P are shown in Figure 3.29 and results of the ANOVAS are given in Table 3.16. Results are similar to what we would expect given the data for MBC, MBN and MBP, as indicated above. MB C:N, MB C:P and MB N:P vary seasonally and peak in October 2003, May – June 2004 and July 2003, respectively. MB N:P peaked in July 2003 because of low microbial P at that time. The P effects for MB C:P ($F_{1, 44.3} = 22.18$, P <0.0001) and MB N:P ($F_{1, 44} = 19.60$, P <0.0001) are due to differences on only a few sampling days, but they do not constitute an overall seasonal trend.

3.4.2 Seasonal changes in soil phosphatase activities in response to NP-fertilization

Acid and alkaline phosphatase activities also significantly increased after the ground was frozen (AcidPases: $F_{5, 54} = 23.82$, P <0.0001; AlkPases: $F_{5, 51,1} = 13.82$, P <0.0001) (Figure 3.30, Table 3.17). AcidPase activities were highest in early spring (June 2004) at a mean of 2.7 mg *p*-nitrophenol released per gram dry weight of soil per hour. AlkPase activities also peaked in June 2004 at a mean of 2.5 mg *p*-nitrophenol released per gram dry weight of soil per hour. Although AcidPases also responded to N-fertilization over time, the general response of AcidPases to N-fertilization does not follow a similar pattern.

From June 2003 to July 2004, ratios of AcidPase and AlkPase activities changed (MANOVA: $F_{7, 62} = 2.917$, P =0.0105), so that Bonferroni-type adjustments ($\alpha = 0.025$) were made to the multi-way ANOVAs of AcidPase and AlkPase activities that are described above (Figure 3.31, Table 3.17). ANOVA of the ratio of activities of AcidPase to AlkPase reveals a significant effect of N-fertilization ($F_{5, 52.1} = 4.56$, P =0.0375), but again, this effect is not consistent over time.

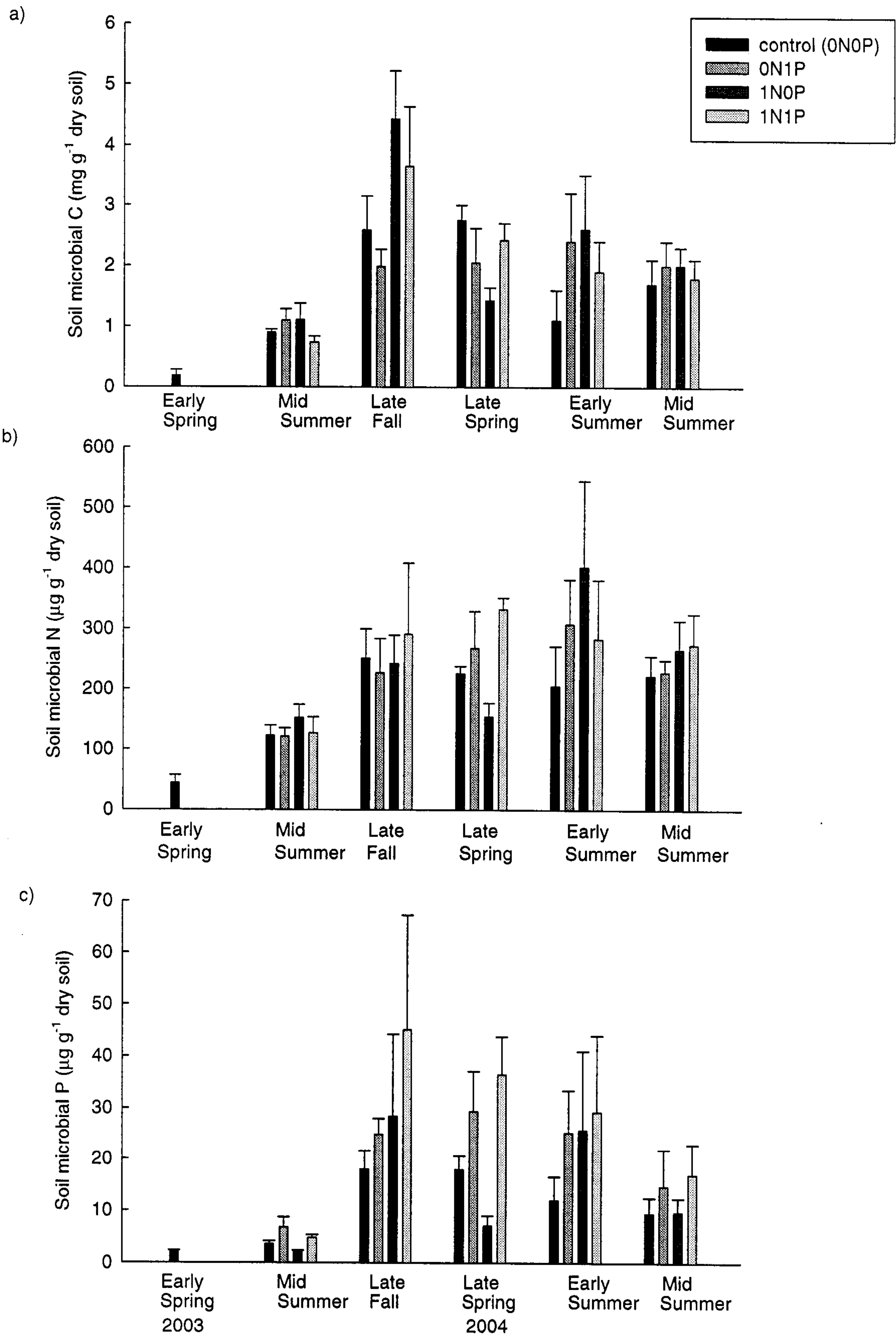

a)
Soil microbial C (mg g^{-1} dry soil)
0
1
2
3
4
5
6
control (0N0P)
0N1P
1N0P
1N1P
Early Spring
Mid Summer
Late Fall
Late Spring
Early Summer
Mid Summer
b)
Soil microbial N (µg g^{-1} dry soil)
0
100
200
300
400
500
600
Early Spring
Mid Summer
Late Fall
Late Spring
Early Summer
Mid Summer
c)
Soil microbial P (µg g^{-1} dry soil)
0
10
20
30
40
50
60
70
Early Spring
2003
Mid Summer
Late Fall
Late Spring
2004
Early Summer
Mid Summer

Figure 3.28 Seasonal changes in soil microbial (a) carbon (MBC), (b) nitrogen (MBN) and (c) phosphorus (MBP) in control and fertilized subplots on the western intertidal marsh at La Pérouse Bay, Manitoba between 3 June 2003 and 9 July 2004. Soil was collected at rooting depth (5cm). Means and standard errors (+/- SEM) are shown (n=3). Final calculations of MBC, MBN and MBP were corrected for using extractability factors $k_{EC} = 0.35$, $k_{EN} = 0.40$ and $k_{EP} = 0.40$, respectively, and a P fixation factor, $k_{fix} = 0.77$.

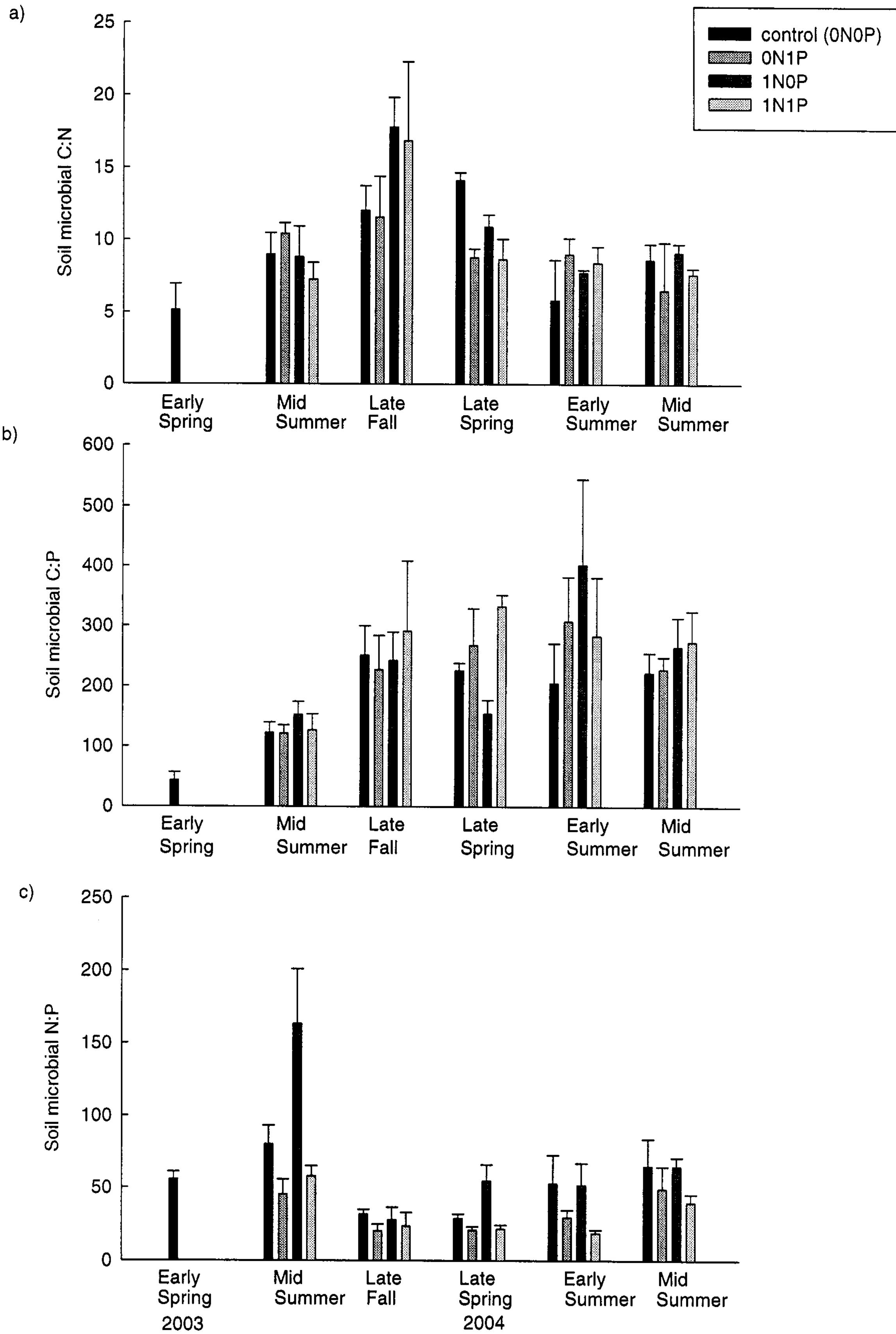

a)
25
20
15
10
5
0
Soil microbial C:N
control (0N0P)
0N1P
1N0P
1N1P
Early Spring
Mid Summer
Late Fall
Late Spring
Early Summer
Mid Summer
b)
600
500
400
300
200
100
0
Soil microbial C:P
Early Spring
Mid Summer
Late Fall
Late Spring
Early Summer
Mid Summer
c)
250
200
150
100
50
0
Soil microbial N:P
Early Spring 2003
Mid Summer
Late Fall
Late Spring 2004
Early Summer
Mid Summer

Figure 3.29 Seasonal changes in soil microbial (a) C:N, (b) C:P and (c) N:P in control and fertilized subplots on the western intertidal marsh at La Pérouse Bay, Manitoba between 3 June 2003 and 9 July 2004. Soil was collected at rooting depth (5cm) and ratios were calculated on an atomic basis. Means and standard errors (+/- SEM) are shown (n=3).

Table 3.16 Multi-way ANOVAs of soil microbial C, N and P in response to NP-fertilization on the western intertidal marsh at La Pérouse Bay, Manitoba from June 2003 to July 2004. ** P<0.05; *** P<0.0001.

Data	Fixed effects	Num df	Den df	F	P	
log(Microbial C) (g^{-1})	Time	6	53	12.19	<0.0001	***
	Nitrogen	1	53	1.24	0.2696	
	Phosphorus	1	53	0.02	0.8966	
	Nitrogen x Time	5	53	0.96	0.4524	
	Phosphorus x Time	5	53	0.65	0.6624	
	Nitrogen x Phosphorus	1	53	0.70	0.4057	
log(Microbial N) (g^{-1})	Time	6	55	15.50	<0.0001	***
	Nitrogen	1	55	1.27	0.2644	
	Phosphorus	1	55	0.10	0.7571	
	Nitrogen x Time	5	55	0.20	0.9621	
	Phosphorus x Time	5	55	0.99	0.4335	
	Nitrogen x Phosphorus	1	55	0.01	0.9095	
log(Microbial P) (g^{-1})	Time	6	51.1	18.70	<0.0001	***
	Nitrogen	1	51	0.00	0.9618	
	Phosphorus	1	51	11.81	0.0012	**
	Nitrogen x Time	5	51	1.07	0.3877	
	Phosphorus x Time	5	51	1.40	0.2413	
	Nitrogen x Phosphorus	1	51	0.56	0.4573	
Microbial C:N	Time	6	53	3.90	0.0027	**
	Nitrogen	1	53	0.00	0.9478	
	Phosphorus	1	53	0.01	0.9262	
	Nitrogen x Time	5	53	0.55	0.7388	
	Phosphorus x Time	5	53	1.14	0.3531	
	Nitrogen x Phosphorus	1	53	0.17	0.6817	
log(Microbial C:P)	Time	6	44.4	8.45	<0.0001	***
	Nitrogen	1	44.9	0.25	0.6177	
	Phosphorus	1	44.3	22.18	<0.0001	***
	Nitrogen x Time	5	44.3	0.39	0.8500	
	Phosphorus x Time	5	44.3	1.20	0.3243	
	Nitrogen x Phosphorus	1	44.5	2.79	0.1018	
log(Microbial N:P)	Time	6	44.1	9.79	<0.0001	***
	Nitrogen	1	44.1	0.51	0.4782	
	Phosphorus	1	44	19.60	<0.0001	***
	Nitrogen x Time	5	44	0.99	0.4320	
	Phosphorus x Time	5	44.1	0.66	0.6588	
	Nitrogen x Phosphorus	1	44.1	1.57	0.2166	

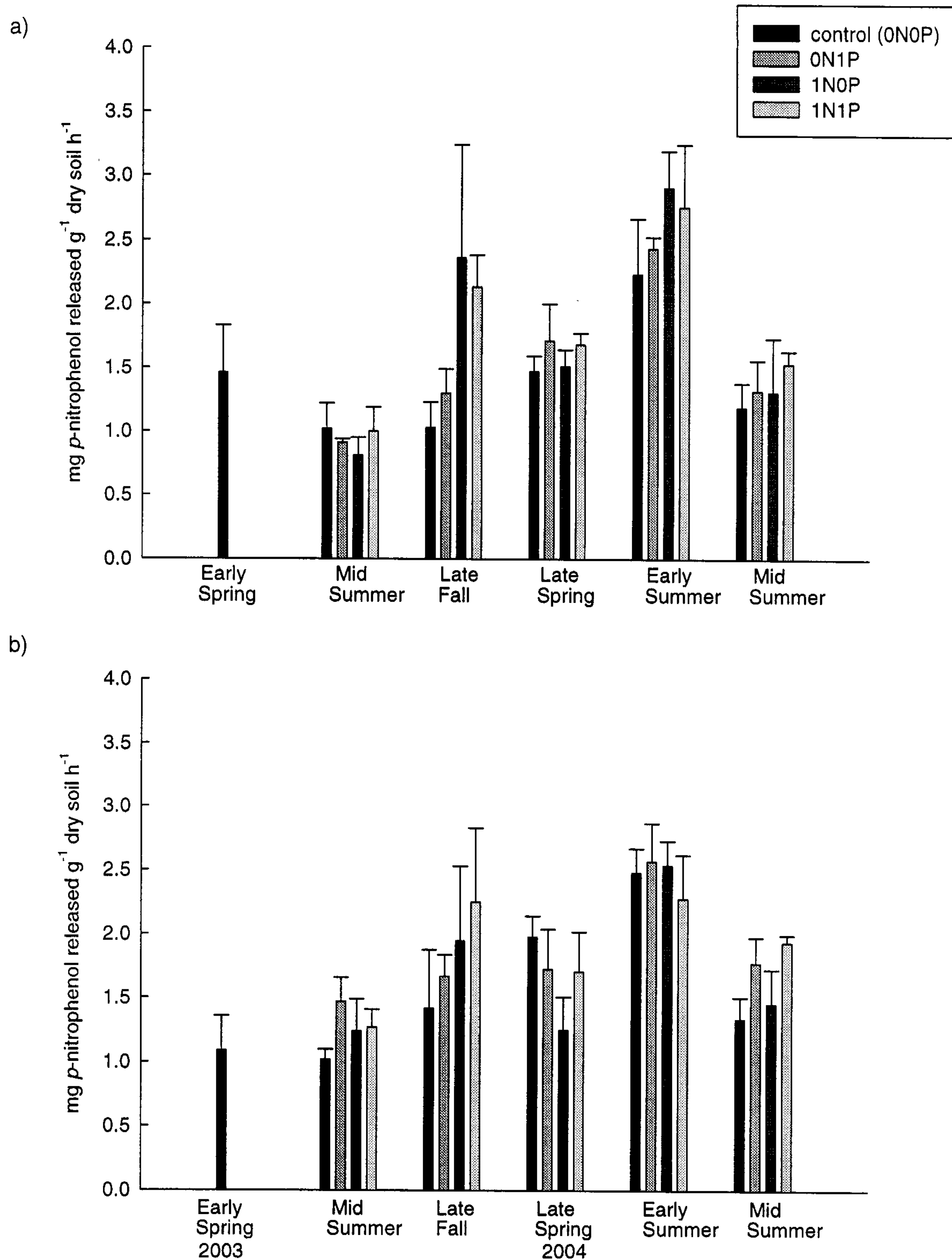


Figure 3.30 Seasonal changes in (a) acid and (b) alkaline phosphatase activites from control and fertilized plots on the western intertidal marsh at La Pérouse Bay, Manitoba. Activities were measured in early summer (3 June), mid-summer (14 July), late fall (25 October) 2003, and late spring (19 June), early summer (29 June) and mid-summer (9 July) 2004. Soils were incubated for 60 minutes. Means and standard errors (+/-SEM) are shown (n=3).

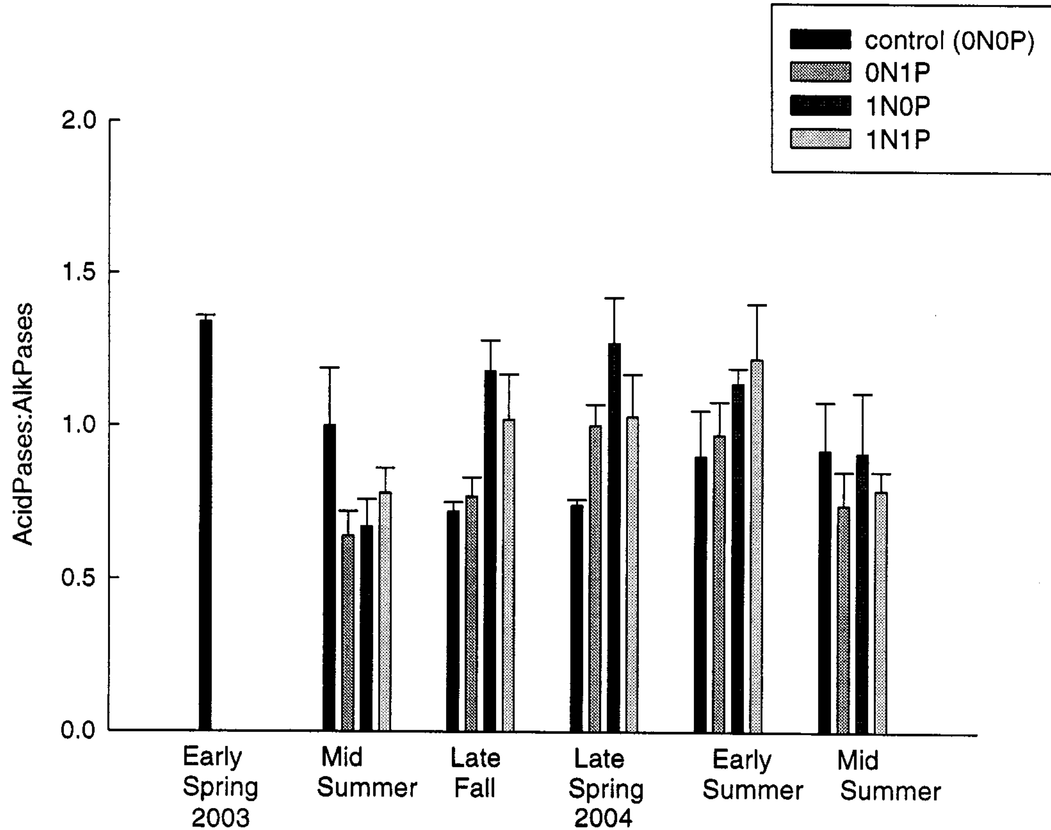


Figure 3.31 Seasonal changes in the ratio of acid phosphatase activities (AcidPases) and alkaline phosphatase activities (AlkPases) in fertilized and control plots on the western intertidal marsh at La Pérouse Bay, Manitoba from June 2003 to July 2004. Soils were collected from the top 5 cm of the soil profile and incubated for 60 minutes. Means and standard errors (+/-SEM) are shown (n=3).

Table 3.17 Multi-way MANOVA and ANOVAs of acid and alkaline phosphatase activities (AcidPases and AlkPases, respectively) in response to NP-fertilization on the western intertidal marsh at La Pérouse Bay, Manitoba from June 2003 to July 2004. * P<0.05; *** P<0.0001.

Analysis	Data	Fixed effects	Num df	Den df	F	P	
MANOVA	log(Pases) (g^{-1})	Whole Model	7	62	2.917	0.0105	*
ANOVA	log(AcidPases) (g^{-1})	Time	5	54	23.82	<0.0001	***
	α = 0.025	Nitrogen	1	54	6.33	0.0148	*
		Phosphorus	1	54	1.73	0.1934	
		Nitrogen x Time	4	54	3.35	0.0160	*
		Phosphorus x Time	4	54	0.11	0.9797	
		Nitrogen x Phosphorus	1	54	0.08	0.7743	
ANOVA	log(AlkPases) (g^{-1})	Time	5	51.1	13.82	<0.0001	***
	α = 0.025	Nitrogen	1	51.1	0.41	0.5260	
		Phosphorus	1	51	3.14	0.0825	
		Nitrogen x Time	4	51	2.03	0.1036	
		Phosphorus x Time	4	51	0.69	0.6020	
		Nitrogen x Phosphorus	1	51	0.05	0.8282	
ANOVA	AcidPases: AlkPases	Time	5	52.1	2.23	0.0649	
	α = 0.05	Nitrogen	1	52.1	4.56	0.0375	*
		Phosphorus	1	52.1	0.44	0.5118	
		Nitrogen x Time	4	52.1	2.32	0.0693	
		Phosphorus x Time	4	52	0.40	0.8057	
		Nitrogen x Phosphorus	1	52.1	0.31	0.5774	

3.5 The seasonal effect of goose grazing on the western intertidal marsh from June 2003 to July 2004

3.5.1 Soil microbial C, N and P in response to goose grazing

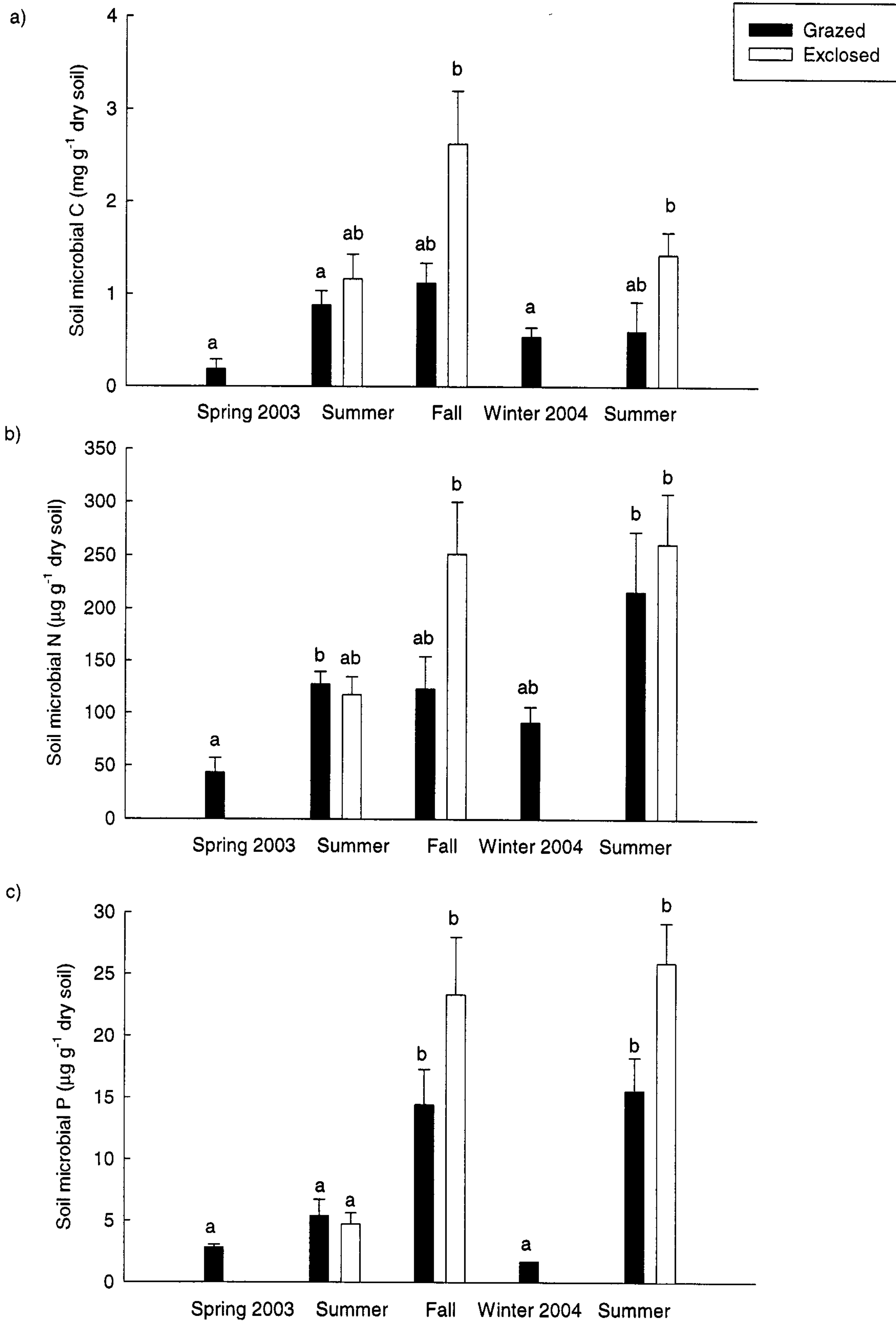
Results of microbial C, N and P measured in soil from beneath NP-fertilized exclosures and grazed, intact swards of *Puccinellia phryganodes* on the western intertidal salt marsh in July and October 2003, April and July 2004 are as follows (Figure 3.32, Table 3.18). All data are log-normally distributed. MBC, MBN and MBP significantly changed over time, and all were lowest in early June 2003. MBC in soil under exclosed (0N0P subplots) and intact sites peaks in frozen soils in October 2004 ($F_{4, 19}$ = 5.17, P =0.0054) and, although there was no significant effect of grazing ($F_{1, 19}$ = 2.72, P = 0.1155), MBC in soils under exclosed sites was higher than that in soils beneath grazed sites at this time. MBN and MBP were highest in October 2003 and in July 2004 ($F_{4, 19}$ = 7.44, P =0.0010). Similar to MBC results, MBN and MBP in soils under exclosed sites were higher (but not significantly different) than those in soils beneath grazed sites. Note that, because of a late season and low nesting densities of geese in the area, there was relatively little grazing in the summer of 2004.

There were no significant differences in atomic MB C:N in soils beneath exclosed and intact grazed sites over time ($F_{4, 19}$ = 1.54, P = 0.2328) (Figure 3.33, Table 3.18), although values did peak in October 2003. For atomic MB C:P, there was a significant effect of time ($F_{3, 19}$ = 4.44, P = 0.0188) because of a peak in MB C:P in April 2003 (winter 2004). However this effect was not apparent with Tukey-Kramer multiple comparisons on log-normal MB C:P because n=1 (Figure 3.33). Similarly, atomic MB N:P changed significantly over time ($F_{4, 19}$ = 29.25, P <0.0001) with a peak mean of 203 in April 2004. Average MB N:P in soils under exclosed and intact grazed sites on all other sampling dates was between 23 and 78.

3.5.2 Soil phosphatase activities in response to goose grazing

For both AcidPases and log-transformed AlkPases, mg *p*-nitrophenol released per gram dry weight per hour remained the same between sites and among dates, except for soils beneath grazed sites in the summer of 2003 that account for the significant treatment effects (AcidPases: $F_{1, 24}$ = 10.50, P = 0.0035; AlkPases: $F_{1, 24}$ = 18.63, P = 0.0002) (Figure 3.34, Table 3.19). Activities beneath grazed sites in the summer of 2003 peaked at a mean of 2.6 mg *p*-nitrophenol released g^{-1} of soil for AcidPase activities and at a mean of 2.9 mg *p*-nitrophenol released g^{-1} of soil for AlkPase activities, based on an incubation of 60 minutes.

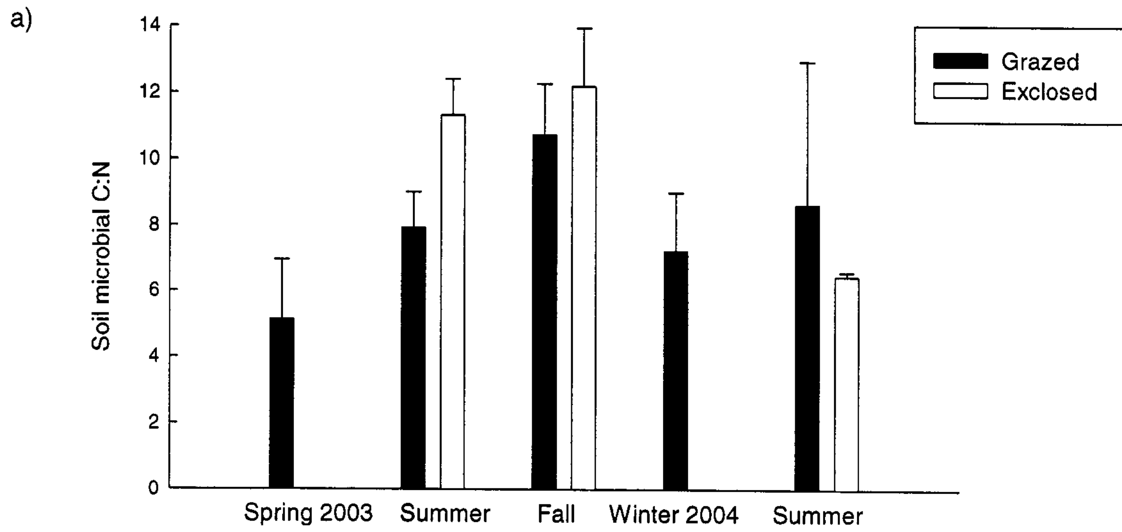
MANOVA of the effect of grazing over time on the activities of AcidPases and AlkPases was insignificant ($F_{3, 27} = 0.867$, $P = 0.4730$), indicating that the response of the two enzyme complexes to grazing over time was statistically similar. Therefore, ratios of AcidPase to AlkPase were not examined further. Note that there was little grazing in the summer 2004.



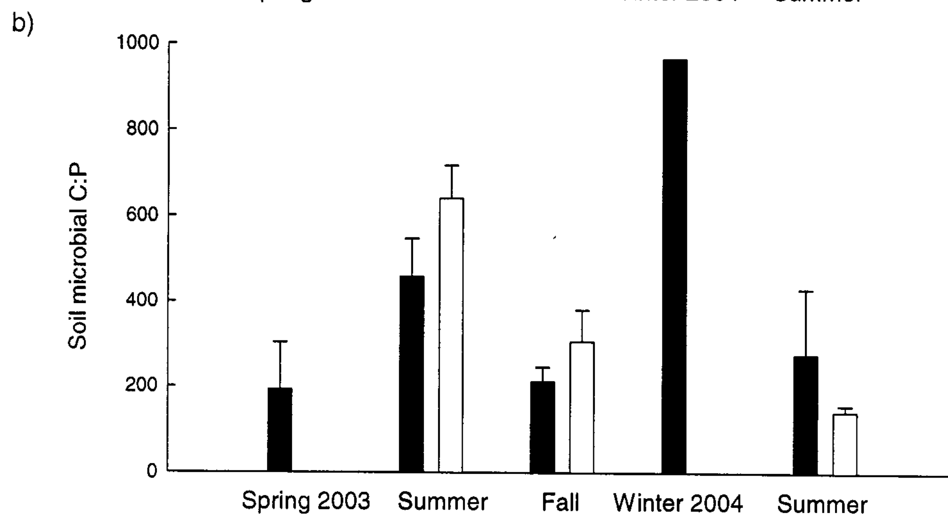
a)
Grazed
Exclosed
Soil microbial C (mg g^{-1} dry soil)
0
1
2
3
4
a
a
ab
ab
b
a
ab
b
Spring 2003
Summer
Fall
Winter 2004
Summer
b)
Soil microbial N (µg g^{-1} dry soil)
0
50
100
150
200
250
300
350
a
b
ab
ab
b
ab
b
b
Spring 2003
Summer
Fall
Winter 2004
Summer
c)
Soil microbial P (µg g^{-1} dry soil)
0
5
10
15
20
25
30
a
a
a
b
b
a
b
b
Spring 2003
Summer
Fall
Winter 2004
Summer

Figure 3.32 Soil microbial (a) carbon (MBC), (b) nitrogen (MBN) and (c) phosphorus (MBP) from soil beneath grazed and exclosed (0N0P subplots) sites on the western intertidal marsh at La Pérouse Bay, Manitoba between 3 June 2003 and 28 July 2004. Soil was collected at rooting depth (5cm). Because of a late summer, there was relatively little goose grazing in the summer of 2004. Means and standard errors (+/-SEM) are shown (n varies from 3, 4 and 5). Final calculations of MBC, MBN and MBP were corrected for using extractability factors k_{EC} = 0.35, k_{EN} = 0.40 and k_{EP} = 0.40, respectively, and a P fixation factor, k_{fix} = 0.77.

a)



b)



c)

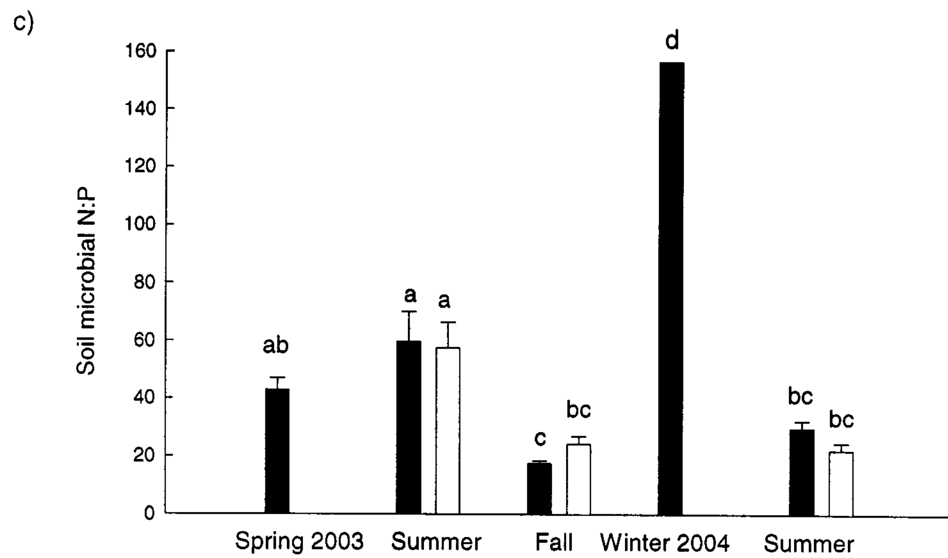


Figure 3.33 Soil microbial (a) C:N, (b) C:P and (c) N:P from soil beneath grazed and exclosed (0N0P subplots) sites on the western intertidal marsh at La Pérouse Bay, Manitoba between 3 June 2003 and 28 July 2004. Soil was collected at rooting depth (5 cm) and ratios were calculated on an atomic basis. Because of a late summer, there was relatively little goose grazing in the summer of 2004. Means and standard errors (+/-SEM) are shown (n varies from 3, 4 and 5).

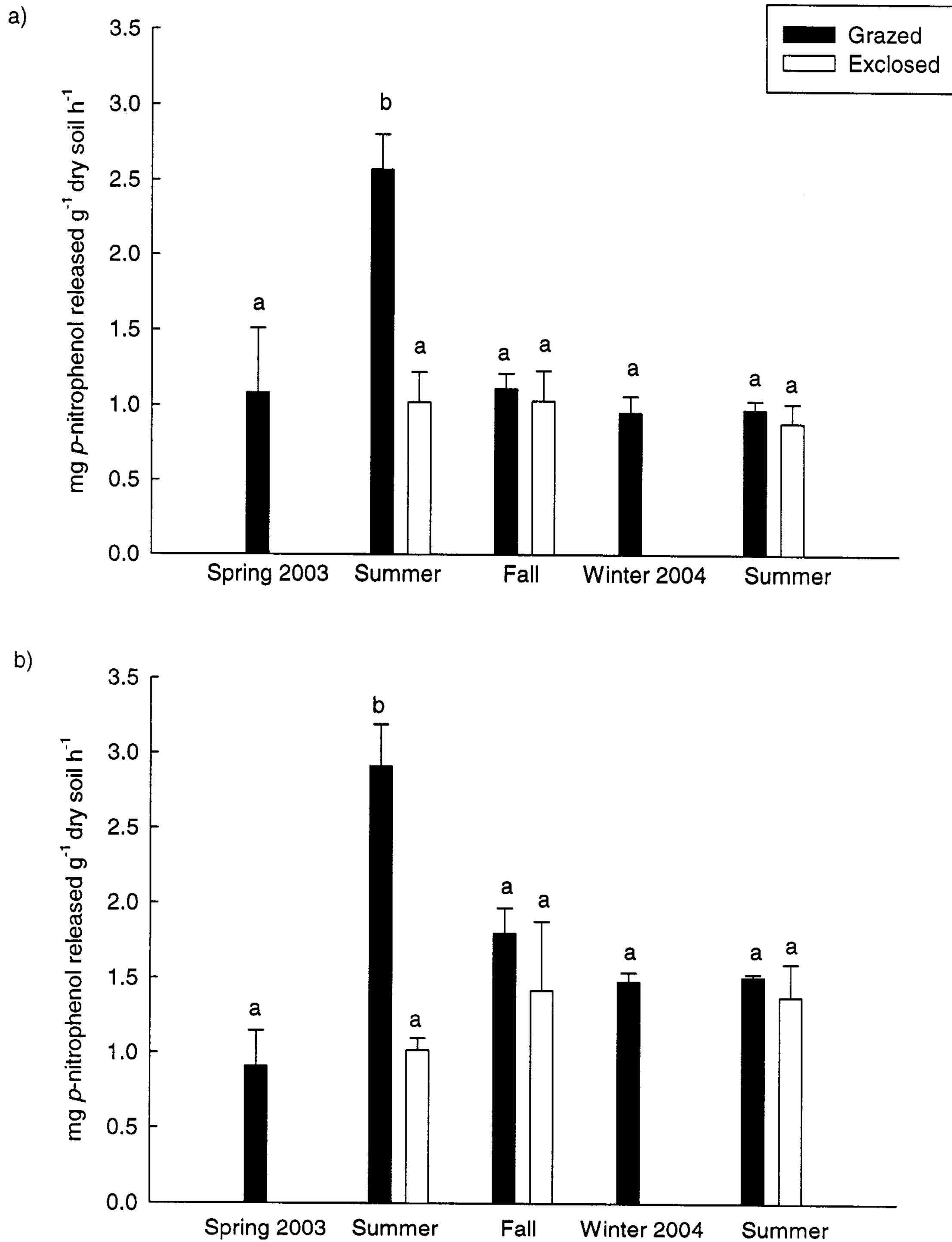


Figure 3.34 (a) Acid and (b) alkaline phosphatase activities from beneath exclosed (0N0P subplots) and grazed sites on the western intertidal marsh at La Pérouse Bay. Activities were measured on 14 July (exclosed) and 25 July (grazed), and fall (25 October) 2003, and in late winter (25 April, grazed only), and summer (28 July) 2004. Means and standard errors (+/-SEM) are given. The number of replicates varied from n=3, n=5 and n=8. Common lower case letters denote a lack of significance.

Table 3.18 Two-way factorial ANOVAs of soil microbial C, N and P from soil beneath intact and exclosed (control subplots) on the western intertidal marsh at La Pérouse Bay, Manitoba from June 2003 to July 2004. * P<0.05; ** P<0.01; *** P<0.0001.

Data	Fixed effects	Num df	Den df	F	P	
log(Microbial C) (g^{-1})	Time	4	19	5.17	0.0054	**
	Grazing	1	19	2.72	0.1155	
	Time x Grazing	2	19	0.38	0.6884	
log(Microbial N) (g^{-1})	Time	4	18	7.44	0.0010	**
	Grazing	1	18	2.86	0.1082	
	Time x Grazing	2	18	2.14	0.1465	
log(Microbial P) (g^{-1})	Time	4	15	22.33	<0.0001	***
	Grazing	1	15	3.46	0.0813	
	Time x Grazing	2	15	1.36	0.2843	
log(Microbial C:N)	Time	4	18	1.54	0.2328	
	Grazing	1	18	0.73	0.4043	
	Time x Grazing	2	18	0.08	0.9202	
log(Microbial C:P)	Time	3	16	4.44	0.0188	*
	Grazing	1	16	0.42	0.5256	
	Time x Grazing	2	16	0.34	0.7191	
log(Microbial N:P)	Time	4	15	29.25	<0.0001	***
	Grazing	1	15	0.00	0.9854	
	Time x Grazing	2	15	2.92	0.0848	

Table 3.19 Two-way factorial MANOVA and ANOVAs of acid phosphatase activities (AcidPases) and alkaline phosphatase activities (AlkPases) beneath intact and exclosed (control subplots) on the western intertidal marsh at La Pérouse Bay, Manitoba from June 2003 to July 2004.
** P<0.01.

Data	Fixed effects	Num df	Den df	F	P	
MANOVA (g^{-1})	Whole Model	3	27	0.867	0.4730	
AcidPases (g^{-1})	Time	4	24	8.58	0.0002	**
	Grazing	1	24	10.50	0.0035	**
	Time x Grazing	2	24	8.56	0.0016	**
logAlkPases (g^{-1})	Time	4	24	8.36	0.0002	**
	Grazing	1	24	18.63	0.0002	**
	Time x Grazing	2	24	6.83	0.0045	**

Chapter 4: Discussion

4.1 Variation in soil sampling

The majority of the measurements in this study were based on samples that need to be processed at several different stages (i.e. sample collection, extraction, chemical analysis) and the samples were taken from soils, which were heterogeneous at the microscale (< 1 cm) in distribution, supply rate and forms of different nutrients. Generally, heterogeneity in resource distribution in soils arises as a result of organic inputs and their subsequent microbial decomposition (Hodge 2004). The source of variation of final measurements can be confounded when the sampling processes and analytical procedures are tiered, as in this study. In order to account for soil heterogeneity, the maximum number of replicates permitted by the patchiness of intact swards on the intertidal marsh was sampled and equalled three. Because replication at the level of the whole intertidal marsh was relatively low, two replicates within each site were taken from the main plots in the NP-fertilization experiment when possible. In order to reduce variation within a single soil core, all soil was thoroughly mixed so that a representative sample of the soil from the entire rooting depth was taken. For the smaller cores (2.2 x 6 cm), a representative sample of soil was taken by hand from fresh soil from the centre of the core. In order to account for variation associated with the chemical analyses of the samples, 3 analytical replicates were measured and the mean of these replicates was used in final calculations. Samples were reanalyzed if variation among the analytical replicates was greater than 5-10% of the mean. Although the above-mentioned protocols were used to reduce the variation caused by sampling or analysis, variation of the measurements taken from soil samples, such as microbial C, N and P and phosphatase data for example, remained high. The result of this high variability was that the measurements made in this study only show major differences, so that interpretation of the results was difficult at times.

The assumption that variation within a single subplot was relatively homogeneous in comparison to other subplots was verified visually from the plant response to nutrient addition, which was uniform within subplots. Furthermore, standard errors of results of mean above-ground biomass from subplots on a single sampling day were generally low. The assumption that variation within a single exclosure was similar throughout the different subplots of the exclosure is supported by work of Jackson and Caldwell (1993a,b). In these studies, the authors used geostatistical analysis to demonstrate that within the rooting zone of an individual plant there can be as much variation in nutrient availability as within an entire 120 m^2 plot. However, the Jackson and Caldwell (1993a,b) measured variation in nutrient supplies in a

sagebrush steppe in Utah so that scaling up from soil heterogeneity at a plant-root level to the entire intertidal salt marsh at La Pérouse Bay remains unknown. Since soil heterogeneity, and thus variation in nutrient supplies, is an important factor controlling plant and microbial nutrient uptake (e.g. Schimel and Bennett 2004), variation within subplots, and within and between exclosures and sites, should be investigated more thoroughly in future studies. Geostatistical analysis, such as interpolated soil contour plots, and multivariate statistics could be used to look at changes in variation in soil physical and chemical properties at different scales, and results from these studies could greatly improve overall interpretation of the results.

4.2 Soil nutrient status

4.2.1 The effect of soil physical properties on soil nutrient status

The intertidal marsh at La Pérouse Bay is strongly influenced by the sub-Arctic/Arctic climate that results in frozen soil for about 8 months of the year. The climate and the marine environment affect soil nutrient status and soil properties, including pH and redox potential (E_h), which are used to characterize conditions under which biogeochemical processes take place (Patrick 1996). The soil pH values from the intertidal salt marsh during the summer of 2003 are nearly neutral to slightly basic and are similar to those found by Wilson and Jefferies (1996) and Ngai (2003) (Table 3.6). Redox potentials are similar to those found by Ngai (2003) on the eastern intertidal salt marsh during the summer of 2002 (Table 3.6). They are slightly lower than those characteristic of aerated soils (+400 to +700 mV) but the soils are still classified as aerobic at pH 7, and therefore are unlikely to negatively affect rates of microbial mineralization that can decrease at reduced redox potentials (+100 to -300 mV) (Patrick 1996). The redox potential of the soil also affects the solubility of iron - phosphate complexes. Ferric iron compounds, which are insoluble, are more common in well-aerated soils, while ferrous iron compounds form the dominant iron compounds in soils with lower redox potentials and are comparatively more soluble.

Salinity of the soil solution in the intertidal marsh was similar to values found by Ngai (2003) but much lower than those reported by Buckeridge (2004) in the degraded supratidal marsh (Table 3.6). Salinity can affect the availability of exchangeable phosphate. As salinity of the soil solution approaches that of seawater, the contribution of iron- and aluminum-bound phosphate to total sediment P decreases but the concentration of calcium-bound P increases (Paludan and Morris 1999). Although the concentration of sodium ions in soil solution from the intertidal marsh was lower than that of seawater during most of the study, differences in P

availability along salinity gradients suggest that less saline soils may adsorb P more tightly. This may, in part, explain the relative P-limitation of freshwater marshes (Ngai 2003). Bulk density of the soil of the intertidal marsh was higher than bulk density previously measured in soils from the freshwater marsh (Ngai 2003). The moderately packed soil of the intertidal marsh is a result of a relatively higher proportion of mineral substrates and less accumulation of organic matter. Bulk density increased slightly throughout the summer of 2003, as a result of compaction due to human and goose trampling.

4.2.2 Soil chemical properties and predictions of nutrient limitation

The nutrient status of the soil is an important factor regulating plant and microbial growth. Examining the content of N and P in different soil pools from the intertidal marsh at La Pérouse Bay provides insight into factors regulating N and P cycles and plant and microbial uptake of these elements. Results of total soil N were lower than those measured previously on the eastern intertidal marsh (Wilson and Jefferies 1996, Ngai 2003). In soil, when the C:N ratio is above 30:1, heterotrophic bacteria are N-limited and below this value they are more likely to be C-limited (Kaye and Hart 1997). Accordingly, results from analyses of total soil C and N on a mass ratio and atomic ratio basis during the summer of 2003 indicate that heterotrophic soil bacteria in intertidal soils are probably N-limited. Furthermore, net immobilization of soluble P is most likely to occur at total soil C:P ratios greater than 300:1, while net mineralization is likely to occur at below C:P ratios of 200:1(Bray and Weil 2002). The average total C:P ratio (Table 3.6, mean C:P mass ratio of 203) suggests that net immobilization of P may be occurring, which is an indication that bioavailable P may be limiting for plant and microbial growth.

Measurements of KCl-exchangeable ammonium and nitrate from the NP-fertilization experiment during the summer 2003 are consistently low and values are comparable to results from previous studies on the intertidal marsh (Bazely and Jefferies 1989b, Henry and Jefferies 2003, Buckeridge 2004). These previous studies also show a flush of ammonium (Henry and Jefferies 2003, Buckeridge 2004) and nitrate ions (Buckeridge 2004) at post-thaw in early spring. In this study, concentrations of ammonium and nitrate in 2M KCl-extracts were variable among treatment plots but they remained low during the summer of 2003 (Figure 3.11 and 3.12). Ammonium and nitrate concentrations are positively, but weakly correlated (Table 3.10). At this site, concentrations of ammonium and nitrate in soil solution constitute less than 10% each of the total soil soluble N mid-summer (Henry 2003).

During the early and mid-growing season when there was a rapid increase in plant above-ground biomass production, extractable phosphate concentrations in the soil were low

(Figure 3.9 and 3.10). After this initial and rapid growth of above-ground biomass, measurements of total extractable soil P peaked in the middle of the growing season (17 July 2003), both in control (0N0P) and NP-fertilized subplots, indicating that the increase in extractable P is not due to the second fertilization addition on 10 July 2003. No clear peak in soil extractable P was detected using Olsen's solution. Both Bray's- and Olsen's extractable phosphorus values were higher than values measured previously on the eastern intertidal marsh (Ngai 2003). However, the results are lower than the range of values found using Olsen's extractable P in temperate salt marshes (15 - 200 μg P g^{-1} (Hazelden and Boorman 1999)) and lower than KCl- extractable P measured in an alpine wet meadow (around 75 μg g^{-1}, Bowman et al. 1993). Bray's- and Olsen's extractable P accounted for approximately 2 - 6% of the total soil P, which was higher than the percentage found in soils in *Carex*-dominated northern wetlands in Alaska (0.7%) (Giblin et al. 1991). Results of Bray's extractable P were consistently higher (around 10 μg P g^{-1} dry weight of soil higher) than Olsen's extractable P, and this is in contrast to results from Ngai (2003). The differences between the two studies are likely due to differences in the ratio of soil to extractant. A lower soil to extractant ratio was used in her study suggesting that the extractants may be saturated at the lower ratio (1:5). In addition, the mineral composition of the intertidal soils was different. The sand fraction is greater in the eastern intertidal marsh compared to the western intertidal marsh, which has a higher clay content (Jefferies et al. 1979; Jefferies, unpublished). Two grams fresh weight were extracted with 40 mL Olsen's extract buffered at pH 8.5, while Bray's extractant was used in a 1 gram fresh weight to 35mL extract ratio in order to maintain a pH level <2.9 during the extraction for better P extraction (Kuo 1996). As a result of the low soil to extractant ratio, the average values were generally in the high category of extractable P for both Bray's (values exceeding 30 μg P g^{-1} are high) and Olsen's (values around 10 μg P g^{-1} are high) (Kuo 1996). Although both solutions extracted P in similar amounts, the correlation between Olsen's and Bray's extractant solutions was weak (Table 3.10).

Moreover, the lack of response of extractable P to P-fertilization suggests that the extractants used in this study were not ideal for extraction P from the intertidal soil. Bray's extractant is best on acid soils, as the fluoride present in Bray's solution complexes with aluminum, thereby increasing P-Al desorption. Olsen's extractant is most often used on calcareous because it increases the solubility of calcium phosphates by promoting the precipitation of $CaCO_3$. However, the intertidal soil during the summer of 2003 was near neutral and below the pH at which calcium carbonate should be found in excess (pH 7.6) (Thomas 1996). This soil-extractant incompatibility could, in part, account for the lack of response of

extractable P to P-fertilization. Future studies should test the response of the intertidal soils to P application. This could be done in the laboratory at the University of Toronto by applying inorganic phosphate at varying concentrations and then extracting immediately with various extractants appropriate to the soil pH, and at a variety of soil to extractant ratios. The extracting solution and extractant to soil ratio with the best P-recovery should be chosen as the extractant used in field studies. An additional extractant that could be tested is the Mehlich-1 P test, which is comprised of dilute concentrations of strong acids (H_2SO_4 and HCl) and which extracts large amounts of nonlabile P in non-calcareous soils that have pH greater than 6.0 (Kuo 1996).

Organic P (soluble and insoluble) usually constitutes the majority of the total soil P. This is especially true in Arctic soils where rates of mineralization are low and the organic content of soils is often high. Although P is not necessarily biologically available when present as soluble organic P, the size of the soluble organic P pool is still important because it represents the potential amount of P available for mineralization within the system. The only reported value for tundra soil solution organic phosphorus concentrations is the study by Barèl and Barsdate (1978), who measured DOP and found it to be 23 times higher than inorganic P in the soil solution. This is in comparison to fertilized agricultural land in Argentina where Olsen's extracted organic P was only slightly higher than Olsen's extracted inorganic P (Picone et al. 2003). In this study, DOP in the soil solution was measured for the first time at La Pérouse Bay and values were unexpectedly lower than the results of total extractable P by an order of magnitude, and there was no response of DOP to NP-fertilization (Figures 3.8 and 3.9). DOP may not be as high as previously reported in the Alaskan tundra because the study site is coastal and therefore soil nutrient content is influenced by tidal activity. This could increase leaching of soluble organic P, which is very mobile because it is not so readily adsorbed by organic-rich clays as P_i (Brady and Weil 2002). The method used (acid persulfate oxidation) is both the original method developed for seawater extraction of soluble organic P (Koroleff 1983) and a standard method in ecological studies (Lajitha et al. 1999). It was chosen because percent recovery, although not as great as that of high temperature oxidation (Ormaza-González 1996), is high and ranges from around 80 -95% (Cembella 1986, Sharp 2002). Possible reasons for these results for DOP could be one or several of the following. Several procedures call for DOP oxidation within two hours of sampling (Koroleff 1983), however Ridal and Moore (1990) found no significant difference among total dissolved orthophosphate and total dissolved phosphorus in samples tested immediately after sampling, one week and 5 months after collection. Another procedure that could have contributed to an underestimation of DOP in soil solutions is that the samples were not acidified before they were frozen.

Acidification of samples before storage is called for in many DOP procedures (Koroleff 1983, Ridal and Moore 1990), presumably to maintain solubility of organic P particles in solution and to limit microbial activity. As a precaution, soil solutions should be acidified before they are frozen for storage. Other common problems with DOP analysis involve interference by silicate and arsenate with the formation of a blue colour-complex in the molybdate-blue method, and subsequent overestimation of dissolved P in a sample. However, neither arsenate nor silicate is present in significant concentrations in the soil solution. Future studies should measure organic P as the increase in inorganic P in Olsen's extracts after nitric and perchloric acid digestion (Shaw 1959), and as the total organic P of dried soil as measured after extraction with 1N H_2SO_4 (Saunders 1955, Picone et al. 2003). Values of soluble organic P based on a variety of methods would clarify whether acid persulfate oxidation is reliable. Although negatively correlated, there are no strong or significant trends in the relationship between extractable P_i and DOP (Table 3.10). Results of total extractable P and DOP will be discussed in light of phosphatase activities in sections 4.3.2 and 4.5.3.

It is generally believed that N-availability is more limiting to primary productivity in marine ecosystems, whereas P-availability is more limiting in freshwater habitats (Pomeroy 1970, Smith 1984). The results of the soil nutrient analyses in the intertidal marsh appear consistent with the above assertion. Total soil N:P ratios of the total amounts of these elements were lower than those found at sites where P-limitation dominates. Soil extractable concentrations of inorganic N in control (0N0P) subplots were lower than soil extractable phosphate in control (0N0P) subplots, further indicating that vegetation fertilized on the intertidal marsh will respond more to additions of inorganic nitrogen than to inorganic phosphate. Furthermore, values of soil exchangeable nitrate in control subplots did not change throughout the summer of 2003 (Figures 3.11 and 3.12), in contrast to soil extractable P which increased slightly in control subplots (Figures 3.9 and 3.10, Table 3.9), suggesting that nitrate and ammonium ions were in high demand throughout the summer. High concentrations of inorganic P and low values of organic P led to the prediction that the response of soil phosphatase activities to fertilization were unlikely to be large.

4.3 The use of the soil phosphatase assay at La Pérouse Bay, Manitoba

4.3.1 Testing the factors affecting the soil phosphatase assay

Factors affecting the soil phosphatase assay will be discussed first followed by a discussion on the use of the soil phosphatase assay at La Pérouse Bay. At La Pérouse Bay,

soil was assayed for acid phosphatase activity at pH 6.5 and for alkaline phosphatase activity at pH 10.5 (Figure 3.1, Table 3.1). Choice of buffer pH in this study is consistent with other studies that showed that maximal enzymatic hydrolysis of *p*-Nitrophenyl phosphate occurred at or near neutral pH 6.2 – 7.0 (Tabatabai 1969). A maximal pH of 10.5 for the assay of alkaline phosphatase activities is in between the range of pH values suggested by Jansson (1988) (pH 9 – 10) and the pH value outlined in the general assay procedure (pH 11) (Tabatabai 1994). Although maximal pH for the hydrolysis of acid phosphatases on the root surface of *Puccinellia phryganodes* were not explicitly measured, buffering the roots at pH 6.5 was consistent with the idea that root surface phosphatases should have maximal activity at pH values around the prevailing soil pH (Kroehler and Linkins 1988), which was approximately a pH of 7.4. Because the soil pH on the intertidal salt marsh is intermediate to the optimal pH values of AcidPases and AlkPases complexes, secretion of Pases by both plants and soil microbes should not be affected by the pH of the environment. Pase enzymes are still active at non-optimal pH values (Jansson et al. 1988) and plant are able to acidify the rhizosphere through the release of organic acids. The correlation between AcidPases and AlkPases was relatively strong and significant (Table 3.10). Other assumptions of the assay, including that the reaction is linear with respect to time (Figure 3.2) and that diluting samples does not affect final concentrations of *p*-nitrophenol released (Figure 3.3), were tested for a single sample for AcidPases only, but the results are consistent with previous studies (Tabatabai 1969, Kroehler and Linkins 1988, Tabatabai 1994, Johnson et al. 1999).

Generally, apparent K_m values for AcidPases and AlkPases are within the range of 1.3 to 4.5 mM substrate and 0.4 to 4.9 mM substrate, respectively (Tabatabai 1994). Apparent K_m values at La Pérouse Bay during the summer of 2003 are at the high end of this range, and are 4.59 mM *p*-Nitrophenyl phosphate (*p*-NPP) at 37°C for AcidPases and 4.93 mM *p*-Nitrophenyl phosphate at 37°C for AlkPases (Table 3.2), indicating that the phosphatase enzymes in the soil have a relatively low substrate affinity compared to Pase enzymes elsewhere. The corresponding values of apparent V_{max} are 0.785 mg *p*-nitrophenol released g^{-1} dry soil h^{-1} for AcidPases and 1.18 mg *p*-nitrophenol released g^{-1} dry soil h^{-1} for AlkPases. The K_m values reported in this study are lower than the K_m estimated by Kroehler and Linkins (1988) for acid phosphatase on the root surface of *E. vaginatum*, which was 9.23 mM *p*-NPP. Differences in substrate affinities at different sites may be partially explained by differences in incubation temperature. For example, the considerably low substrate affinity of enzymes released by roots of *E. vaginatum* in Alaska (Kroehler and Linkins 1988) may be a result of the low incubation temperature, which was12°C (the mean air temperature at their field site during the growing

season). Furthermore, K_m values are more uniform among soils when the soil-substrate mixture is shaken than when static incubation techniques are employed, thus the K_m values obtained for phosphatases are affected by the incubation technique used. Besides procedural differences, variation in apparent K_m values is due to a variety of other factors. Several assumptions must be made when applying the Michaelis-Menten equation to soil systems, and all assumptions are based on a simple one-substrate enzyme system (Tabatabai and Dick 2002). Although the Michaelis-Menten equation is still used to describe substrate affinity of a complex of enzymes, such as the phosphatase complex, the overall apparent K_m in soil solution is based on an additive effect and will vary depending on the suite of enzymes specific to each soil. Substrate structure has a significant effect on the reaction rate, just as the structure of the product released affects its extractability from soils (Tabatabai and Dick 2002). Interactions between enzymes and their substrates will also affect enzyme kinetics. For example, the stability of phosphatases as been correlated with organic C in surface soils and soil profiles, so that enzyme activity is characterized in relation to humus-enzyme complexes (Nannipieri et al. 1988). Paul and McLaren (1975) proposed that a three-dimensional network of clay and humus complexes exists in which active enzymes become incorporated. Adsorption of phosphatases on clay minerals significantly alters their kinetic parameters (Dick 1987).

Although extracellular phosphatases are commonly measured, few studies have been conducted in Arctic soils where enzyme properties, particularly at low temperatures below the snow pack in frozen soil, should play an important role in determining rates of mineralization. One of the few studies to address the importance of changes in enzyme kinetics in tundra soils is by Kroehler and Linkins (1988). In this study, the authors tested their hypothesis that phosphatases on the root surface of *Eriophorum vaginatum* would be active at low temperatures (1°C) in order for the plant to take advantage of the flush of soluble organic P, which has been estimated to be nearly half the mean annual pool size of P in the soil solution (Chapin et al. 1978) returned to the soil at snowmelt at that site. From a plot of root surface phosphatase activity and temperature (1 to 48°C), 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, common summer soil temperatures on the Alaskan tundra where the study took place. Root incubations below freezing were not possible but tundra soil Pase activities have been measured at -25°C (Herbein 1981), so it is evident that phosphatases are active at these low temperatures which is of ecological significance in Arctic soils.

4.3.2 Ecological significance of soil phosphatase activities

The measurement of soil phosphatase activities is a relatively straight-forward method used to quantify coarse rates of phosphorus mineralization expressed as concentration of substrate released per unit soil (or per root area) per incubation time. Although inferior, the soil phosphatase assay can also act as a surrogate for rates of turnover, or plant and microbial P immobilization and mineralization, when radioactive P isotope studies are not possible. Unlike isotope studies, which trace the movement of the labelled nutrient ions into the plant, microbial and soil compartments, measurements of phosphatase activities represent a single point in time and must be interpreted with caution. Measurements of phosphatase activities depend on plant and microbial P demand, the amount of organic phosphate (substrate) that can be hydrolyzed in the soil and the concentration of enzymes present in the soil, which in turn depend on a variety of factors, including the amount of orthophosphate already available in the soil solution, plant and microbial energy budgets, and rates of soil nutrient cycling. It is difficult to determine whether decreased phosphatase activities in soil solution, for example, are an indication of reduced phosphatase release because of sufficient inorganic P (or C and N) supply, or if they are an artifact of a decrease in the amount of DOP available for hydrolysis even though biological demand may be high.

Generally, an increase in Pase activities is indicative of P-limitation in some component of the system. For example, a comparison of the N-limited salt marsh and the P-limited freshwater marsh (Ngai and Jefferies 2004) demonstrates that phosphatase activities can act as an indicator of P-limitation of the system (Figure 3.7). Based on the fact that AcidPases (excreted by plants and soil microbes) but not AlkPases (excreted only by microbes, Figures 3.5 and 3.6) increased in soils from the freshwater marsh, it is clear that plants are P-limited in this system. However, whether or not plants and/or soil microbes immobilize the hydrolyzed P_i is, however, impossible to determine without the use of isotope tracers. Nonetheless, most investigators assume that an increase in Pase activity corresponds to an increase in P demand or an increase in the ability of the plant to produce AcidPases, as a result of N-fertilization, for example (Treseder and Vitousek 2001). This assumption is valid if the DOP pool is never limiting. However, Kroehler and Linkins (1988) found that the substrate concentration at which AcidPases of *Eriophorum vaginatum* were saturated was higher than the average DOP concentration in the same system. This is likely because organic phosphate compounds appear in the soil solution in pulses, so that a K_m of an enzyme complex larger than the average substrate concentration is geared to these seasonal pulses of substrate (Price and Stevens 1982). Similarly, the enzymatic saturation level of substrate *p*-NPP measured in this study,

about 50 µmol, is much higher than the average DOP in the soil. AlkPases were positively correlated to concentrations of DOP in soil solution, but this correlation was not significant (Table 3.10). There was no clear relationship between AcidPases and DOP in soil solution (Table 3.10). Although concentrations of inorganic P in the soil solution are less than the concentration at which phosphatases are likely to be fully inhibited as a result of a negative feedback resulting from the build-up of the concentration of the end-product (P_i). The high ratio of inorganic to organic P in soil solution may be partially inhibiting phosphatase activities at La Pérouse Bay. Correlations between extractable P_i and phosphatase activities provide little insight because the strengths of the relationships were low (Table 3.10). Moreover, the acquisition of N in an N-limited system increases allocation of energy to phosphatase release (Treseder and Vitousek 2001), so that strong N-limitation in a system may inhibit phosphatase activities. In general, correlations between concentrations of nitrate and ammonium ions in soil solution and acid and alkaline Pase activities were negative, but weak and insignificant (Table 3.10).

Apart from substrate concentrations and enzyme inhibition, comparing phosphatase activities in samples taken at different soil temperatures remains a problem. The effect of temperature in the experimental assay as compared with the actual activity in soil will be important any time the soil temperature differs from the incubation temperature of 37°C. Thus, it may not be possible to compare absolute activity measured at 37°C with the activity in soils at different temperatures, but the detection of Pase activities alone indicates that there is a demand for P in the system.

Phosphatases were measured for the first time at La Pérouse Bay in the summer of 2003. Because there were no difficulties with the procedure used or criticisms in the literature, it is evident that this assay is a useful index of the amount of phosphate mineralization via biological activity. However, the proper interpretation of Pase values remains a challenge. Studies should always test maximum activities in relation to pH values, and characterize enzyme kinetics (K_m and V_{max}). Since Pase activity in frozen soil may be particularly important but lower than when the soil is incubated at 37°, future studies of phosphatase activities at La Pérouse Bay should include experiments on temperature dependence (Q_{10}) and investigators should consider incubating soil at soil temperature in the summer following Kroehler and Linkins (1988). Further studies could also examine the effect of shaking samples during incubation on estimates of enzyme activity and enzyme kinetics (Tabatabai 1994). Since phosphatase values were so variable, it may be useful to explore multivariate discriminant analysis with soil physical

and chemical factors and microbial factors (Tscherko et al. 2003) to tease apart sources of variation.

4.4 Testing the assumptions of chloroform-fumigation-extraction for measurement of soil microbial C, N and P

4.4.1 Discussion of the general assumptions of chloroform-fumigation-extraction

Since its introduction in the 1970s, the chloroform-fumigation extraction (CFE) has become a method used to quantify many biogeochemical soil processes because it is a relatively easy way to estimate soil microbial biomass, measured as organic carbon, and soil microbial immobilization of N and P, as described in section 2.4.4. However, the method is not without criticisms, most of which concern the handful of methodological assumptions. The most important assumptions surround fumigation time, the effect of the chloroform vapour and kill-efficiency. There have also been changes made to the first “direct extraction” (Brookes et al. 1982, Brookes et al. 1985a, Brookes et al. 1985b) that must be addressed formally.

In the method proposed by Brookes and colleagues in the 1980’s, soil microbial C, N and P are calculated as the difference between chloroform-labile and non-chloroform-labile C, N and P_i in a soil and the fumigation is performed in a desiccator under a vacuum. More recently, estimates of soil microbial biomass have been made on fumigated samples at atmospheric pressure, and this modification makes the CFE method more practical in remote field locations. When compared, estimates of soil extractable C and N from samples fumigated under vacuum pressure were greater (6-7%) than soil extractable C and N estimated from samples fumigated in air-tight bottles at atmospheric pressure (Witt et al. 2000). In addition, in a previous study on the supratidal marsh at La Pérouse Bay (Buckeridge 2004), the fraction of soil microbial C and N in soil did not differ significantly under vacuum or atmospheric pressure. Further, with the use of $^{15}NH_4$-N isotope label, both methods produced the same amount of label (~50% of the amount originally injected) in the microbial biomass after one week, probably indicating that both fumigation procedures attack similar soil communities (Buckeridge 2004).

Another common modification of chloroform-fumigation-extraction (CFE) has been a reduction in incubation time from a 5-day period to a 24 hour period (Brookes et al. 1985b). Originally, it was thought that the maximum flush of microbial biomass was reached after 5 days so that a shorter incubation must assume that a constant, well-correlated proportion of the population is killed after 24 hours. This assumption has been tested by several researches (Brookes et al. 1985b, Witt et al. 2000, Haubensak 2002), and over half the soils tested required

only 1 day of fumigation, including the sub-Arctic wetland soils taken from the supratidal marsh (Buckeridge 2004). Similar to the procedural modification from vacuum to atmospheric pressure that was necessary for work in a field laboratory, in this study it was also necessary to manually shake samples frequently as opposed to continuous mechanical shaking. This final assumption was also tested by Buckeridge (2004) and results showed that frequent manual shaking provided sufficient agitation for extraction of microbial C and N equal to that obtained on a mechanical shaker at the University of Toronto laboratory. Microbial P was not tested explicitly but it is assumed, based on results of microbial C and N, that the method of shaking does not affect MBP extraction. This assumption is further supported because total extractable P was extracted efficiently using frequent manual shaking with Bray and Olsen's extractants. Although the above-mentioned assumptions were tested on soil from the supratidal marsh at La Pérouse Bay, it is valid to extrapolate the results to soils from the intertidal marsh because supratidal and intertidal soils are similar.

In addition to the methodological assumptions, one of the most important assumptions of the CFE is that the application of a standard extractability factor for incomplete extraction of microbial biomass is applicable to all soil types at all seasons. Extractability factors were originally estimated for the fumigation-incubation method (Jenkinson 1976) by adding a suite of microorganisms to non-fumigated and fumigated soils and calculating the percent recovery of C, N and P by comparing the concentrations of these nutrients in soil extracts with the known nutrient content measured after dry-ashing and digesting the microbial cultures (Brookes et al. 1982). The organisms used were culturable yeasts, fungi, actinomycetes and bacteria but they were not necessarily native to the soil fumigated, nor to the soils used in future studies in which these values have been used (Jenkinson 1976). Although extractability factors do vary (e.g. k_{EP} from Brookes et al. 1982 and Hedley and Stewart 1982), many changes to the original extractability factors (Voroney and Paul 1984, Brookes et al. 1985a,b, Cheng and Virginia 1993) have led to a general consensus in the literature that extractability factors for incomplete extraction of microbial biomass within the range of 0.35 to 0.40 are the most reasonable (Jonasson et al. 1996, Jenkinson et al. 2004). Still, changes to extractability factors have not been made in light of the evidence that the composition of microbial communities changes with season (Schadt et al. 2003), so the application of a single extractability factor to both summer and winter estimates is highly assumptive. Extractability factors calculated using soil microbial communities specific to different soil types, and that incorporate the effects of seasonal changes in microbial communities, will improve the ecological meaningfulness of this useful method. For

the most part, these improvements involve the use of genetic and molecular techniques and are beyond the scope of this study.

4.4.2 Testing the assumptions for measuring soil microbial phosphorus

Similar to extractability factors, a common assumption made for measurements of microbial phosphorus is that the large majority (over 90% after 24 hour incubation) of microbial P released during extraction and fumigation is released as orthophosphate (Brookes et al. 1982). This is presumably because phosphatase activities continue throughout incubation. This assumption has been tested by Brookes et al. (1982) in temperate soils and by Jonasson et al. (1996) in Arctic tundra soils and it is generally assumed than an extractability factor (k_{EP}) of 0.40 is valid. In addition to the correction for total microbial phosphate recovery using the extractability factor, it is also important to correct for P_i fixation by soil colloids (Brookes et al. 1982). This is done by adding a known amount of orthophosphate to soil extractions and calculating the percent recovery using Bray's or Olsen's extractants. The percent fixation specific to each soil is then incorporated into the final calculations (Brookes et al. 1982). In this study, the fixation factor was found to be 0.77 so that the percent of inorganic P that was fixed to soil colloids was found to be 23% (Table 3.5). Fixation factors for P have been reported in very few studies, despite the fact that it an important part of the MBP measurements. In relation to the values reported by Picone et al. (2003) and Brookes et al. (1982), the fixation factor of k_{fix} = 0.77 calculated in this study is comparable.

In all studies involving soil extractions, it is important to find the optimal extractant specific to each site and nutrient, as well as the optimal extractant to soil ratio (Wu et al. 2000). In the past, 0.5M K_2SO_4 has been used in CFE for estimations of microbial C and N. The first study estimating microbial P used Olsen's bicarbonate extract (Brookes et al. 1982) but since then investigators have used both Olsen's extract and potassium sulfate to extract phosphate from soils. Jonasson and colleagues exclusively use potassium sulfate for extractions of C, N and P in Arctic soils, although in one study (Jonasson et al. 1999) they later found that microbial P results were underestimated because of a low extractability of 0.5M K_2SO_4. Bowman and colleagues (1993) found that KCl was an effective extractant of inorganic P in soil solution in an alpine wet meadow and extracted around 75 μg P g^{-1} of dry soil. Differences in microbial P extractability are found in this study. A comparison of results from the NP-fertilization experiment using 0.5M K_2SO_4 extract (Figures 3.18 and 3.19) and the CNP-fertilization experiment using Olsen's bicarbonate (Figures 3.21 and 3.22) indicates that MBP results from the NP-fertilization were most likely underestimated. The underestimation is not because the

assumption that microbial P is released as orthophosphate is invalid, but rather because of the low extractability of 0.5M K_2SO_4 compared to that of 0.5M $NaHCO_3$. Furthermore, results of total extractable phosphate (Figure 3.9) reveal that Bray's extractant may provide even better estimates than Olsen's extract, which is similar to results obtained on acid soils by Wu et al. (2000). This investigation led to the measurement of microbial phosphorus for the first time at La Pérouse Bay, so the standard 0.5M K_2SO_4 extractant was used because of its applicability to a variety of soil types. Future studies should compare microbial P estimates using a variety of different extractants (e.g. 0.5M K_2SO_4, Olsen's extractant following Brookes et al. (1982), Bray's extractant following Wu et al. (2000) and refer to section 4.2.2), and optimal extract to soil ratios should be determined, since reliability and consistency of results may depend on this ratio (Wu et al. 2000).

4.5 Plant and microbial nutrient limitation

4.5.1 N and P co-limitation of primary production on the intertidal marsh

The vegetation in the intertidal salt marsh, mostly *Puccinellia phryganodes*, is most strongly limited by nitrogen, but phosphorus availability rapidly becomes limiting once the community is released from N-limitation (Figure 3.13). The response to both nitrogen and phosphorus indicates a co-limitation of primary productivity in the marsh and is consistent with previous results at La Pérouse Bay (Cargill and Jefferies 1984a, Ngai and Jefferies 2004) and results from temperate salt marshes (N- and P–limitation, Van Wijnen 1999; N-limitation Kiehl 1997). The results are expected given the soil nutrient status, which has relatively low P_i and even lower inorganic N supplies. Although the sharp increase in above-ground biomass in 1N0P and 1N1P subplots at day 44 (Figure 3.13) is coincident with the period immediately following the second nutrient addition, this increase in standing crop is less likely a direct result of the second fertilization event and more likely a result of enhanced growth as a result of soil warming due to high temperatures in the middle of the summer. Similar results were seen by Cargill and Jefferies (1984a), who added fertilizers twice monthly from mid-June to mid-August 1979 and 1980 and reported in both years a sharp increase in the standing crop in N- and NP-fertilized plots in mid- to late July. In general, the growth response to N-fertilization during the summer of 2003 is similar to the responses of *Puccinellia phryganodes* (Cargill and Jefferies 1984b, Bazely and Jefferies 1985), *Carex ramenskii* (Ruess 1997), and *Festuca rubra* (O 2003) to goose grazing and faecal deposition at moderate goose densities. This leads to the formation of grazing lawns with increased productivity.

It could be argued that the lack of response to the P-alone treatment is because the inorganic phosphate is bound to soil particles or that it is immobilized by soil microorganisms before plant uptake. The immediate growth response to N and P additions and increased root and shoot P content in samples taken from P-fertilized subplots indicate that the inorganic fertilizer added to the soil is indeed taken up by the plants (Figures 3.14 and 3.15, Table 3.12). This is an indication that plants are able to take advantage of flushes of nutrients that may occur in spring and fall, for example. Furthermore, when shoot and root pool sizes were examined on an area basis (Table 3.12) it is clear that N-fertilization increased N content in shoots and roots and that P-fertilization similarly increased P content in shoots and roots. However, the ability of the plants to acquire phosphorus was enhanced more by N-alone fertilization than by P-alone fertilization. This indicates that, in the intertidal salt marsh, plant uptake of P is first limited by N availability and then by P availability.

According to the Koerselman and Meuleman study (1996) of 45 European wetlands, there is 'critical' N:P mass ratio between 14 and 16, so that vegetation is said to be P-limited if N:P ratios are above 16 and N-limited when N:P ratios are below 14. Although not definitive, the critical N:P mass ratio of 14 can act as a general guideline and the study from which it arose is a rigorous example of how to combine the growth response of plants to fertilization with changes in N:P ratios in plant tissue (Güsewell 2004). In my study, the pattern in the response of above-ground biomass to NP-fertilization is consistent with the N:P ratios of shoots and roots (Figures 3.16 and 3.17). Throughout the summer of 2003, the average atomic N:P ratios in control subplots was in the range of 15 to 25 (approximately 6 to 11 N:P mass ratio) for shoot N:P and from 12 to 17 (approximately 5 to 8 N:P mass ratio) for root N:P, indicative of potential N-limitation as verified with the growth response to N-fertilization. These results are similar to those found by Bedford et al. (1999) in a survey of North American wetlands, who found the mean N:P mass ratios of marsh vegetation and all vascular herbs of around 10 was a consistent indicator of N-limitation within the systems. Changes in the average N:P ratios in both shoots and roots in the fertilized subplots were also consistent with N and P limitation within the control subplots. P-alone fertilization caused a reduction in shoot N:P ratio, N-alone fertilization to plots caused a large increase in shoot N:P ratio, and NP-fertilization balanced the effect of N and P fertilization.

The rapid growth response of *Puccinellia phryganodes* is because it is relatively shallow-rooted with roots concentrated within the top 3 cm of the soil profile, which facilitates root uptake of nutrients after fertilization. *P. phryganodes* also exhibits stoloniferous growth throughout the growing season, with a new leaf being produced on average once every 11 days (Bazely and

Jefferies 1989a), thus allowing for continuous growth responses throughout the growing season. Although growth was continuous over time, shoot N per gram dry weight in N-enriched subplots decreased over time but shoot P per gram dry weight in all treatment plots remained relatively static. Nutrient-use efficiency is the amount of plant biomass that is produced by a given intake of nutrient (Aerts and Chapin 2000) and is the inverse of biomass N and P "concentrations" (contents) (Chapin 1980). Hence, fertilization had the community-level effect of decreasing nitrogen-use efficiency (NUE_N) and phosphorus-use efficiency (NUE_P), as previously reported by Bowman (1994). The effect of fertilization on NUE_N and NUE_P is likely due to luxury consumption of nutrients beyond immediate demands for plant growth. Higher NUE is thought to be advantageous under conditions of low soil fertility because it allows for greater biomass production given a limited supply of nutrients (Aerts and Chapin 2000). Bowman (1994) reported higher nitrogen-use efficiency (NUE_N) in a N-limited dry meadow, as compared to a NP co-limited wet meadow, which had a higher phosphorus-use efficiency (NUE_P). Given the low concentrations of 2M KCl-exchangeable nitrate and ammonium ions in the soil solution it is not surprising that NUE_N in all subplots increased throughout the growing season. NUE_P of shoots in all subplots remained relatively constant throughout the summer of 2003. This is not a direct result of the relatively high extractable P in soil solution, but rather because of the low concentrations of ammonium and nitrate ions in the soil solution (Figure 3.11 and 3.12) since plant uptake of P is limited by N-availability.

4.5.2 Variation in response of microbial C, N and P to NP- and CNP-fertilization

The response of microbial biomass carbon (MBC) and microbial immobilization of N and P (MBN and MBP, respectively) to nutrient additions were varied. Measurements of MBC, MBN and MBP were consistent with previous results from the intertidal salt marsh (Henry and Jefferies 2003, Buckeridge 2004), but were generally lower than measurements in other tundra soils. Measurements of microbial biomass C in this study were about 0.8 mg C g^{-1} dry soil, while measurements from other studies range from 4.4 mg C g^{-1} dry soil (Jonasson et al. 2004), to between 20 and 38 mg C g^{-1} dry soil (Cheng and Virginia 1993, Jonasson et al. 1999). Schmidt et al. (2000) reported values of MBC between 5.9 to 11 mg C g^{-1} dry soil and these correlated well with estimates obtained using phospholipid fatty acid (PLFA) analysis. Global estimates of microbial nitrogen, as reported by Rosswall (1976), are approximately 4 g m^{-2}. Since then, values of MBN have ranged from 3.6 to 9.5 g N m^{-2} (Jonasson et al. 1996) and from between 0.66 (Jonasson et al. 2004) to 2 mg N g^{-1} (Jonasson et al. 1999). Results from this study are only slightly lower, with the average value during the summer of 2003 of around

0.8 mg N g^{-1}. Results of MBP, however, are much lower than values previously reported. Jonasson et al. (1996, 1999) reported values around 2.84 g P m^{-2} or approximately 0.6 mg P g^{-1} dry soil, compared to the value of 0.004 mg P g^{-1} dry soil (0.5M K_2SO_4 –extracted) and 0.04 mg P g^{-1} dry soil (0.5M $NaHCO_3$–extracted) measured for the first time on the intertidal salt marsh. Taking into account the fact that Bray's extractant is best on the intertidal marsh and extracts about 2 times more inorganic P than Olsen's extractant, and given the scenario that up to 35% of the microbial P could potentially be in organic forms and therefore not extractable without oxidation, estimates of microbial biomass are still lower than previously reported.

Results from the CNP-fertilization experiment indicate that soil microbial biomass (MBC) is limited primarily by carbon (Figure 3.21). In contrast, results from the NP-fertilization experiment indicate that microbial C, N and P are not primarily limited by additions of N and P (Figure 3.18). Soil microbial N:P ratios did not significantly increase over time (Figure 3.20), implying that the relative N and P demand by the soil microbial community did not change and that the microbial turnover of these nutrients may be high. Moreover, microbial biomass (MBC) increased throughout the summer as temperatures increased and plant senescence provided inputs of available carbon later in the season. The ability of the soil microbes to sequester N during the summer of 2003 was coupled with increasing MBC over time, but ability of the soil microbes to immobilize P was independent of MBC and MBN and remained consistently low throughout the growing season.

4.5.3 Plant and microbial phosphatase release in response to NP- and CNP-fertilization

Previous measurements of phosphatase activities in Arctic systems have measured root surface acid phosphatases but not soil phosphatase activities. When compared to soil phosphatase activities in temperate regions, activity levels measured in this study, approximately 1 mg *p*-NP released g^{-1} dry soil h^{-1} at 37°C are mid-range. Colvan et al. (2001) measured AcidPase activities of 0.7 mg *p*-NP released g^{-1} dry soil h^{-1} at 37°C and AlkPase activities of 0.3 mg *p*-NP released g^{-1} dry soil h^{-1} at 37°C, whereas Johnson et al. (1998) recorded values of approximately 0.2 mg mmol *p*-NP released g^{-1} dry soil h^{-1} at 37°C. Tabatabai and Bremner (1969) measured peak phosphatase activities of approximately 2 µmol *p*-NP released g^{-1} dry soil h^{-1} at 37°C, but measurements by Criquet et al. (2004) in an oak forest were much higher, at about 10 mg *p*-NP released g^{-1} dry soil h^{-1} (at 50°C).

Overall, there was no response of acid and alkaline phosphatase activities to NP-fertilization (Figures 3.23 - 3.26) and CNP-fertilization (Figure 3.27), and phosphatase activities

remained static throughout the summer of 2003. This is in contrast to what was generally expected after N and P fertilization. For example, a study in a Hawaiian rain forest (Treseder and Vitousek 2001) reported a decrease in root surface AcidPases in response to P-fertilization and an increase in root surface AcidPases in response to N-fertilization. Similarly, Johnson et al. (1998) measured higher Pase activities in N-fertilized plots than in control plots. Several studies have shown a strong correlation between concentrations of ammonium ions in the soil solution and phosphatase activities (Johnson et al. 1999). This is likely because of a reallocation of resources to phosphorus acquisition once N is readily available. As mentioned in section 4.3.2, there were negative, but weak relationships, between concentrations of nitrate and ammonium ions and AcidPases and AlkPases (Table 3.10). Since ammonium and nitrate concentrations in soil solution are limited and consistently low, changes in Pase activities enhanced by N additions did not occur during the summer of 2003. Previously reported correlation with extractable inorganic P is variable. Colvan et al. (2001) reported suppression of AcidPases with increased concentrations of orthophosphate, but Johnson et al. (1999) found no clear correlation between the two. In this study, there were some subtle changes in Pases activities that correspond with insignificant changes in DOP and Bray's extractable P (Table 3.10). These trends may have been better defined with increased replication. For example, there was a slight peak in AcidPases and AlkPase activities per mL of soil solution at the end of the growing season (fifth sampling day) and a slight decrease in Bray's extractable phosphate on the same day. Results of DOP also increased at this time, suggesting there was a greater substrate pool on which the Pases were potentially acting.

At moderate levels of goose grazing, like during the summer of 2003, there is increased turnover of nutrients measured as increased net N mineralization and demonstrated in a similar snow goose-grazed Arctic salt marsh (Zacheis et al. 2002) and in an Arctic system grazed by reindeer (Stark et al. 2002). As a result of the increased turnover, substrate pools increase along with total N pools because goose faeces fertilize the soil with organic N inputs. However, goose faecal matter returns little P to the soil (Ngai and Jefferies 2004), so that soils under moderately grazed sites have a higher substrate to the P ratio. Although above-ground biomass and shoot and root P contents were not measured in grazed sites, the effect of increased turnover is evident from increased phosphatase activities in grazed sites in the summer of 2003. Thus, it would appear that plant and microbial phosphatase activities are limited by availability of organic substrates.

4.6 Nutrient partitioning among plants, microorganisms and soil and the effect of goose grazing.

The only consistent responses to NP-fertilization on the intertidal salt marsh during the summer of 2003 were changes in the plant above-ground biomass and in shoot and root nutrient concentrations. Plant above-ground biomass and MBN and MBC increased over time. MBP, ammonium, nitrate, orthophosphate and dissolved organic phosphate concentrations, and soil phosphatase activities remained relatively static throughout the growing season. The majority of studies of plant and microbial responses to NP or NPK-fertilization in tundra soils have reported that the supply of nutrients to plants is regulated by microbial nutrient immobilization (Jonasson et al. 1996, Jonasson et al. 2004), such that soil microbes act as strong sinks for N and P. However, some studies have also reported that a large proportion of the nutrients added as fertilizer were taken up by plants and that microbial nutrient immobilization increased only after plant NUE declined (Jonasson et al. 1999). From these studies, we would expect that nutrient sequestration of N and P in soil microbes would increase when the sink strength of N and P in plants declined, as indicated by an increase in shoot and root nutrient content in N- and P-fertilized subplots. However, the low level of responses of microbial C, N and P were independent of plant nutrient-use efficiency or sink strength, and MBP was independent of MBC and MBN.

Due to the fact that the rise in MBC during the summer of 2003 was coupled with a rise in MBN, it is apparent that soil microbes are limited by N once carbon availability increases. Competition for N (NH_4^+-N) between plants and soil microbes on the intertidal marsh was unequivocally demonstrated through the use of a shared resource using ^{15}N stable isotopes (Buckeridge 2004). Under extremely N-poor conditions, like 0N0P and 0N1P subplots, both plants and microbes rely on organic compounds for their N (Schimel and Bennett 2004). Amino acid uptake has been shown to represent a substantial portion of N-uptake by roots of *P. phryganodes* in the intertidal zone at La Pérouse Bay in summer (Henry and Jefferies 2003). In these situations, soil microorganisms congregate around microsites of relatively high N-availability and thus are likely to gain access to more N than plants based on uptake kinetics (Schimel and Bennett 2004). However, plants tend to allocate resources to belowground root proliferation when they are in interspecific competition for organic patches containing a finite local supply of mixed N sources (Hodge 2004), so that they are able to take up N diffusing across microsites. This means that, on a community-level basis, plants were strong sinks for N under conditions of low N availability because total plant N increased with increasing above-ground biomass throughout the summer of 2003. When N was readily available (1N0P and

1N1P subplots), shoot and root N contents increased but MBN and soil inorganic N concentrations remained unchanged, thus implying that plants, but not microorganisms, used readily available N efficiently. Relatively high N-availability is analogous to moderate goose grazing, when the intertidal swards are fertilized with organic N inputs from goose feces and plant above-ground biomass and total nitrogen content increased in grazed sites as compared to ungrazed sites (Cargill and Jefferies 1984b).

Similar to the response of nitrate and ammonium concentrations in the soil solution to N-fertilization, P-fertilization did not affect the soil inorganic P concentrations. And, like the plant and microbial response to N, the P added as fertilizer was taken up by plants, as seen in the increased P content of shoots and roots in 0N1P and 1N1P subplots (Figure 3.14 and 3.15, Table 3.12), but not by microbes (Figures 3.18). Moreover, MBP did not increase during the summer of 2003 and microbes did not sequester "extra" P once carbon was availability (Figure 3.21). *Puccinellia phryganodes* is able to take-up P under conditions of N-surplus and N-limitation, likely because these graminoids are known to be mycorrhizal (Buckeridge 2004). The overall lack of response of MBP, in light of the fact that there was a surplus of inorganic P available in the soil solution, suggests that the soil microbial community on the intertidal salt marsh is not limited by P. This is in contrast to the temperate coastal salt marsh system studies by Sundareshwar et al. (2003). Moreover, Colvan et al. (2001) reported a strong positive correlation between MBP and extractable P in unfertilized plots, implying that microbial immobilization of P in the Colvan study was limited by P. However, Colvan et al. (2001) also reported that the correlation between MBP and extractable phosphate was uncoupled once microbial immobilization of P was released from P-limitation, as seen as from the lack of correlation between MBP and extractable P after P-fertilization. On the intertidal salt marsh, Bray- and Olsen's extractable P and microbial immobilization of P were insignificantly correlated neither with P-treated subplots (0N1P, 1N1P subplots) nor with the subplots that were not fertilized with P (0N0P, 1N0P subplots) (Table 3.10).

For plants, goose grazing at moderate densities increases turnover of fine root hairs in response to increased growth demands, thereby presumably providing a labile source of C for plant and microbial growth. However, competition for this C is strong such that microbial communities did not expand in response to increased turnover in grazed sites during the summer of 2003, since MBC was statistically indistinguishable between grazed and exclosed (control subplots) sites at this time (Figure 3.32). Similar to the lack for response to additions of N and P fertilizer, MBN and MBP in soil from beneath grazed and exclosed (control) subplots was statistically similar in the summer of 2003 (Figure 3.32). This means that C made available

from the turnover of fine root hairs and 'extra' N made available from goose faecal matter were taken up mostly by plants and the phosphorus made available from phosphatase hydrolysis was taken up by roots and microbial populations but P pools in these compartments did not change since little P is returned in goose faecal matter (Ngai and Jefferies 2004).

4.7 Plant and microbial growth and phosphatase activities vary seasonally

Arctic soil processes are strongly regulated by seasonal extremes, and until recently it was thought that nutrient cycling via microbial activity ceased during the long and harsh winter. It is it now known that soil microbial communities are active in frozen soil and under the snow pack. Respiratory release of CO_2 occurs at reduced rates throughout the winter in many alpine and Arctic soils (Oechel et al. 1997, Grogan et al. 2001). The discovery of this continued activity has directed a handful of studies which have sought to understand the seasonal dynamics of nutrient cycling. Annual patterns in N and P supply are characterized by a substantial increase in soluble N (Schimel and Chapin 1996, Lipson et al. 1999) and P (Chapin et al. 1978) pools at spring thaw. This flush of nutrients is important because it is thought to regulate the pool of nutrients available to plants during the growing season. This is also evident in that there is seasonal displacement of nutrient uptake, whereby plant communities gain access to nutrients during the summer months and microbial communities gain access to most of their nutrients after plant senescence in autumn and throughout the winter months (Jaeger et al. 1999).

From previous studies using ^{15}N isotope tracer, it is know that plant uptake of ^{15}N-labelled ammonium ions post-freeze-up in October and into late winter (April 2004) is marginal (Buckeridge 2004), indicating that plant nutrient uptake is greatly reduced in the Arctic autumn and throughout the winter. Overall, the fluctuations in microbial population growth correlate with carbon availability, whereby plant root release of low molecular weight carbon compounds may prime organic matter decomposition in the summer, and the decomposition of dead plant material releases soluble nutrients to fuel microbial growth in the winter. Results from soil samples taken after freeze-up in October 2003 and before thaw in April 2004, together with spring and summer data from 2003 and 2004, illustrate this phenomenon. Microbial C and microbial P were highest in October 2003 and remained relatively high until early summer, after which time they decreased. The peak in microbial C coincided with plant senescence and MBP corresponded with an increase in phosphatase activities, which presumably reflects the breakdown of dead plant material and the release of phosphate to the cryogenic microbial community. Acid and alkaline phosphatase activities were generally high throughout the winter

months and peaked in early summer of 2004. This result is consistent with Criquet et al. (2004). The detection of phosphatase activities during the winter, regardless of the potential temperature-dependent decrease in activities, indicates breakdown of organic P and the release of orthophosphate to soil microbes. Although soil inorganic P and DOP pools were not measured at this time, the peak in phosphatase activities in the second summer (2004) is likely a result of the breakdown of accumulated dead plant matter as a result of the fertilization in the summer of 2003. Microbial N peaked in late fall and this peak continued into early summer of 2004, when plant primary production was suppressed because of a late start to the growing season. Microbial C, N and P were all higher in the summer of 2004 than in the summer of 2003. This is probably because of the large amount of inputs of dead plant material from the fertilized plots in 2003.

4.8 Future research

Studies linking above-ground and below-ground processes are increasingly recognized as some of the most important to our future understanding of ecosystem ecology. At La Pérouse Bay, Manitoba, plant-herbivore relations are well-studied and have been applied in the context of managing the increasing snow goose population. In contrast, the study of plant-microbe interactions in this system is relatively new but is of particular importance because Arctic soils provide a unique and highly nutrient-limited environment in which to study the factors regulating nutrient cycling. Until now, most studies at this site have been conducted at the field laboratory, thus providing ease of sample collection and initial sample processing. Future studies will primarily require the use of new techniques so that it will be necessary to process samples at the laboratory at the University of Toronto. Since all filtrates from soil extractions can be preserved by freezing them for long periods of time, the relative inconvenience of displacing in time the sample collection and the subsequent analysis does not outweigh the importance of future studies on soil microbial communities at La Pérouse Bay for an overall understanding of above-ground and below-ground processes.

Although nutrient limitations of microbial biomass and immobilization of nutrients were investigated in this study, microbial activity is a more powerful measurement as it relates directly to rates of nutrient cycling. The most straightforward method for investigating the nutrient limitation of soil microbial activity is with the use of thymidine and leucine incorporation using a homogenization-extraction technique (Alden et al. 2001). Radioactive incorporation of thymidine as a measure of microbial activity has been successfully used in aquatic systems and soil systems (Söderberg and Bååth 1998). Full-factorial experiments can be performed in the

laboratory by addition of C, N and P to soil samples and measuring microbial activity after incubations of one to five days in each treatment. Studies examining the nutrient limitation of microbial activity will also provide insights into the future nutrient limitation of the salt marsh system, as it is becoming increasingly degraded and devoid of vegetation.

The use of isotope tracers can be used to establish the utilization of a shared resource. In a nutrient limited system such as at La Pérouse Bay, isotope studies, therefore, allow for the assessment of plant-microbe competition. In order to improve the understanding of plant-microbe competition for nutrients in this nutrient-limited system as well as turnover times of C, N and P within plant and microbial communities, these nutrients should be studied simultaneously using the stable isotopes ^{13}C and ^{15}N and the radioactive isotope ^{32}P as tracers of inorganic N and P and organic sources of C, N and P. Studies in intact sites will improve the understanding of plant-microbe interactions, while studies in degraded sites will improve the understanding of the contribution of ecosystem degradation to nutrient loss of a system. Because primary productivity in the adjacent freshwater marshes at La Pérouse Bay is P-limited, similar studies in these marshes could be used to compare the control of plant nutrient-limitation on overall nutrient cycling.

In order to expand the understanding of the composition of soil microbial communities and the possible consequences of shifts in microbial structure, phospholipid fatty acid analysis (PLFA) and DNA sequencing methods can be used to characterize changes in bacterial: fungal ratios. Changes in the community structure between intact and degraded or N- and P-limited systems, as given by bacterial: fungal ratios and genetic composition, will allow for a better understanding of the ability of specific groups of microorganisms to regulate plant access to nutrients. These techniques should also be used to look for seasonal differences within the soil microbial community, as reported by Lipson and colleagues (Schadt et al. 2003).

Along with different measurements of C, N and P in soil compartments, further studies should examine plant roots directly. Root surface phosphatases (acid phosphatases) can be measured with little modification to the procedure outlined in this study. Direct measurement of root phosphatase activity, as opposed to bulk soil phosphatase activities, may provide a better estimate of plant demand for P since the rhizosphere is likely a 'hotspot' for activity due to acidification by plant roots. Measurements of root exudation may also provide insight into plant-microbe interactions in the rhizosphere since carbon that is released by plant roots as they grow is thought to be a critical source for C-limited soil microorganisms (Alden et al. 2001).

Chapter 5: Conclusions

Plant primary production on the western intertidal salt marsh at La Pérouse Bay, Manitoba is co-limited by nitrogen (N) and phosphorus (P), which corresponds to low KCl-exchangeable nitrate and ammonium concentrations in soil solution and relatively higher total P-extractable concentrations. Acid and alkaline phosphatase activities were limited by available substrate and it is apparent that resources in this N-limited system are being allocated, not to the acquisition of P via Pase release, but presumably to the acquisition of N. Moreover, the relatively high concentrations of orthophosphate in the soil solution compared to the concentration of DOP in soil solution moderated the activity of these soil enzymes.

Soil microbial biomass, measured as microbial carbon (MBC), is primarily limited by carbon inputs but not inputs of N and P. Moreover, MBC and microbial activity (measured as MBN) on the intertidal salt marsh are regulated by C and N but not P. Although competition was not explicitly demonstrated in this study, comparisons of plant and microbial pool sizes reveal that, under conditions of low N-availability soil microbes may outcompete plants at a micro-scale. However, when viewed at the macro-scale or whole-soil level, changes in the relative partitioning of N between plants and microbial communities were largely controlled by plant uptake. In contrast to the summer months when nutrient uptake is dominated by plants, microorganisms immobilize a significant amount of C, N and P in the winter months and, after the cryogenic microbial die in spring—caused by spring freeze-thaw cycles, these nutrients again become available for plant uptake in the summer. Phosphatase activities were higher during the winter months as microbes were presumably able to gain access to inorganic P and organic and inorganic N after mineralizing dead plant material. Overall, seasonal shifts in the allocation of C, N and P within the community indicate a switch in the relative sink strength of plants and soil microbes, from a microbial dominated system in the winter to a plant-dominated system in the summer.

Appendix 1: Kjeldahl digestion of plant tissue

Reagents

1. Zinc powder
2. 98% H_2SO_4
3. K_2SO_4

Procedure

1. Weigh out 20 mg dried, ground tissue into test tubes.
2. Add 25 mg zinc powder.
3. Add 2 glass beads.
4. Add 1 mL 98% H_2SO_4.
5. Cap with drip bulb or marble. Place capped test tubes in a digestion block.
6. On a hotplate, heat slowly to 500°C to prevent acid from boiling. Once at 500°C, digest for 20 minutes.
7. Let cool and then add 100 mg K_2SO_4.
8. Heat slowly to 500°C until solution clears (around 1 to 7 days). Let cool.
9. Analyze for phosphate concentration following Appendix 2.

(modified from Grimshaw et al. 1989)

Appendix 2: Analysis of phosphate in plant tissue digests

Reagents

1. Plant tissue digest
2. NaOH, 6M
3. 0.42% ammonium molybdate in 1N H_2SO_4: add 28.6 mL concentrated H_2SO_4 to approximately 800 mL deionized water. Add 4.2 g ammonium molybdate. Top up to 1L. Mixture is stable at room temperature.
4. 10% (w/v) ascorbic acid. Refrigerate for up to 1 month.
5. Ammonium molybdate - ascorbic acid reaction mixture: add 1 part of 10% (w/v) ascorbic acid to six parts of 0.42% ammonium molybdate in 1N H_2SO_4. Keep on ice for up to one day.
6. KH_2PO_4, 1mM

Procedure

Dilution and neutralization of plant tissue digest

The acid digest solutions must be neutralized before phosphate analysis to ensure full colour development (Grimshaw 1989).

1. Add 4mL of deionized water to 1 mL acid from plant tissue digestion (Appendix 1).
2. Pipette 1 mL of diluted plant digest into glass scintillation vial.
3. Add 0.98 mL deionized water and 1.02 mL 6M NaOH to the diluted plant digest in the scintillation vial.

Phosphate analysis

At least three analytical replicates are desired.

1. Pipette 0.3 mL of this 3 mL neutralized digest into another glass scintillation vial.
2. Pipette 0.7 mL ammonium molybdate - ascorbic acid reaction mixture into the 0.3 mL of sample solution.
3. Cap scintillation vial and incubate at 45°C for 20 minutes.
4. Let sample cool. Read absorbance on spectrophotometer at 820 nm.

Standard curve

The standards are 0 - 1.86 μg phosphate.

1. To construct standard curve use 1 mL acid (heated with 25mg zinc powder and 100 mg K_2SO_4 in the same manner as samples) in glass scintillation vial.

2. Add 1.02 mL 6m NaOH, 0 - 0.6 mL 1mM KH_2PO_4, and add enough deionized water to make up to 3.0 mL total volume.

3. Pipette 0.3 mL standard into another scintillation vial.

4. Proceed as with sample.

(modified from Ames 1966 and Grimshaw 1989)

Phosphate concentration of a sample

P (mg g^{-1} dry weight plant tissue) = μg P x $mL_{dilution}$ x (1000 mg / (20 mg x 1000 μg))

where μg P = μg P measured in 0.3 mL of the diluted digest after conversion using the standard curve

$mL_{dilution}$ = dilution factor (mL), e.g. (3 x 5)/ 0.3 mL

Appendix 3: Analysis of dissolved organic phosphorus by acid persulfate oxidation

Reagents

1. Soil solution
2. H_2SO_4, 0.9 M
3. $K_2S_2O_8$
4. Organic P standard, p-Nitrophenyl phosphate stock solution: 1 mg P / mL

Procedure

1. Wearing latex gloves, squeeze soil pore water through a Whatman #1 filter. Prepare blank with deionized water. Freeze samples in plastic scintillation vials until analysis.
2. Analyze inorganic phosphate using 0.3 mL of the soil solution and 0.7 mL reaction mixture (Appendix 4).
3. Pipette an additional 5 mL sample into a 50 mL volumetric.
4. Add 10 mL 0.9 M H_2SO_4 and 0.8 g $K_2S_2O_8$.
5. Cap volumetric with aluminum foil and weigh.
6. Autoclave at 121°C for 50 minutes.
7. Let cool and reweigh sample.
8. Pipette 1 mL of the autoclaved sample into glass scintillation vial and neutralize with 1 mL 0.6 M NaOH.
9. Neutralize sample and analyze total dissolved phosphorus (Appendix 4).

Preparation of standards

Standards are 0.05 - 0.25 μg P mL^{-1}.

1. Pipette 7.5 mL standard stock solution into a 100 mL volumetric and dilute to volume with deionized water.
2. Pipette 1 mL of this into another 100 mL volumetric and dilute to volume with deionized water to make a 0.75 μg P mL^{-1} solution.
3. Pipette 1-, 2-, 3-, 4-, or 5 mL of the 0.75 μg P mL^{-1} solution into a 50 mL volumetric and add deionized water to make up to 5 mL total volume.

4. Proceed as with sample (step 2 of Procedure).

(modified from Lajitha et al. 1999)

Total dissolved organic phosphorus (DOP) in a sample

DOP (unit) = $P_T - P_i$

where P_T = total phosphorus, µg P mL^{-1} of soil solution after oxidation
P_i = inorganic phosphorus, µg P mL^{-1} of soil solution before oxidation
DOP= dissolved organic phosphorus

Appendix 4: Analysis of phosphate in soil extracts and soil solution

Reagents

1. Soil extract or soil solution
2. 0.42% ammonium molybdate in 1N H_2SO_4: add 28.6 mL concentrated H_2SO_4 to approximately 800 mL deionized water. Add 4.2 g ammonium molybdate. Top up to 1L. Mixture is stable at room temperature.
3. 10% (w/v) ascorbic acid: refrigerate for 1 month.
4. KH_2PO_4, 1mM
5. Ammonium molybdate - ascorbic acid reaction mixture: add 1 part of 10% ascorbic acid to six parts of 0.42% ammonium molybdate in 1N H_2SO_4. Keep on ice for up to one day.
6. HCl, 6M: for neutralization
7. NaOH, 0.6M: for neutralization

Procedure

Neutralization

Acid and alkaline sample solutions must be neutralized before phosphate analysis to ensure full colour development (Grimshaw 1989). For un-oxidized soil solution and near neutral extracts such as 0.5M K_2SO_2 and Bray's extractant, proceed directly to Phosphate analysis below.

1. To neutralize 0.5M $NaHCO_3$ extractant buffered at pH 8.5, add 1 part 6M HCl and 10 parts extract.

2. To neutralize soil solution after acid persulfate oxidation, add 1 part extract and 1 part 0.6M NaOH

Phosphate analysis

At least three analytical replicates are desired.

1. Pipette 0.3 mL sample (neutralized extract or soil solution where applicable) into scintillation vial.
2. Pipette 0.7 mL ammonium molybdate - ascorbic acid reaction mixture to the 0.3mL of sample solution.
3. Cap scintillation vial and incubate at 45°C for 20 minutes.
4. Let sample cool. Read absorbance on spectrophotometer at 820 nm.

Standard curve

The standards are 0 - 1.86 µg phosphate.

1. To construct standard curve use extract, neutralized in same manner as samples when applicable.

2. Add 0-, 0.4-, 0.8-, 1.2-, 1.6-, 2 mL aliquots 1mM KH_2PO_4 to 10mL volumetrics. Dilute to 10 mL with extract.

3. Pipette 0.3 mL standard into scintillation vial.

4. Proceed as with sample.

(modified from Ames 1966 and Grimshaw 1989)

Phosphate concentration of a sample

Of soil extracts: **P (mg kg^{-1} OR µg g^{-1} dry weight soil) =**

(µg P – blank) x $mL_{dilution}$ x ($mL_{extract}$ / FW_{sample}) x (FW/DW)

where µg P = µg P measured in 0.3mL of the sample, after conversation using the standard curve.
blank = µg P in 0.3 mL field blank
$mL_{dilution}$ = dilution factor (mL), e.g. (1/0.3) mL for and 0.5M K_2SO_2, Bray's extractant, and unoxidized soil solution; (1.1/0.3) mL for 0.5M $NaHCO_3$ buffered at pH 8.5.
$mL_{extract}$ = total volume of soil extractant used for extraction (mL)
FW_{sample} = fresh weight of soil used for extraction (g)
FW = fresh weight of soil used for fresh weight to dry weight ratio (g)
DW = dry weight of soil used for fresh weight to dry weight ratio (g)

Of soil solution (refer to Appendix 3): **P (µg mL^{-1} soil solution) =**

(µg P – blank) x (($mL_{initial}$ – mL_{lost}) / mL_{sample}) x $mL_{dilution}$

where µg P = µg P measured in 0.3mL of the sample, after conversation using the standard curve.
blank = µg P in 0.3 mL field blank
$mL_{initial}$ = volume in volumetric before autoclaving, if applicable (mL), e.g. 15 mL
mL_{loss} = volume lost during autoclaving (mL), if applicable
mL_{sample} = volume of soil solution added to volumetric before autoclaving, if applicable (mL), e.g. 5 mL
$mL_{dilution}$ = dilution factor (mL), e.g. (1/0.3) mL before oxidation and (2/0.3) mL for after oxidation

Appendix 5: Estimation of microbial C, N and P using chloroform-fumigation-extraction

Reagents

1. Soil extract, K_2SO_4, 0.5 M

2. Ethanol-free chloroform: Using a separating funnel, wash 100 mL chloroform with 100 mL 5% H_2SO_4, and then rinse three times with 100 mL deionized water.

Procedure

1. Weigh a portion of fresh soil (5 – 10 g). Place in drying oven at 50 - 60°C and reweigh after 48 hours for fresh weight to dry weight ratio.

2. For each replicate, collect two 70 g fresh weight subsamples. Wearing latex gloves clear subsamples of live plant material and break up into small, dime-sized chunks.

3. Immediately add 140 mL 0.5 M K_2SO_4 and handshake frequently for 1 hour.

3. Filter through Whatman GF/A filter paper. Freeze extracts in a plastic scintillation vial until nutrient analysis.

4. Place the second subsample in a 250 mL glass Schott bottle. Add 2 mL ethanol-free chloroform and seal immediately.

5. Incubate in the dark at room temperature for 24 hours.

6. Following 24 hour incubation, open the Schott bottle in a well-ventilated area and allow chloroform to evaporate for 30 minutes.

7. Extract as with first subsample (steps 3 & 4).

8. Include extract blanks. For estimation of microbial carbon, 2 mL ethanol-free chloroform should be incubated (no soil) for 24 hours and extracted to account for carbon contamination.

9. Measure total extractable P, C and N as described in Appendices 4, 6 and 7, respectively.

(modified from Witt et al. 2000)

Estimation of microbial biomass

Microbial carbon = $(C_{fum} - C_{unfum}) \times (1/k_{EC})$

where C_{fum} = organic carbon measured (Appendix 6) in the fumigated sample
C_{unfum} = organic carbon measured (Appendix 6) in the non-fumigated sample
k_{EC} = Extractability factor for estimation of microbial carbon (see text)

Microbial nitrogen = $(N_{fum} - N_{unfum}) \times (1/ k_{EN})$

where N_{fum} = total nitrogen measured (Appendix 7) in the fumigated sample
N_{unfum} = total nitrogen measured (Appendix 7) in the non-fumigated sample
k_{EN} = Extractability factor for estimation of microbial nitrogen (see text)

Microbial phosphorus = $(P_{fum} - P_{unfum}) \times (1/ k_{EP}) \times (1/k_{fix})$

where P_{fum} = phosphorus measured (Appendix 4) in the fumigated sample
P_{unfum} = phosphorus measured (Appendix 4) in the non-fumigated sample
k_{EP} = Extractability factor for estimation of phosphorus (see text)
k_{fix} = P fixation factor (see text)

Appendix 6: Organic carbon estimation by dichromate digestion

Reagents

1. Soil extract
2. $K_2Cr_2O_7$, 0.07 M
3. 98% H_2SO_4
4. 88% H_3PO_4
5. $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, 0.01 N in 0.4M H_2SO_4 titrate: Add 22.2 mL 98% H_2SO_4 to approximately 700 mL deionized water. Dissolve 3.92 g $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in solution. Dilute to 1 L with deionized water. Wrap in aluminum foil and refrigerate for up to 2 days.
6. N-phenylanthranilic indicator: Dissolve 106 mg Na_2CO_3 in just under 100 mL deionized water. Dissolve 100.2 mg N-phenylanthranilic acid in solution. Make to 100 mL with deionized water for a final concentration of 4.7 mM N-phenylanthranilic acid and 0.01M Na_2CO_3. Wrap in aluminum foil and refrigerate for up to 2 days.
7. Glucose standard, 4 mM: 0.3605 g $C_6H_{12}O_6$ dissolved in 500 mL soil extract

Procedure

1. Pipette 1 mL soil extract into a test tube.
2. Add 1 mL 0.07 M $K_2Cr_2O_7$, 2 mL 98% H_2SO_4, 1 mL 88% H_3PO_4.
3. Add two glass beads and cap with drip bulb or marble.
4. Heat samples, hot blanks and standards in digestion block to 150°C and digest for 30 minutes. Let cool. Re-digest any samples that over-boil.
5. Transfer digested solution to a beaker and rinse test tube into beaker with 10 mL deionized water.
6. Add 120 µl indicator N-phenylanthranilic indicator.
7. Titrate samples with 0.01 N $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ in 0.4M H_2SO_4, using a 50-mL burette calibrated at 0.1-mL intervals on a magnetic stirrer with Teflon coated stirring bar, until sample flashes green.

Calibration standards

Each digestion run should be calibrated with a replicated glucose standard.

1. Pipette 1 mL glucose standard into test tube.
2. Proceed as with sample.

Blanks

For each digestion run, 5 hot blanks and 5 cold blanks are desired.

1. For preparation of HB, add 1 mL 0.5 M K_2SO_4 blank and proceed with digestion as with sample.

2. For preparation of CB, titrate1 mL of undigested 0.07 M $K_2Cr_2O_7$.

(modified from Nelson and Sommers 1996)

Organic carbon concentration in a sample

$C_{sample} = [(mL_{HB} - mL_{sample}) \times ((mL_{cB} - mL_{HB}) / mL_{cB}) + (mL_{HB} - mL_{sample})] - mL_{blank}$

C (mg g^{-1} dry weight soil) =

$C_{sample} \times (0.01\ mol/L) \times (3\ g\ g^{-1}) \times (1L / 1000\ mL) \times 100\% \times (10\ mg / \%) \times (mL_{extract} / FW_{sample}) \times (FW/DW)$

where C_{sample} = amount of organic C present in the digested sample
mL_{HB} = mean volume of hot blank titrate (mL)
mL_{sample} = volume of sample titrate (mL)
mL_{CB} = mean volume of cold blank titrate (mL)
mL_{blank} = volume of field blank titrate (mL)
0.01 mol/ L = concentration of the Fe^{2+} in the titrate
3 g g^{-1} = assumed organic C equivalent when reacted with dichromate from soil solution (Grimshaw 1989)
$mL_{extract}$ = total volume of soil extractant used for extraction (mL)
FW_{sample} = fresh weight of soil used for extraction (g)
FW = fresh weight of soil used for fresh weight to dry weight ratio (g)
DW = dry weight of soil used for fresh weight to dry weight ratio (g)

Appendix 7: Total N determination using alkaline persulfate oxidation

Reagents

1. Oxidizing agent: Dissolve 25 g low-N $K_2S_2O_8$ and 15 g H_3BO_3 in 50 mL of 3.75 M NaOH and making up volume to 500 mL with deionized water.

Procedure

1. Pipette 3 mL of soil extract and 3 mL oxidizing agent into a glass tube and seal immediately with a screw cap containing a PTFE liner. Maintain a consistent headspace for all samples.
2. Combine one part extract to one part oxidizing agent to use as blank solution during nitrate analysis.
3. Weigh tube. Unscrew cap one half twist immediately before it is placed in autoclave for 30 minutes at 120°C.
4. Retighten screw cap and reweigh tube to determine water loss.
5. Analyze nitrate concentrations of solution.

(modified from Cabrera and Beare 1993)

Total N in a sample

N (µg mL^{-1}) = (A x (($mL_{initial}$ - mL_{loss}) / mL_{sample}) - blank)

where A = ppm of NO_3^- returned from auto analysis, ppm = µg mL^{-1}
$mL_{initial}$ = volume in glass tube before autoclaving (mL), e.g. 6 mL
mL_{loss} = volume lost during autoclaving (mL)
mL_{sample} = volume of sample extract added to glass tube before autoclaving (mL), e.g. 3 mL
blank = concentration of field blank from auto analysis (ug mL^{-1})

N (µg g^{-1} dry weight soil) = N (µg mL^{-1}) x ($mL_{extract}$ / FW_{sample}) x (FW/DW)

where $mL_{extract}$ = total volume of soil extractant used for extraction (mL)
FW_{sample} = fresh weight of soil used for extraction (g)
FW = fresh weight of soil used for fresh weight to dry weight ratio (g)
DW = dry weight of soil used for fresh weight to dry weight ratio (g)

N (mg L^{-1} soil solution) = N (µg mL^{-1}) x ($mL_{extract}$ / FW_{sample}) x (1 / (1 - (DW/FW))

where $mL_{extract}$ = total volume of soil extractant used for extraction (mL)
FW_{sample} = fresh weight of soil used for extraction (g)
FW = fresh weight of soil used for fresh weight to dry weight ratio (g)
DW = dry weight of soil used for fresh weight to dry weight ratio (g)

Appendix 8: Assay of soil phosphatase activity

Reagents

1. Toluene
2. NaOH, 1M (for MUB stock solution)
3. Modified universal buffer (MUB) stock solution: dissolve 12.1 g tris(hydoxymethyl) aminomethane (THAM), 11.6 g maleic acid, 14.0 g citric acid, 6.3 g boric acid into 488 mL 1N NaOH and adjust volume to 1 L with deionized water. Refrigerate.
4. HCl, 0.1 M (for MUB titration)
5. MUB, pH 6.5: Place 200 mL MUB stock solution in a 500 mL beaker. Titrate solution to pH 6.5 using 0.1 M HCL and adjust volume to 1L with deionized water.
6. NaOH, 0.5M (for assay and MUB titration)
7. MUB, pH 10.5: Place 200mL MUB stock solution in a 500mL beaker. Titrate solution to pH 10.5 using 0.5 M NaOH and adjust volume to 1L with deionized water.
8. *p*-Nitrophenyl phosphate solution (*p*-NPP), 0.05 M, pH 6.5 or pH 10.5: Dissolve 0.840 g disodium - nitrophenol phosphate tetrahydrate in about 40 mL MUB pH 6.5 (or MUB pH 10.5) and dilute to 50 mL with MUB pH 6.5 (or MUB pH 10.5). Refrigerate.
9. $CaCl_2$, 0.5M
10. Standard *p*-nitrophenol solution (*p*-NP): Dissolve 1.0 g *p*-Nitrophenol in about 700 mL deionized water. Dilute to 1 L with deionized water. Refrigerate.

Procedure

2. Weigh a portion of fresh soil (5 – 10 g). Place in drying oven at 50 - 60°C and reweigh after 48 hours for fresh weight to dry weight ratio.
3. Wearing latex gloves, crumble 1g fresh weight soil into a 125 mL Erlenmeyer flask.
4. Add 0.2 mL toluene, 4 mL MUB pH 6.5 (or MUB pH 10.5), 1 mL *p*-NPP pH 6.5 (or *p*-NPP pH 10.5).
5. Swirl flask for a few seconds and stopper the flask or cover the flask with parafilm.
6. Place in water bath at 37°C for one hour.
7. Remove from water bath and add 1 mL 0.5 M $CaCl_2$, 4 mL 0.5 M NaOH to stop phosphatase activities and develop the colour. Swirl flask.
8. Filter soil suspension through Whatman No. 1 filter paper.

9. Centrifuge for 5 minutes if a precipitate remains after filtering.

10. Read absorbance on spectrophotometer at 410 nm. If necessary, dilute samples with deionized water until the absorbance read is below that of 50μg standard.

Soil controls

Controls should be run for each soil analyzed to detect trace amounts of *p*-nitrophenol in the pNP and for extraction of trace amounts of coloured soil material by the $CaCl_2$-NaOH treatment.

1. Follow the procedure as above, but make the addition of 1 mL *p*-NPP *after* the reaction has stopped, i.e. incubate soil with only 4 mL MUB, and add 1 mL *p*-NPP after adding 0.5 M $CaCl_2$ and 0.5 M NaOH.

Standard curve

The standards are 0 - 50 μg p-nitrophenol.

1. Dilute 1 mL of standard *p*-NP to 100 mL with deionized water in a volumetric and mix thoroughly.

2. Pipette 0-, 1-, 2-, 3-, 4-, 5 mL aliquots of the diluted standard solution into 125 mL Erlenmeyer flasks.

3. Adjust total volume to 5 mL with deionized water.

4. Proceed as described for the incubated sample (add 1 mL 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH 0.5 M and filter).

(modified from Tabatabai 1994)

Amount of *p*-nitrophenol phosphate in sample

***p*-NP (mg g^{-1} dry weight soil) =**

$$[(\mu g\ p\text{-NP released } g^{-1} \text{ fresh weight soil} \times mL_{dilution} \times (FW/DW)) - C_{soil}] \times (1\ mg / 1000\ \mu g)$$

where μg *p*-NP released g^{-1} fresh weight soil = the amount of *p*-NP released after conversion from sample absorbance using standard curve.
$mL_{dilution}$ = total volume of the diluted sample (mL).
FW = fresh weight of the sample taken for fresh weight to dry weight ration (g)
DW = dry weight of the sample taken for fresh weight to dry weight ratio (g)

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