THE ALLOCATION OF INORGANIC NITROGEN ($^{15}NH_4^+$) TO SOIL, MICROBIAL AND PLANT BIOMASS IN AN ARCTIC SALT MARSH

by

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ABSTRACT

This study has demonstrated, through the shared distribution of a single resource ($^{15}NH_4^+$), that plants and soil microbes compete for inorganic N in a N-limited system. At La Pérouse Bay, soil microorganisms out-compete plants for this resource, but their competitive advantage is dampened relative to microorganisms in other Arctic ecosystems by the effects of goose grazing that promote plant growth via the addition of faeces. There is a seasonal displacement of N allocation, as microbes continue to mineralize and slowly immobilize nitrogen in the winter. Loss of vegetation, as a result of goose grubbing, has led to changes in soil characteristics, including high salinity and low redox potentials. These edaphic conditions may dampen N-uptake by soil microorganisms in these disturbed soils. Seasonal and grazing effects interact to contribute to a large potential N loss from these soils, although this is minimized by the apparent abiotic fixation of inorganic N.

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INTRODUCTION

Nitrogen in the environment

Poor access to sources of nitrogen that can be used for growth and reproduction limits the abundances of living organisms at different trophic levels (White 1993). Although nitrogen is a major component of the tropospheric atmosphere (78%), it consists mostly of inert dinitrogen gas (Sprent 1987) and only a small fraction of this nitrogen (110-130 Tg yr^{-1}) is added to the biosphere each year in a reactive form, largely as a result of nitrogen fixation by prokaryotic organisms. A roughly equivalent amount (135 Tg. yr^{-1}) is added from anthropogenic sources (Vitousek 1994). About half of the combined nitrogen in the biosphere consists of organic nitrogen, most of which is bound in plant litter, organic matter in soils, and particulate matter in aquatic systems and is generally unavailable for uptake by plants and microorganisms. In contrast, the remainder is present as inorganic nitrogen, especially ammonium and nitrate ions that can be readily taken up by plants and microorganisms. Their demand for nitrogen is high relative to other mineral nutrients, despite the relative scarcity of nitrogen in available forms suitable for uptake (Epstein 1965).

Nitrogen and plant and microbial growth

The productivity of most ecosystems is limited either by the availability of nitrogen (N), or phosphorus (P), or both of these elements are co-limiting (Vitousek and Howarth 1991, Aerts and Chapin 2000, Wardle 2002). In Arctic soils there is evidence of both nitrogen and phosphorus limitation (Nadelhoffer *et al.* 1992, Shaver

and Chapin 1995, 1980). Several researchers, however, have also proposed that Arctic soil microorganisms may be limited by carbon (C) (Jonasson *et al.* 1996, Vance and Chapin 2001). This has implications for nutrient availability to plants, as these microorganisms are responsible for mineralizing N and P. An element is considered limiting if an increase in its availability increases the productivity of the system (Chapin *et al.* 1986). The availability of N and P are largely determined by biological processes, particularly microbial activity in soils and aquatic systems. In the case of nitrogen, the different valency states of forms of inorganic nitrogen are regulated largely by microbial processes linked to mineralization of organic nitrogen or the use of oxides of nitrogen as electron acceptors in microbial respiration (Fenchel and Blackburn 1979).

As indicated above, much of the total N in soils is present in organic matter and is recalcitrant as it is bonded tightly to carbon compounds, resulting in organic matter that is resistant to decomposition by microorganisms. As a result, this recalcitrant nitrogen has very long turnover times, which can be the order of decades (Vitousek and Howarth 1991, Vitousek *et al.* 2002). Due to the low decomposition rates in the Arctic that are primarily a result of low soil temperatures and a short growing season, a large portion of the total nutrients present in soils is held in soil organic material (Hobbie *et al.* 2002). Only about 5% of the total N pool in most soils, including Arctic soils, is actively turning over (half-time ~3 days), and much of this represents N in dead root hairs and microbial necromass (Sprent 1987).

Concentrations of inorganic nitrogen in soil solutions of non-agricultural soils, which are largely the result of the mineralization (ammonification followed by nitrification) of low molecular weight organic polymers, are usually between 10^{-5} and

10^{-4} M. Rates of net mineralization of nitrogen by soil microorganisms are dependent on litter quality, particularly the carbon/nitrogen ratio of the plant and microbial litter (Chapin *et al.* 1993). Where this ratio is greater than 25-30, most organic N that is mineralized is immobilised by microorganisms, resulting in low rates of net mineralization of N. At lower C:N ratios in litter, net mineralization rates are higher because there is an excess of nitrogen relative to the demand for carbon by microorganisms, hence inorganic N is released into the soil. In spite of this, because of the low temperatures and short growing season in the Arctic, soil net 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} $year^{-1}$ for temperate systems (Nadelhoffer *et al.* 1992). However, in the last decade it has been shown that both microorganisms and plants can take up low molecular weight organic N compounds, such as amino acids (Kielland 1994, 1995, Schimel and Bennett 2004), in addition to inorganic N. Furthermore, it has been shown that plants can compete with soil microorganisms for inorganic and organic N, and that they are not reliant on microbial "leftovers" (Kaye and Hart 1997). Hence, net mineralization rates are an inadequate description of available sources of N for plant and microbial growth. The rate-limiting process in the generation of bioavailable N is seen as the availability and activity of exoenzymes (peptidases), which are secreted by plants and microbes, including mycorrhizae, and can attack organic polymers in the soil (Schimel and Bennett 2004). The organic monomers (e.g. amino acids) that are produced can be taken up directly by plants and microorganisms, thereby by-passing the need for nitrogen mineralization that leads to the production of ammonium and nitrate ions. Concentrations of free amino acids

in Arctic coastal soils are about 10^{-5} M, similar to the concentration of ammonium ions (Henry and Jefferies 2002).

Roots of tundra plants are able to absorb nutrients at lower soil temperatures than the roots of temperate plants (Chapin and Bloom 1976, McCowan 1978, Kielland and Chapin 1992), and they have a high absorption capacity (Chapin 1974a,b). New roots of *Eriophorum vaginatum* closely follow the retreating boundary of frozen soil as the active layer develops at spring thaw (Chapin 1974a,b). Arctic plants also effectively recycle nutrients by translocating a substantial amount of N and P (50-85%) from senescing above-ground tissues to below-ground organs at the end of the growing season, where they are stored for use the following spring (Chapin 1978, Chapin *et al.* 1980, Berendse and Jonasson 1992). When pools of available soil N are high, leaf tissue N as a percentage of the dry weight of the tissue may reach values of between 4%-5%, although more often values are between 1.5% and 2.5%, particularly late in the growing season when supplies of available N become short (White 1993). A N concentration of 1.5% of plant dry weight is considered adequate for plant maintenance, but not for growth (Epstein 1972). However, these percentages can vary considerably between plants with different growth forms. For example, amounts of N in evergreen tissues are lower than corresponding values for forbs and graminoids on account of the high amount of structural carbon in leaf tissues that "dilutes" the N amount per unit mass. Nitrogen levels as a percentage of dry weight tend to be high in young tissues, some storage organs and root hairs as high amounts of structural carbon are not present in these organs (Larcher 1995).

Plant and microbial interactions and carbon and nitrogen cycling

Plants, as primary producers, are a source of organic carbon and nitrogen for soil decomposers and they also provide resources from root exudates and within roots that enable symbiotic mutualists to function and survive. The decomposers, in turn, break down dead plant material and indirectly regulate plant growth by determining the supply of nutrients available to plants. Because plant species differ in both the quality and quantity of resources that they return to the soil, individual plant species may be expected to have an important effect on the soil biota and the processes that they regulate that bears no relation to the abundance of the plants in a community. Furthermore, this returned supply may be diverted or delayed by the presence of mosses (Kotanen 2002). In grasslands and forests, different microbial communities and differences in the soil fauna that feed on the microbes occur in the rhizosphere around the roots of different species (Bardgett 1999, Yeates 1999, Porazinska *et al.* 2003).

Above-ground trophic interactions also have indirect effects on soil biota by affecting the quantity and quality of resources that plants produce (Wardle 2002). Foliar herbivory can trigger the release of root exudates rich in carbon that alleviates carbon limitation for soil microorganisms. This can result in increased nitrogen availability for plant growth as a result of enhanced rates of mineralization (Hamilton and Frank 2001, Bardgett *et al.* 1998). Herbivores may also promote compensatory plant growth following defoliation as a result of the addition of faecal material to soil, rather than as recalcitrant plant litter (Hik and Jefferies 1990). The faecal material contains readily available organic and inorganic N sources (amino acids, NH_4^+) for plant growth. Aside from the negative effect of defoliation and a potential loss of plant productivity, herbivory may

also lead to induced production of secondary defence compounds and the promotion of succession by favouring the dominance of unpalatable species (Pastor *et al.* 1993). An increase in plant litter rich in secondary chemicals is likely to reduce soil microbial activity (Chapin *et al.* 1994). In addition, foliar herbivory can also result in a reduction in rates of mycorrhizal infection of roots, possibly because of lack of sustained carbon allocation to roots (Wardle *et al.* 2004).

Those plant species with a high intrinsic relative growth rate, which flourish under fertile conditions, often can support high herbivore densities where more than 50% of the net above-ground primary production (NAAP) enters the soil as faecal material containing available nutrient sources for plant growth (Wardle *et al.* 2004). In contrast, under infertile conditions nearly all of the NAPP is returned to the soil as recalcitrant plant litter, because rates of herbivory are low (Grime 2001, Wardle *et al.* 2004). Litter is low in N, often high in phenolics and other secondary compounds and high in structural carbohydrates, including lignin. In fertile soils, a bacteria-based energy channel functions, where the rate of turnover of material is rapid, and the C:N ratio of plant litter low (<20). Typically, there is a low net accumulation of carbon in these soils. Aside from the presence of bacterial communities, a rich microfauna consists of different species of nematode and protozoa (Coleman *et al.*1983; Wardle *et al.* 2004). Infertile soils, in contrast, are dominated by fungi, enchytraeid worms and arthropods, including mites, springtails and millipedes. The soil food web is dominated by a fungal-based energy channel and soil processes are characterised by low soil mixing, slow rates of decomposition and nutrient mineralization, high soil carbon sequestration and low nutrient supply rates (Wardle 2002).

The mechanistic basis by which net primary production is regulated by below-ground interactions between plants and microorganisms is reasonably well known. Plant parasites, pathogens, and root herbivores can directly remove C and N from plant tissues and reduce the capacity of roots to take up nutrients from the soil, which has a negative impact on plant growth (Bever *et al.* 1997). Root pathogens and root-feeding invertebrates do not affect adversely all plants in the community equally and thus their presence in soil can lead over time to quantitative differences in plant community composition and in above-ground trophic relationships (Bezemer *et al.* 2003, Masters *et al.* 2001). The presence of these organisms can lead to the successional replacement of species (De Deyn *et al.* 2003). Similarly, although mycorrhizal fungi increase the access of a plant to limited supplies of N and P, these plant-fungal mutualisms do not affect all plants in the community equally, and in some cases plants do not benefit from these mutualisms (Klironomos 2002). However, the hyphae potentially can exploit a larger volume of soil than the plant root system and they also secrete exo-enzymes such as peptidases and phosphatases that catalyse the production of low molecular weight forms of organic N and inorganic phosphate respectively in the soil (Smith and Read 1997).

Seasonal controls on nutrient dynamics in alpine and Arctic soils

In alpine and polar regions weather conditions enforce a profound seasonal effect on plant and microbial dynamics and on the flow of nutrients in soil systems. Investigations of microbial activity (Brooks *et al.* 1998) and community structure (Schadt *et al.* 2003) have revealed a genetic and functional shift in alpine soil microbial communities between summer and winter (Schadt *et al.* 2003).

Recently, a model has been proposed by Lipson and his co-workers (Lipson *et al.* 2002, Schmidt and Lipson 2004) which describes annual changes in seasonal availability of nitrogen in alpine soils (and by inference Arctic and sub-Arctic soils: c.f. Hobbie and Chapin 1996, Grogan and Jonasson 2003) and the interactions between microbial and plant growth and litter decomposition in promoting these changes. The model indicates that plant and microbial litter inputs at the end of the summer growing season provide substrates for the growth of cryogenic microorganisms in late autumn and winter. These organisms can grow in soil under a snow pack at temperatures around the freezing point (-5 °C to + 3 °C) and they deplete the soil of labile nitrogen and carbon derived from plant litter. Their activity depends very much on the depth of the snow pack. If snow is blown free from sites exposing the underlying vegetation or soils, soil temperatures may be well below -5°C and microbial activity is much reduced. Where snow has accumulated during the winter, the spring freeze-thaw cycles lead to a reduction in the cryogenic microbial biomass, as a result of starvation from low nutrient availability (C and N) at the end of the winter and an intolerance of these organisms to higher soil temperatures. The lysis of microbial and plant necromass releases soluble nitrogen and carbon into the soil system. These nutrients are utilized by plant roots and the summer microbial community, which are tolerant of higher temperatures (5°C - 20°C) (Schmidt and Lipson 2004). In addition, these microorganisms utilize plant root exudates, especially carbon sources, during the summer. Hence, there is a temporal coupling of soil biological activity between winter and summer that regulates the availability of soil nitrogen for plant and microbial growth (Schmidt and Lipson 2004). Early plant growth and post-thaw microbial communities are believed to provide a vernal "dam" (Zak *et al.* 1990, Tessier

and Raynal 2003) for nutrients released from these winter microorganisms by spring freeze-thaw cycles (Schimel and Clein 1996). Thus, the spring release of nutrients is effectively retained in the system. There is additional evidence that fungi probably make up most of the microbial biomass in winter and that bacteria dominate in summer (Lipson *et al.* 2002, Schadt *et al.* 2003). In the 2002 study by the Lipson group, shifts in species composition between pre-and post-melt communities were detected using reciprocal hybridization of community DNA, fungal/bacterial ratios as measured by direct microscopic counts, and by substrate-induced respiration experiments with specific inhibitors for the respective groups. All values were higher in soils in winter compared to soils in summer (Lipson *et al.* 2002).

Ecosystem disturbance and nitrogen cycling in an Arctic salt marsh

Very little is known about how disturbances to soil and vegetation affect soil microbial communities. Sources of disturbance vary, as do the duration and severity of their effects. Although disturbances are a ubiquitous phenomenon in ecosystems, the relative importance of edaphic versus biotic controls on microbial biomass and nutrient cycling is poorly understood. Whereas it is recognised that microbial communities are larger and more diverse in the rhizosphere zone than in bulk mineral soils, the effect of vegetation removal on these communities is known only from a qualitative standpoint. Heavily disturbed ecosystems generally show decreases in microbial taxonomic diversity and reductions in rates of nutrient cycling due to altered rates of decomposition (Allen 1999).

A disturbance that is widespread in coastal marshes of the eastern Arctic of North America is the effect of foraging by populations of breeding and moulting geese that visit these marshes in summer and winter in the southern United States of America and northern Mexico. These effects have been extensively reviewed recently (Jefferies, Rockwell and Abraham 2003, 2004). In particular, the Mid-Continent population of lesser snow geese (*Chen caerulescens caerulescens* A.O.U.), which breeds in these Arctic coastal marshes, together with populations of Canada geese (*Branta canadensis* A.O.U.) that are composed of both breeding birds and moult migrants, have contributed to this disturbance, as a result of their foraging activities. The Mid-Continent population, in particular, has shown a near geometric increase in numbers, largely as a result of increased foraging on agricultural crops on the wintering grounds and along the flyways (Jefferies, Henry and Abraham 2003). This increase in herbivore density in these Arctic coastal marshes has resulted in two different outcomes to the vegetation, depending on the herbivore density, which can be represented by two positive feedbacks. When the population of lesser snow geese at the intensive study site at La Pérouse Bay on the south-west coast of Hudson Bay was approximately 3500 pairs of birds in 1978 (Cooke *et al.* 1995), goose faecal matter provided inorganic and organic N fertilization to salt-marsh swards that were N-limited (Cargill and Jefferies 1984, Bazely and Jefferies 1985). In spite of grazing by the geese, the fertilization stimulated plant growth and accelerated N turnover rates, so that net primary production was higher in grazed areas compared with ungrazed areas (Cargill and Jefferies 1984). The second type of positive feedback is associated with high populations of geese and it has led to the degradation of coastal marshes at La Pérouse Bay and elsewhere (Srivastava and Jefferies 1996, Jano *et al.*

1998). At the time of the last census in 1997, the local snow goose population had reached 44,500 pairs at La Pérouse Bay (Abraham, Rockwell and Ross, unpublished data). The degradation is the outcome of the grubbing of the vegetation by lesser snow geese (and Canada geese) in spring after the surface of the ground has thawed, but before the start of above-ground plant growth. The birds remove roots and rhizomes from the thawed ground, which leaves only a few shoots. The exposed sediments dry out in summer, and the sediments can become hypersaline (~ 3x the salinity of sea water). At these salinities, the forage plants in these salt marshes are unable to re-establish and, over several years, the surface layers of sediment are eroded by wind and water (Iacobelli and Jefferies 1991, Srivastava and Jefferies 1996). These degraded areas represent an alternate stable state (Hik, Jefferies and Sinclair 1992), where high soil salinities, compaction, low soil N, low redox potentials, and an impoverished seed bank, all contribute to prevent the re-establishment of vegetation (Jefferies, Rockwell and Abraham. 2003, 2004).

Previous research at La Pérouse Bay has shown that degraded sites in a supratidal marsh (two tidal inundations every three years, on average), which are also a consequence of goose grubbing, exhibit decreased rates of N mineralization relative to rates at sites with intact graminoid swards (Wilson and Jefferies 1996). The dominant form of available N for plant growth in these marsh soils is NH_4^+, which is present as exchangeable ammonium ions bound to fixed, negatively charged sites in soil and as ammonium ions in the soil solution. Nitrogen also may be present as nitrate ions, especially late in the growing season, and as soluble organic nitrogen in the soil solution (Wilson and Jefferies 1996; Henry and Jefferies 2003). The latter form of nitrogen

includes amino acids that also may be bound to exchange sites in soil. Nitrifying bacteria that convert ammonium ions to nitrate are inactive under cold, waterlogged conditions, and it is only at the end of summer, when soils are warmer and less reduced, that nitrate is present in the soil solution in detectable amounts. (This excludes the very short-lived pulse of nitrate in the soil solution that occurs at melt and is limited to one or two days). In spring, the exposed sediments are often colonised by algal mats consisting of cyanobacteria and diatoms, provided that the thin veneer of organic-rich sediment at the surface of these exposed soils has not been eroded (McLaren and Jefferies 2004). The mats and the surface layers of the sediment dry out in summer and the mats are blown away by wind. This process results in the gradual erosion of the organic-rich layer over two to three years until the mats fail to establish on a mineral substratum where the N and C contents of the upper layers of soil are much reduced (McLaren and Jefferies 2004).

In keeping with Arctic soils in general, intertidal and supratidal soils of the coastal marshes at La Pérouse Bay are primarily nitrogen-limited, although addition of nitrogen to sediments quickly leads to phosphorus limitation, hence both elements may be regarded as co-limiting for plant growth in these soils (Cargill and Jefferies 1984 , Ngai and Jefferies 2004). Loss of vegetation, as a result of grubbing, leads to loss of N, further limiting the supplies of this element for plant and microbial growth (McLaren and Jefferies 2004), and this N loss may be contributing to the maintenance of N limitation in the marsh soils. Hence, competition between plants and microorganisms may be expected to occur for this limited resource. The rationale for this study, therefore, was based on the need to determine the partitioning of nitrogen between microbial and plant biomass and the soil components in a system that is N- limited, in order to examine the extent to which

there was competition for nitrogen between microorganisms and plants. The investigation was based on a series of linked questions. Much of the study involved the introduction at the rooting depth (0-6 cm) of $^{15}NH_4^+$ into soil cores, in order to establish the partitioning of the isotopic label between microbial and plant biomass and the soil matrix after 24 hours of incubation. The study included an investigation of soil with intact turfs of the salt-marsh forage grass, *Puccinellia phryganodes,* which is eaten by the geese, and disturbed, degraded soils devoid of vegetation which are described above. Two marshes were selected for the study. The first was a young intertidal marsh dominated by *P. phryganodes,* located in the western intertidal marsh at La Pérouse Bay. The second was an older supratidal marsh adjacent to the Mast River which drains into La Pérouse Bay and which had the same type of vegetation as the younger marsh. However, overall, it represented vegetation at a later stage of development than that in the intertidal marsh, as shown by the deeper (15 cm) and better-developed surface organic layer. At both sites, soils from disturbed sites (grubbed sites, hereafter called "degraded") were examined in order to determine the effect of the above-ground trophic influence of the geese on the partitioning of pulses of added nitrogen between microbial biomass and soil in the absence of vegetative cover. The results were compared to similar data from soils in which the sward was intact (hereafter called "intact"). As indicated above, microbial activity may show strong seasonal trends. In early winter and at spring melt microbial biomass may be high. In summer, by contrast, carbon limitation may curtail growth in soils. Because of this, soil microbial activity in the two sites was examined throughout the year, although the number of samples that were collected in winter was limited for logistical reasons.

Research questions examined in this study:

1. Do the nitrogen dynamics and the partitioning of ^{15}N between microbial biomass, plant biomass and the soil compartments differ between the younger intertidal marsh site and the older supratidal marsh?
2. Do the nitrogen dynamics of soils with vegetative cover differ substantially from soils where vegetation is absent as a result of grubbing?
3. Is there strong seasonal variability in soil microbial biomass and microbial activity measured in the snow-free season versus when the ground is snow-covered?
4. During the snow-free season do plants compete effectively with microorganisms for NH_4^+, and is the nitrogen rapidly transferred from the roots to the shoots?

METHODS

Site description

La Pérouse Bay (58°04'N, 94°03'W) is located on the south-west coast of Hudson Bay, approximately 30 km east of Churchill, Manitoba (Fig. 1). It is part of the Hudson Bay Lowland, an isostatically rebounding region (0.5-1.2 m 100 y^{-1}) (Andrews 1973).

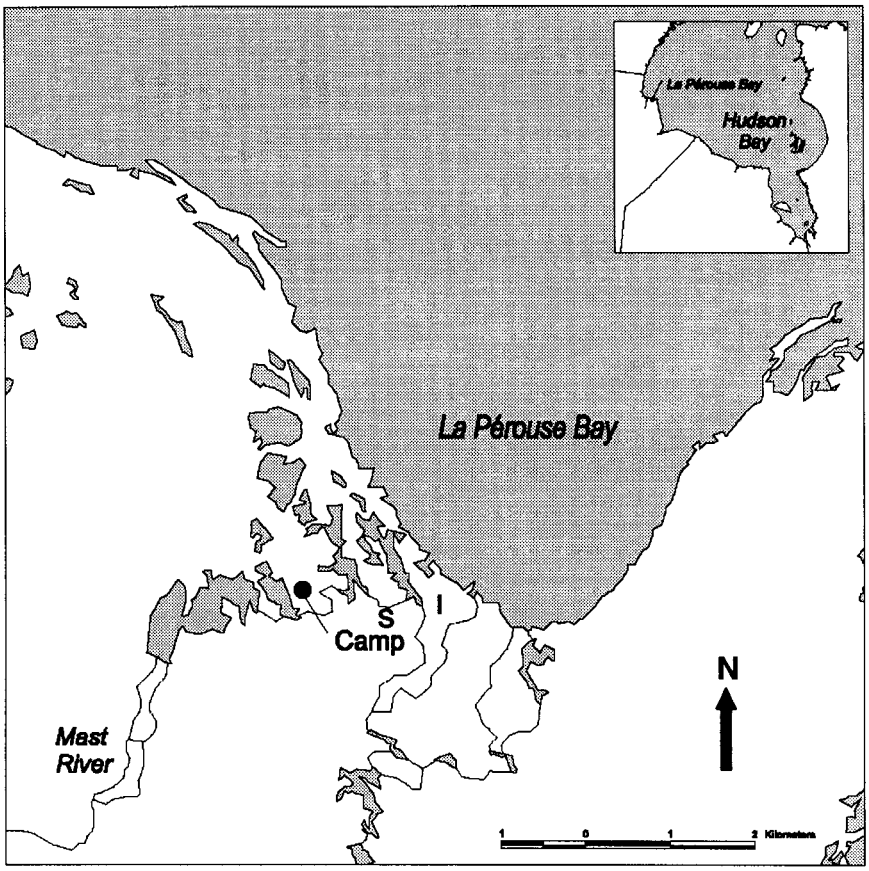

FIG. 1 Location of experimental sampling sites, within the coastal salt marsh of La Pérouse Bay, MB, on the west coast of Hudson Bay. **I** represents the location of the intertidal marsh, **S** represents the supratidal marsh. **Camp** is the location of the field camp. Scale, 1.1 cm = 1 km.

La Pérouse Bay is characterized by a low, flat coastal plain, consisting of sandy sediment, marine clays and glacial gravel and erratics, below which lies limestone bedrock

(Jefferies *et al.* 1979). At La Pérouse Bay a delta has formed at the confluence of the braided, freshwater Mast River and the more saline water of Hudson Bay. The inshore water in coastal marine areas is brackish with a salinity of less than 12 g of solutes L^{-1}, as a result of the outflow of fresh water from rivers (Srivastava and Jefferies 2002). One of the main controls on landscape heterogeneity is frost-heaving, which results in an ephemeral hummock and pool or stream topography that is well developed in the supratidal marsh and in the upper levels of the intertidal marsh (Jefferies *et al.* 1979)(Fig. 2). In the younger intertidal marsh, sediment deposition from tidal activity in the late summer and autumn, and downcutting by ribboned streams, has resulted in raised platforms (c.15-25 cm high) dominated by *Puccinellia phryganodes* and *Carex subspathacea* (plant nomenclature follows Porsild and Cody 1980)(Fig. 2). Further inland in the older supratidal area, in addition to the hummock topography, impeded drainage has resulted in streams and 'abandoned' pools, which merge at the time of spring floods. These pools evaporate by early to mid-summer to leave exposed organic-rich sediments. The hummocks are frequently covered with persistent woody vegetation (*Salix brachycarpa, Myrica gale and Betula glandulosa*), surrounded by swards of *P. phryganodes, C. subspathacea, Festuca rubra and Calamagrostis deschampsioides* at lower elevations (Fig. 2). As the snow goose population has increased in numbers, the graminoid communities in both the young intertidal marsh and the older supratidal marsh have been lost as a result of grubbing. The landscape in both areas is a patchwork of intact vegetation separated by large (supratidal) to extensive (intertidal) mudflats of exposed sediment (Fig. 2).

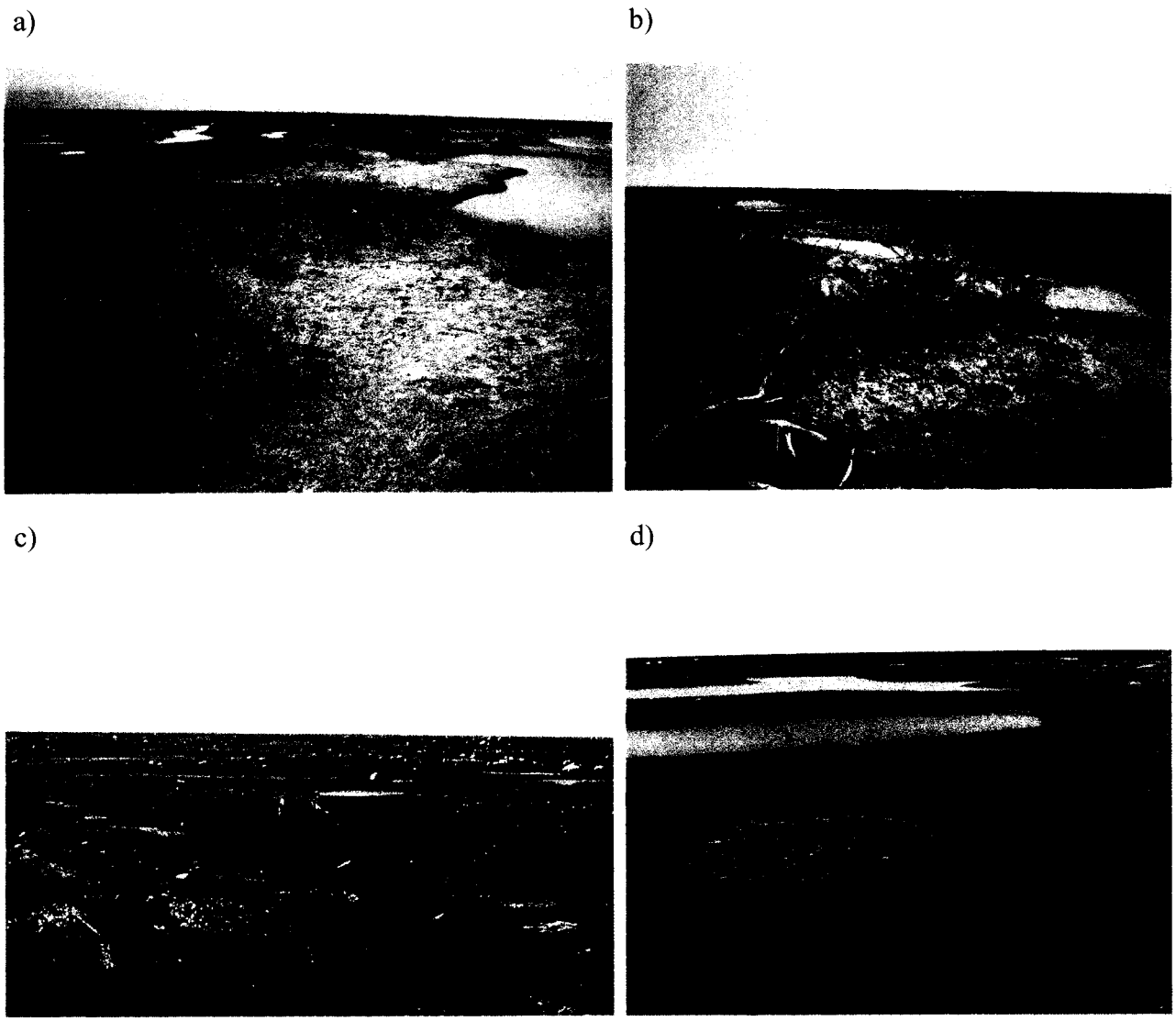

FIG. 2 Photographs of the a) intact and b) degraded supratidal marshes (in the early spring before the grasses have sprouted new shoots) and the c) intact and d) degraded intertidal marshes at La Pérouse Bay, MB. Photographs courtesy of a) and b) Kate Buckeridge, c) Ken Abraham and d) Joan Montagnes.

The soils in the salt-marsh are classified as Regosolic Static Cryosols (Agriculture Canada Expert Committee on Soil Survey 1987, Wilson and Jefferies 1996), characterized by a gleyed mineral horizon (Cg) active layer (up to 30 cm) above permafrost without marked cryoturbation. A thin Ah (humified mineral soil) horizon (0-5 cm) is found in the supratidal sites, but the soils of the intertidal sites had only multiple Cg horizons. In both sites these Cg horizons are formed by striations of sediment, deposited by tides, and partially decayed organic material (Fig. 3) although striations in the intertidal soils are more common within the top 6 cm (i.e. more tidal inundations). Degraded site soils are typically lower in elevation than sites with vegetation, as they are missing the surface layer of rhizomes and roots (0-5 cm). In spring, ephemeral algal mats cover the sediment surface, especially where organic material is still present in the upper layers of sediment. They begin as black crusts, then turn white as they dry-out, and are finally blown away in summer (McLaren and Jefferies 2004).

FIG. 3 Soil block (7.5 x 7.5 cm) next to its hole, collected in the intertidal marsh at La Pérouse Bay in early June 2003. Vegetative cover is *Puccinellia phryganodes,*and spring growth has just begun. Note the striations in the C-horizon from tidal and flood sediment deposition over partially decayed organic material.

Experimental design and sample collection

At both the intertidal and the supratidal sites, samples (with three to five replicates each, varying by date) were removed from areas with intact vegetation ("intact") and from areas devoid of vegetation ("degraded"). Sampling of all four treatments occurred on 30 May, 2 June and 8 June 2003 (n=3). Sample collections were then off-set between the supratidal and intertidal sites to accommodate increased replicates (n=4), with collections on 12 and 16 June, 26 June and 2 July, and 11 and 15 July, respectively. The sample date of 30 May was soon after snow melt, and by 15 July 2003, senescence of leaves of *Carex subspathacea* had begun. An early winter sampling was performed on all treatments on 30 October 2003 (n=5), at which point the snow-covered ground had been frozen for about three weeks. A late winter sampling occurred on 29 April 2004 (n=3) under snow, when the ground had been frozen for almost seven months. The repeated samplings throughout the spring, summer and winter were made to determine the extent of seasonal variability in N dynamics and differences in the partitioning of N in soils beneath grubbed and intact swards at the two sites.

Sample processing

Processing of summer samples occurred at the field laboratory at La Pérouse Bay, a short walk from the sampling sites whereas processing of the winter samples took place in the laboratory at the University of Toronto. On each sampling date, blocks of soil were removed to analyze soil redox potential (mV) and soil pH. The redox potential was determined with a platinum electrode (YSI Model 33 Salinity-Conductivity-Temperature Meter) calibrated with ZoBell's solution (ZoBell 1946), and the pH was measured with a

glass electrode after 15 minutes in a 3:1 (v:v) soil to deionized water slurry (Fisher Accumet Portable pH Meter Model 156). The soils were hand-squeezed to remove soil water, and if sufficient soil water was present salinity was measured in the field. Excess extract was frozen for future analysis of salinity at the University of Toronto.

To monitor short-term fluctuations in N allocation between plants, microbes and soil, a stable isotope (^{15}N) tracer was injected as $^{15}NH_4Cl$ into intact soil cores, and changes in isotope ratios and N concentrations were monitored over 24 hours. On 8, 12, 26 June and 2, 11, 15 July, a pair of soil cores was removed from each treatment using a 7.3 cm-diameter polyethylene tube, cut to a depth of 6.5 cm, wrapped intact in plastic film, and returned to the field laboratory. All cores were stored overnight in the ground near the field laboratory. Soil and air temperature during incubation are recorded in Table 1 and a summary of the sample processing described on the next few pages is depicted in Fig. 4.

Core processing follows the method of Hart *et al.* (1994). The intact soil cores were removed from the ground, inverted, and injected with 7 x 1 ml of 1 mM 98 atom percent $^{15}NH_4Cl$ (Sigma Chemicals, St. Louis) using an 18-gauge double side-port spinal needle (Popper and Sons, Hyde Park, New York) in a 7-point hexagonal + center pattern on the sediment surface at the base of the core. This injection was approximately equal to the background concentrations of soluble NH_4^+, and soil moisture was increased by <6%. The needle was slowly raised and twisted while compressing the syringe to minimize pooling of the salt solution. One core was returned to the storage site to incubate *in situ* for 24 hours. The other core was used as a time 0 control, and both cores were processed in an identical manner The roots and shoots were collected by removing the top 2 cm of

the core and taking a 2.2 cm diameter plug of soil from this section. The living (fine white) plant roots were carefully teased from the top plug of soil, rinsed in deionized water and 0.5 M $CaCl_2$ to clear the isotope present in the Donnan free space, then roots and shoots were dried at ~50° C and stored for future analysis. The remainder of the core was broken up and thoroughly mixed to evenly distribute the tracer. A 24-hour incubation period was chosen to minimize the chance of invalidating the assumption that ^{15}N assimilation during the incubation period is not remineralized (Kirkham and Bartholomew 1954, Davidson *et al.* 1991). Previous work on these soils has found half-lives of ^{15}N turnover from 5 to 35 hours (Henry and Jefferies. 2003). Injecting small amounts of highly enriched $^{15}NH_4Cl$ into an intact soil core allows the investigator to monitor the dilution of the isotopic ratio in the potassium salt-extractable soil solution (see later), while minimizing the fertilizer effect.

TABLE 1 Soil and air temperatures during the 24-hour core incubation at the La Pérouse Bay field site (8 June to 15 July 2003) and at the University of Toronto laboratory (31 October 2003 and 29 April 2004).

	2003								2004
	8 June	12 June	16 June	26 June	2 July	11 July	15 July	31 Oct	29 April
soil temperature during incubation (°C)	2.0 - 6.6	3.5 - 7.9	3.5 - 8.2	4.7 - 7.6	3.8 - 7.5	9.7 - 24.3	10.3 - 6.8	1.0 - 2.0	1.0 - 2.0
air temperature during incubation (°C)	4.0 - 14.1	14.2 -20.4	4.2 - 15.9	6.0 - 11.1	4.1 - 12.7	24.3 -26.5	11.9 -19.2	-2.5 *	-14.9 *
* air temperature during collection									

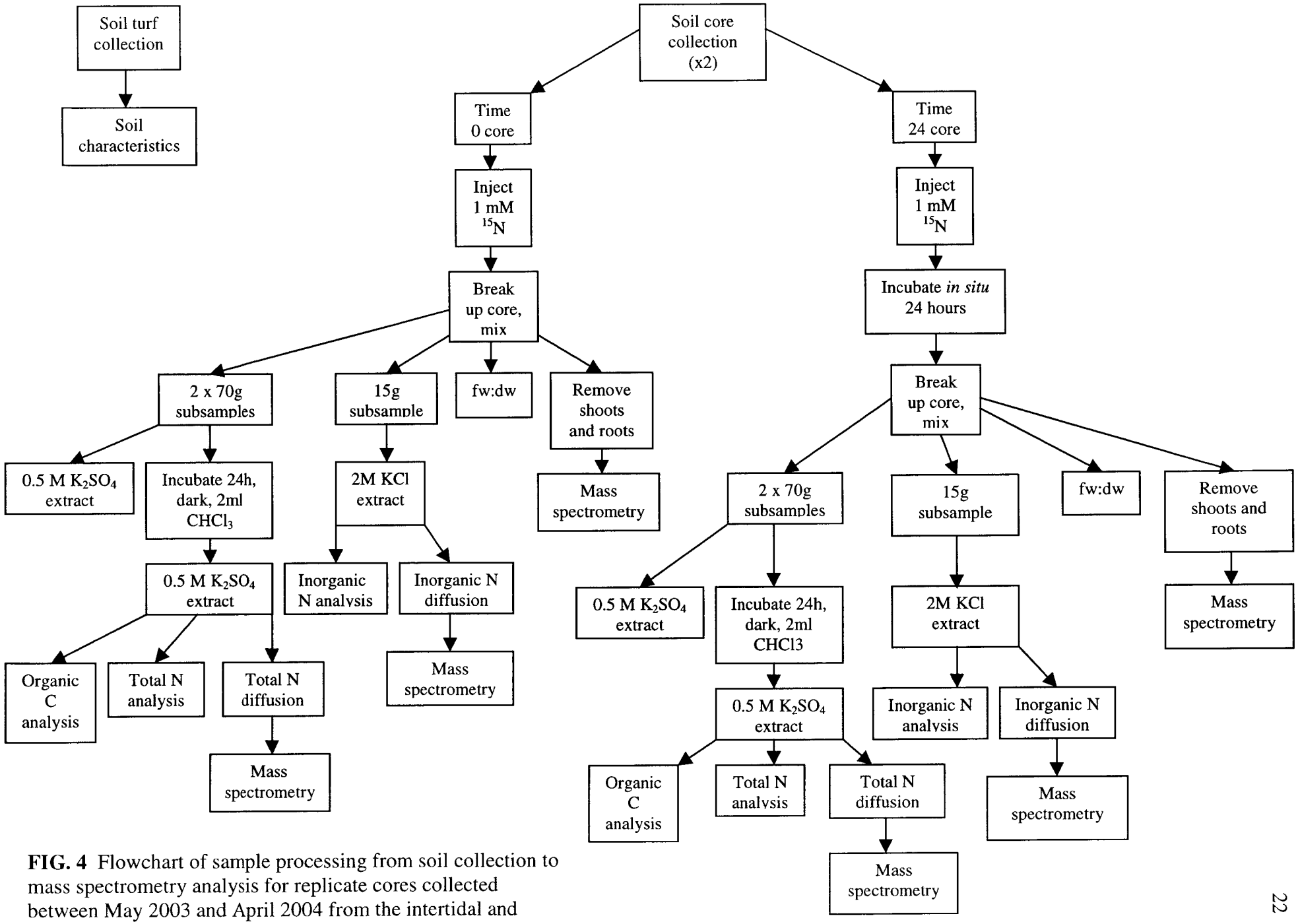

FIG. 4 Flowchart of sample processing from soil collection to mass spectrometry analysis for replicate cores collected between May 2003 and April 2004 from the intertidal and supratidal marsh at La Pérouse Bay, MB.

The mixed soil was sub-sampled for moisture content (~5 g fresh weight soil), for analysis of exchangeable nitrogen (15 g fresh weight soil) (Hart *et al.* 1994), and for estimation of microbial biomass (2 x 70 g fresh weight soil) using a modified chloroform-fumigation-extraction (Appendix 1, Witt *et al.* 2000). The sub-sample for measurement of moisture content was dried at about 50°C for about a week, then re-weighed. The sub-sample for measurement of soil exchangeable nitrogen (largely NH_4^+-N) was extracted in 75 ml of 2 M KCl for 2 hours (hand-shaken frequently), filtered through pre-leached (2 M KCl and deionized water) Whatman #40 filter paper then frozen. Of the two microbial biomass sub-samples, the first was used as a control, and immediately extracted in 140 ml of 0.5 M K_2SO_4 for 1 hour (hand-shaken frequently), filtered through Whatman GF/A filter paper, then frozen. The second sub-sample was incubated in the dark in 250 ml Schott bottles with 2 ml of ethanol-free chloroform. After 24 hours, the Schott bottles were opened for 30 minutes to expel excess chloroform, then extracted in the same manner as the control sample. On the second day, the second core from the injected pair was sub-sampled and extracted in the same manner as the control cores. The difference in microbial C and N content between the control and the fumigated sample at time 0 and after 24 hours indicates C and N immobilization by microbes after 15 minutes (the amount of time required to inject, mix, sub-sample and extract the time 0 core), and over the 24-hour incubation period. This is an estimate of N allocation to microbes, and the amount of microbial biomass (based on microbial C).

Winter samples were collected frozen and transported frozen to the laboratory in Toronto. The soils were thawed in the refrigerator overnight to 0°C, cut into 6.5 cm deep sample replicates (5 replicates x 4 treatments x 2 cores each = 40 cores, each 180 cm^3 in

size collected in October 2003; 3 replicates x 4 treatments x 2 cores each = 24 cores, each 50 cm^3 in size collected in April 2004), injected with 1 mM 98 atom percent $^{15}NH_4Cl$ (5 x 1 ml in October, 4 x 0.4 ml in April), then processed as with the summer samples, with the second core of each replicate incubated for 24 hours at 2°C before extraction.

Biological and chemical analyses

Soil salinity was measured on soil water that had been extracted by hand squeezing. Sodium content was measured with a Perkin-Elmer atomic absorption spectrophotometer (Model 3110, Norwalk, CT) in the flame-emission mode at a wavelength of 589 nm and an air-acetylene flame. These values were then converted to salinity using the regression equation: salinity (g dissolved solids L^{-1}) = 3.59 $[Na^+]$ g L^{-1} + 3.85 (r^2 = 0.96), where $[Na^+]$ is the concentration of sodium ions in solution, determined previously at this site (Srivastava and Jefferies 1995).

The exchangeable inorganic N and that in the soil solution were measured in 2 M KCl soil extracts with the use of an auto analyzer (Technicon AAII, Tarrytown, NY), using the appropriate blanks and standards. Nitrate concentrations were determined by measuring the intensity of colour formed upon complexing with a diazotizing reagent after reduction to nitrite with cadmium (Keeney and Nelson 1982). Ammonium concentrations were determined with the indo-phenol blue method (Keeney and Nelson 1982), which measures the intensity of the colour complex formed when phenol reacts with NH_3 under alkaline conditions.

Soil C:N ratios were determined on oven-dried soil, using an Elemental Combustion System 4010 CHNS-O Analyser (COSTECH International, Valencia, CA).

The total extractable nitrogen in 0.5 M K_2SO_4 soil extracts was determined, to estimate microbial N and dissolved total N. The extracts were treated with alkaline persulfate to oxidize the different forms of N to nitrate (Cabrera and Beare 1993, Appendix 2), The nitrate was measured with the use of an auto analyzer, as described above. The microbial N is the difference between the non-fumigated (dissolved total N) and the chloroform-fumigated N. However, the estimates of microbial N were corrected for assumed incomplete recovery, based upon the use of the chloroform-fumigation extraction by multiplying the values by a factor of 2.5 (k_N = 0.4)(Jonasson *et al.* 1996). This value has been reported as suitable for Arctic soils, and is in the middle of the range of values of extraction efficiency of microbial N by K_2SO_4 (0.3 to 0.54) (Jonasson *et al.* 1996).

Microbial C was estimated by measuring C in 0.5 M K_2SO_4 soil extracts using the dichromate method (Nelson and Sommers 1996, Appendix 3). In this procedure organic C is oxidized with a hot mixture of acid and dichromate, then the excess dichromate is back-titrated with ferric ammonium sulfate using a glucose standard for calibration. The dichromate reduced during the hot digestion is assumed to be equivalent to the organic C in the sample, and the microbial C is the difference in values between the fumigated and non-fumigated samples. The non-fumigated sample measures soil organic C, whereas chloroform vapour is expected to induce lysis of microbial cell membranes and thus soil treated in this manner provides a measure of soil plus microbial C (Witt *et al.* 2000). The estimate of microbial C is corrected by a factor of 2.85 on the basis that only 35% of the C in the original microbial biomass is mineralized (k_C = 0.35)(Jonasson *et al.* 1996). This value is consistent with microbial biomass measured by substrate-induced respiration in a

number of Arctic soils (Cheng and Virginia 1993), and is slightly lower than the value commonly used in temperate, aerobic soils (k_C = 0.38, Vance *et al.* 1987). Soil Fe^{2+} interferes with the dichromate method, but interference at this site has been shown to be insignificant in previous work (Henry and Jefferies 2003).

Soil extractions using potassium salt solutions typically are based upon 1 hour of mechanical shaking (Keeney and Nelson 1982), which was replaced at the La Pérouse Bay field laboratory with 1 hour of frequent, but not continuous, manual shaking. The assumption that this change in procedure did not affect results was examined in a factorial experiment, in which the treatments were either mechanically or manually shaken after chloroform-fumigated or non-fumigated soils were extracted with 0.5 M K_2SO_4 (n = 4). The soils were collected from the supratidal intact sites in late June, equilibrated for one week in the laboratory at the University of Toronto, then the extracts of inorganic N, total N and organic C were frozen for analysis.

The efficiency of fumigation with direct application of chloroform in Schott bottles (Witt *et al.* 2000) was compared to the application of chloroform vapour under vacuum (Brookes *et al.* 1985b), as it is possible that some soil microorganisms may only be susceptible to vacuum pressure, or that the two methods may lyse the cells of different microbial populations. The efficiency of fumigation method was determined by injecting cores from the supratidal intact site with 7mL of 1mM $^{15}NH_4Cl$, incubating for one week in the laboratory in Toronto, then fumigating using both methods and extracting soils for analysis of inorganic N, and microbial N and C, as described above (n = 4). Witt and colleagues determined that 24 hours was the optimum chloroform-fumigation period for microbial biomass estimation for wet soils (Witt *et al.* 2000), and the assumption that this

fumigation period was optimum for La Pérouse Bay soils also was examined. Soils from the supratidal intact sites were fumigated for 18, 24, 30 and 36 hours before they were processed for microbial C and N and inorganic N, as described above. Experiments analyzing the accuracy of assumptions for estimation of microbial biomass did not have a correction factor applied in order that fumigated samples could be compared to non-fumigated, incubated controls.

^{15}N isotopic analyses

Nitrogen was recovered from the sample extractions for mass spectrometry analysis using a modified diffusion technique (Appendix 4, Stark and Hart 1996). This method converts NO_3^- to NH_4^+ to ammonia, which volatilizes and is collected in acidified filter paper traps, which can then be analyzed for ^{15}N content. Acid traps were made by pipetting 2.5 M $KHSO_4$ onto discs cut from pre-leached (2 M KCl and deionized water) and dried Whatman #1 filter paper. The discs were laid upon a strip of PTFE (Teflon) tape, which was folded over and sealed around each disc. The PTFE tape is permeable to gas but not liquid. Due to the low expected concentration of N in these samples and the presence of highly enriched tracer, 8 ml of sample extract was added to 20 ml of a 0.14 mM $^{14}NH_4Cl$ in 2 M KCl carrier solution. In order to recover $^{14+15}$ NH_4^+-N from the extracts, the sample and carrier, an acid trap and MgO were placed into a plastic specimen container and sealed. The MgO makes the solution basic and converts the NH_4^+ to NH_3, which then diffuses to the acid trap. The solutions were mixed daily and the containers were stored upside-down to detect leaks and to minimize gas loss, for 6 days. At the end of the diffusion period, the traps were removed, rinsed with deionized

water, and opened, and the discs were impaled on stainless steel wires and placed in a desiccator over H_2SO_4 for at least 1 day. After drying, the disc pairs were weighed and wrapped in 5 by 9 mm tin capsules and analysed for their $^{15}N/^{14}N$ ratios and N content using an Isochrom continuous flow stable isotope mass spectrometer (Micromass) coupled to a Carla Erba elemental analyser (CHNS-O EA1108) at the Environmental Isotope Laboratory, University of Waterloo. In order to obtain nitrate ($^{14+15}$ NO_3^--N) from the extracts, the samples used to recover NH_4^+-N were left open for 6 days (to prevent NH_4^+ contamination by any NH_4^+-N not yet volatilized), then replacement water, an acid trap, 0.4 g of Devarda's alloy (to reduce the NO_3^- to NH_4^+), and an additional 0.2 g of MgO were added. Additionally, the nitrate samples were spiked with 1 mM 98 atom percent $K^{15}NO_3$ in 0.714 M KNO_3 as carrier and diffused for 6 days before the traps were removed and prepared for analysis, as described above. Very little labelled nitrate was recovered in these soils, and although many samples still await analysis, the assumption is that no nitrate was produced, or that it was rapidly consumed, and therefore not recoverable in the soil solution after 24 hours.

Nitrate ($^{14+15}$ NO_3^--N) was recovered from the alkaline persulphate-oxidized samples to estimate the amount of N taken up by soil microbes over the 24 hour incubation period. Samples were prepared as above, except that 2 ml of sample plus 2 ml of persulfate reagent were autoclaved, then placed in 250 ml Mason jars (Bernardin, Rye, NY) with 20 ml of 0.18 mM $K^{14}NO_3$ in the persulfate reagent and 2 ml of 10 M NaOH added to maintain alkalinity (pH > 13). Samples were left open for 3 days to remove NH_3 in the samples, then replacement water, an acid trap, 0.4 g Devarda's alloy, and an additional 0.3 g of 10 M NaOH were added and the lids tightly closed. Samples were

shaken, kept upright and diffused for 6 days before removing and drying traps, as described above. Conversion of NH_4^+ to NO_3^- was infrequent in these soils, and an ^{15}N signal in samples analyzed for nitrate was difficult to recover. However, some samples (early spring and late winter) were analyzed a second time with twice as much carrier and standard, and values were recovered, suggesting that this method has a much lower recovery rate for NO_3^- than NH_4^+.

^{15}N enrichments were blank-corrected using a calculated blank that does not assume complete recovery of all ^{15}N in the sample (Stark and Hart 1996). This calculated blank was measured by comparing diffused and non-diffused isotope standards (see Appendix 5), where the non-diffused standard was prepared by pipetting ^{15}N as NH_4Cl directly onto paper discs. Blanks, N standards and diffused and non-diffused ^{15}N standards were included with all diffusion analyses.

Gross rates of mineralization, a better indicator of the amount of inorganic N available to plants than net mineralization because it estimates mineralization independent of immobilization, were measured over the 24-hour incubation period based on the isotope-dilution calculation (Appendix 5) (Kirkham and Bartholomew 1954, Davidson *et al.* 1991, Hart *et al.* 1994). This calculation measures the dilution of the known pool size (calculated from the initial core) of $^{15}NH_4^+$, with $^{14}NH_4^+$ as inorganic nitrogen is mineralized and new $^{14}NH_4^+$ is added to the pool. This method assumes that any NH_4^+ removed from the pool via plant or microbial immobilization, nitrification, or volatilization, would not fractionate based upon isotopic differences. A second assumption behind this method is that the incubation time encompasses only one generation of soil microbes, and the associated breakdown of soil organic material.

However, there is some question that this method may be measuring the microbial cycling and recycling of small pools of highly labile, N-rich compounds (Fierer *et al.* 2001). Therefore, ^{15}N results were also calculated as ^{15}N excess in plant, soil and microbial pools, relative to background, and after 24 hours incubation (Appendix 5), as an indicator of the relative activity of the different pools. The dissolved total soil N (DTN) is measured in the non-fumigated sub-samples from the cores that were incubated for 24 hours. This pool represents ^{15}N as soluble organic N, soluble inorganic N and K_2SO_4-exchangeable N that were not immobilized by plants or microbes over the incubation period. Gross microbial immobilization was also calculated with the isotope dilution procedure, coupled with a gross immobilization rate constant that assumes that the ^{15}N enrichment of the NH_4^+ pool declines exponentially as organic-^{14}N is mineralized to $^{14}NH_4^+$, and that the microbial pool is an infinite sink for this immobilized ^{15}N (Davidson *et al.* 1991) (Appendix 5).

Statistical analyses

Results were compared with completely randomized factorial analyses of variance (ANOVAs) (Kirk 1982). Most models incorporated a three-way factorial design, with site (supratidal and intertidal marsh) by treatment (intact and degraded) by date (four to eight dates between 30 May 2003 and 29 April 2004, varying by variable analysed). Exceptions are: plant data, which utilised a two-way factorial model of site by date; soil salinity, which utilised a two-way factorial model of site by treatment; and proportional analysis, which utilised a four-way factorial model with site by treatment by pool by date. Assumptions were analyzed with one-way ANOVAs. All analysis was performed with

JMP 4.0.2 (SAS Institute 1989-2000, Cary, NC). Before running models, the data were confirmed to be robust in terms of normality and outliers by using box plots and normal probability plots. Data were then transformed with logarithmic, inverse or square root transformations, where applicable. Proportional data (total amount of ^{15}N injected that was consumed by each pool) was arcsine-transformed before analysis. As a post-hoc analysis to the ANOVAs, significant differences between means were identified using Tukey-Kramer multiple comparison tests for unequal sample sizes (Zar 1996).

RESULTS

Testing of methodological procedures

The results show that the chloroform-fumigation method for estimating microbial biomass based on procedures of Witt *et al.* 2000 gives similar results over a broad range of incubation times. Amounts of carbon and nitrogen in microbial biomass did not show a significant change when soil was incubated for either 18, 24, 30 or 36 hours (Fig. 5) ($F_{1,10}$ = 1.59, P = 0.24; $F_{1,10}$ = 0.45, P = 0.52, for each element respectively). Similarly, after 24 hours of incubation, estimates of C and N in microbial biomass were not significantly affected by the use of alternative extraction procedures that were based on either frequent manual shaking or continuous shaking on a machine (Fig. 5) ($F_{1,4}$ = 0.02, P = 0.89; $F_{1,4}$ = 4.02, P = 0.12, C and N, respectively). A comparison of estimates of microbial biomass (Fig. 6) was made when either a vacuum was applied during soil incubation in order to allow the added chloroform to enter soil easily and promote the break-up of microbial cells, or when a similar incubation took place at atmospheric pressure. The estimates of microbial carbon, microbial nitrogen or microbial biomass ^{15}N excess ($F_{1,6}$ = 0.38, P = 0.56; $F_{1,6}$ = 1.87, P = 0.22; $F_{1,6}$ = 0.89, P = 0.38, respectively) were not significantly different based upon the use of the two methods. Therefore, we can assume that the two methods are equally effective, and as ^{15}N excess did not differ between the different incubation treatments, we can reject the hypothesis that these two fumigation methods are targeting different microbial populations. Accordingly, in the field laboratory the incubations were carried out at atmospheric pressure.

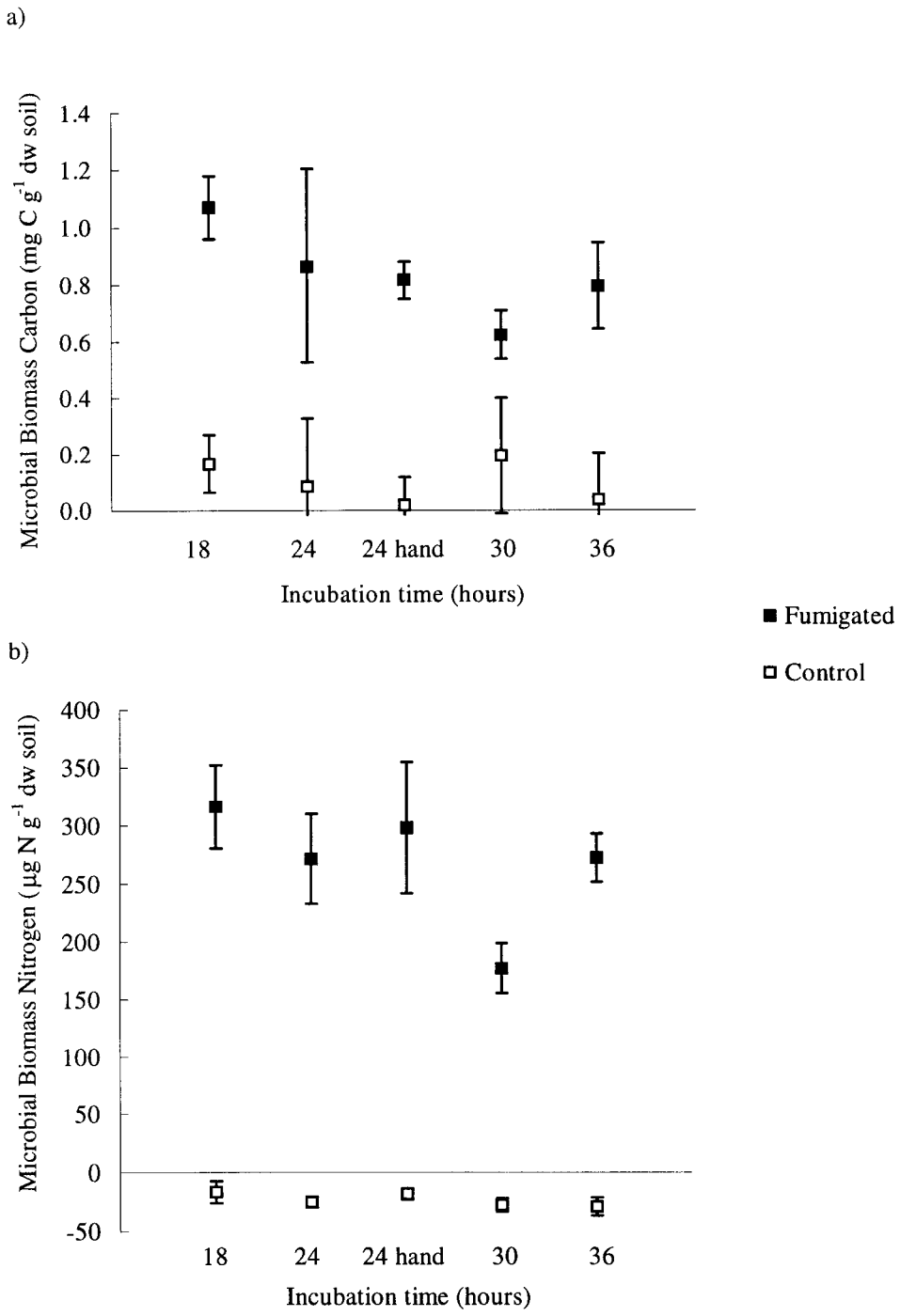

FIG. 5 The effect of incubation time and shaking method (24 = 24 hours, machine-shaken; 24hand = 24 hours, hand-shaken, all other time are machine-shaken) on **a)** microbial biomass carbon and **b)** microbial biomass nitrogen. The soil was removed from the supratidal marsh at La Pérouse Bay, MB in early July 2003 and processed at University of Toronto laboratory. Open squares are non-fumigated control means, and closed squares are chloroform-fumigated means (n=4). Bars are +/- one standard error.

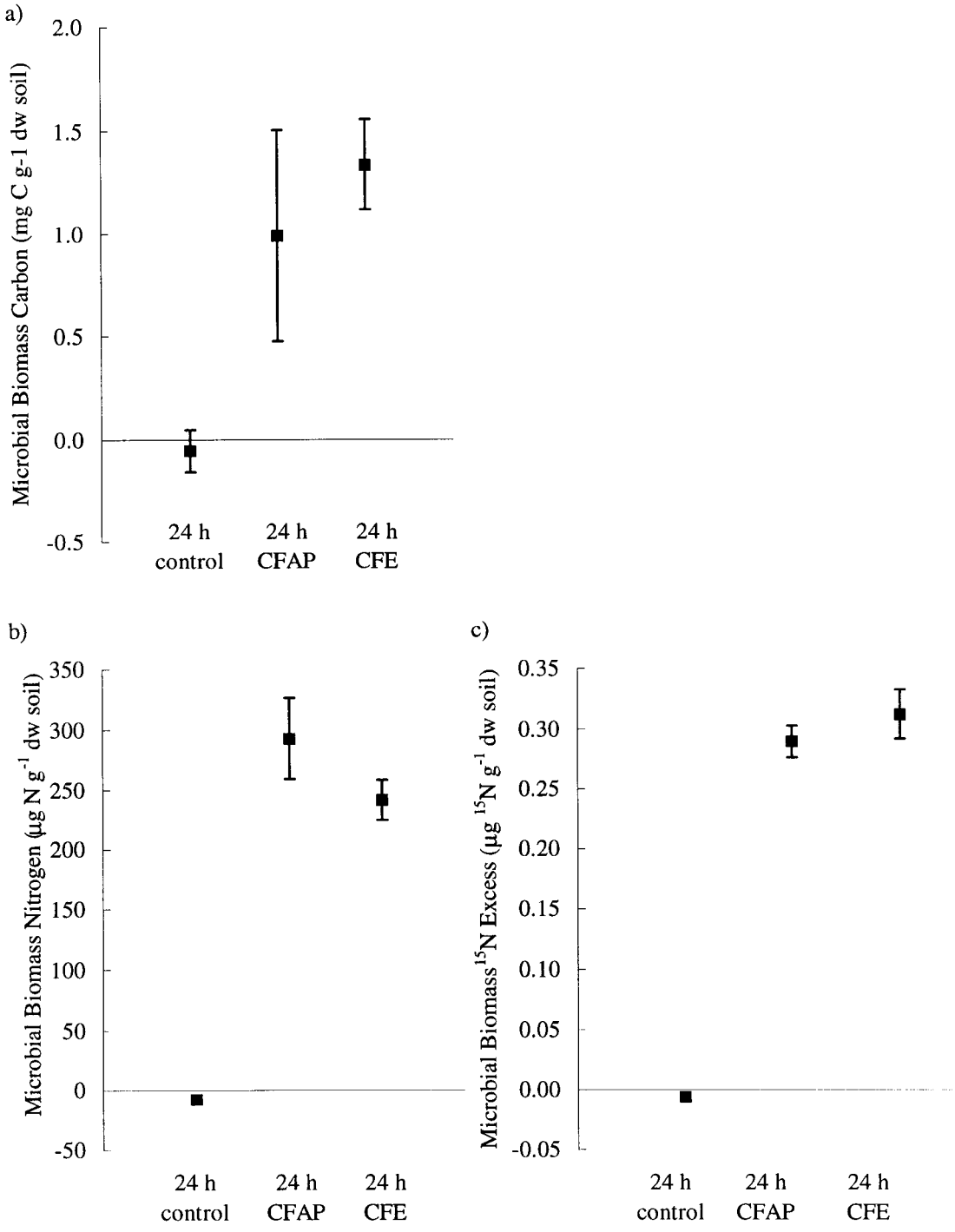

FIG. 6 The effect of a 24 hour vacuum (CFE) versus atmospheric pressure (CFAP) chloroform-fumigation-incubation, with a non-fumigated control soil, incubated at atmospheric-pressure, on **a)** microbial biomass carbon, **b)** microbial biomass nitrogen and **c)** microbial biomass ^{15}N excess, per unit dry weight. The soil was removed from the supratidal marsh at La Pérouse Bay, MB in late July 2003 and processed at University of Toronto laboratory. ^{15}N excess refers to the amount of ^{15}N measured in the microbial biomass, relative to the background natural abundance. Squares and lines represent mean and +/- one standard error, respectively (n = 4).

Seasonal changes in soil characteristics

The intertidal and supratidal sites and the treatment areas (degraded and intact) are clearly differentiated by their respective soil characteristics (Table 3). Soil moisture and salinity were higher within the supratidal marsh than the intertidal marsh ($F_{31,84}$ = 10.83, $P < 0.0001$, $F_{3,66} = 59.77$, $P < 0.0001$, respectively) (Tables 2 and 4). Even by early June, the degraded (grubbed) soils in the supratidal marsh were hypersaline, and the salinity of the soil solution was almost twice that of seawater. The soil salinity beneath intact swards approached that of seawater (~32 g of solutes per litre). In contrast, soils from the intertidal marsh were below 18 g of solutes per litre throughout the study. Soil salinity extracts from later in the season in degraded supratidal sites were not sampled, but it is evident that soil salinity increased in summer. Previous research at this site found that the soil solution salinity of the degraded soils was 75-80 g dissolved solids L^{-1} by the end of July 1991 (Wilson 1993). Soil pH did differ between marshes ($F_{7,20} = 5.42$, P = 0.001) (Table 5), but the variation in pH from neutral was minimal in both marsh soils.

TABLE 2. Three-way factorial ANOVA of soil moisture in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on eight dates between 30 May 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{31,84} = 10.83$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,84	235.31	<.0001
Treatment	1,84	17.53	<.0001
Date	7,84	6.13	<.0001
Site*Date	7,84	2.58	0.02
Site*Treatment	1,84	0.03	0.85
Treatment*Date	7,84	2.81	0.01
Site*Treatment*Date	7,84	1.24	0.29

Site	Treatment	Date	Soil moisture (% of dry weight) n = 3 to 5	Bulk Density ($g\ cm^{-3}$) n = 1 to 5	pH n = 3 to 4	Salinity (g dissolved solids L^{-1}) n = 3 to 5	Redox (mV at 2 cm depth) n = 20	Redox (mV at 5 cm depth) n = 20
Supratidal	Intact	30 May 03	213.91 (8.07)	0.38 (0.02)				
		2 June	121.68 (7.64)		7.13 (0.07)	18.57 (3.14)	314.33 (26.98)	157.00 (22.08)
		8 June	102.32 (21.72)					
		16 June	106.48 (7.00)			26.58 (3.77)	312.50 (19.76)	243.00 (18.38)
		2 July	95.76 (8.59)				193.50 (12.84)	102.50 (6.44)
		15 July	114.44 (10.04)		7.10 (0.12)	27.07 (4.04)	350.50 (8.66)	272.00 (10.50)
		31 October	139.36 (11.29)	0.55 (0.05)		21.84 (2.37)		
		29 April 04	115.20 (19.43)	0.49				
	Degraded	30 May 03	129.91 (11.81)	0.47 (0.01)				
		2 June	89.77 (4.66)		7.10 (0.06)	40.54 (6.82)	140.33 (17.13)	99.00 (21.67)
		8 June	92.74 (17.10)					
		16 June	86.04 (13.07)			59.81(22.61)	205.50 (21.52)	231.00 (14.25)
		2 July	98.36 (12.35)				115.00 (11.80)	178.00 (6.43)
		15 July	96.09 (11.49)		7.10 (0.09)		319.00 (14.01)	341.50 (8.74)
		31 October	76.92 (6.00)	0.86 (0.05)				
		29 April 04	183.24 (6.05)	0.43				
Intertidal	Intact	30 May 03	74.94 (0.45)	0.69 (0.02)				
		2 June	64.89 (3.45)		7.53 (0.03)	7.56 (1.61)	300.00 (8.34)	313.33 (6.52)
		8 June	60.71 (2.80)		7.06 (0.14)			
		12 June	60.78 (2.22)			7.62 (0.31)	333.50 (9.87)	330.00 (10.03)
		26 June	62.79 (6.46)			7.98 (1.16)	152.50 (12.60)	165.00 (10.87)
		11 July	64.25 (1.79)		7.23 (0.05)	8.16 (0.41)	406.50 (11.77)	397.50 (9.26)
		31 October	91.59 (11.93)	0.77 (0.06)		14.30 (0.77)		
		29 April 04	69.82 (1.71)	0.61				
	Degraded	30 May 03	68.78 (1.88)	0.76 (0.04)				
		2 June	50.11 (1.99)		7.53 (0.09)		242.00 (30.76)	274.67 (28.68)
		8 June	78.15 (30.57)					
		12 June	56.65 (1.26)			13.36 (2.69)	168.50 (21.88)	295.50 (17.76)
		26 June	63.48 (1.44)			9.77 (0.78)	112.00 (24.25)	184.75 (17.68)
		11 July	57.76 (1.74)		7.35 (0.03)	9.95 (1.62)	345.50 (14.11)	379.50 (8.16)
		31 October	71.13 (7.08)	0.85 (0.06)		17.99 (1.97)		
		29 April 04	64.90 (2.69)	0.81				

TABLE 3 Moisture contents, bulk density, pH, salinity and redox potential of soils at the supratidal and intertidal marshes beneath intact swards and in degraded plots at La Pérouse Bay, MB, during the study period, May 2003 to April 2004.

TABLE 4. Two-way factorial ANOVA of soil salinity in intact and degraded soils from the supratidal and intertidal marshes at La Pérouse Bay, MB. Site (marsh) is a random effect and treatment is a fixed effect. Whole model $F_{3,66} = 59.77$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,66	150.20	<.0001
Treatment	1,66	38.28	<.0001
Site*Treatment	1,66	10.59	0.002

TABLE 5. Three-way factorial ANOVA of soil pH in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on two dates (2 June and 15 July 2003). Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{7,20} = 5.42$, $P=0.001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,20	28.98	<.0001
Treatment	1,20	0.17	0.68
Date	1,20	5.66	0.03
Site*Date	1,20	4.21	0.05
Site*Treatment	1,20	0.51	0.48
Treatment*Date	1,20	0.51	0.48
Site*Treatment*Date	1,20	0.17	0.68

The large difference in soil moisture between soils from the two marshes is a reflection that soils from the supratidal marsh are rich in organic matter and those from the intertidal marsh are mineral soils as shown by values of bulk density (Table 3). Bulk density and redox potentials at a soil depth of 5 cm are higher at the intertidal site ($F_{11,24} = 7.69$, $P < 0.0001$; $F_{15,284} = 43.00$, $P < 0.0001$, respectively) (Tables 6 and 7). Overall, there is no clear trend in redox potential with increasing soil depth, but in the supratidal site with vegetation, redox values decrease with depth, while in the degraded intertidal site they increase with depth (Table 3). This result may be expected as plant roots will aerate soil.

TABLE 6. Three-way factorial ANOVA of soil bulk density in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on three dates (30 May and 31 October 2003 and 29 April 2004). Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{11,24} = 7.69$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,24	23.36	<.0001
Treatment	1,24	6.85	0.02
Date	2,24	12.52	0.0002
Site*Date	2,24	3.23	0.06
Site*Treatment	1,24	0.00	0.97
Treatment*Date	2,24	1.35	0.28
Site*Treatment*Date	2,24	2.56	0.10

TABLE 7. Three-way factorial ANOVA of soil redox potential at 5 cm depth in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on four dates (2 June to 15 July 2003). Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{15,284} = 43.00$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,284	156.05	<.0001
Treatment	1,284	0.00	0.95
Date	3,284	140.33	<.0001
Site*Date	3,284	13.32	<.0001
Site*Treatment	1,284	6.56	0.02
Treatment*Date	3,284	9.15	<.0001
Site*Treatment*Date	3,284	2.41	0.07

TABLE 8. Three-way factorial ANOVA of soil redox potential at 2 cm depth in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on four dates (2 June to 15 July 2003). Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{15,284} = 31.14$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,284	1.49	0.22
Treatment	1,284	104.88	<.0001
Date	3,284	106.48	<.0001
Site*Date	3,284	3.64	0.01
Site*Treatment	1,284	0.92	0.34
Treatment*Date	3,284	6.47	0.0003
Site*Treatment*Date	3,284	4.53	0.004

Degraded soils, regardless of site, have a lower soil moisture ($F_{31,84} = 10.83$, $P < 0.0001$) (Table 2), higher soil salinity ($F_{3,66} = 59.77$, $P < 0.0001$) (Table 4), higher bulk density ($F_{11,24} = 7.69$, $P < 0.0001$) (Table 6), and a lower redox potential at a depth of 2 cm ($F_{15,284} = 31.14$, $P < 0.0001$) (Table 8) than vegetated soils. Winter sampling generally resulted in wetter, less compact soils.

In soils of both marshes and in treatment areas, amounts of exchangeable and soluble nitrate ions (Fig. 7) decreased from an early spring flush to low levels during the summer. In early winter after 3 weeks of ground temperatures below freezing, the amounts of low soil inorganic N did not change substantially from values in summer. However, by the end of the winter (April 2004), there was a sharp increase in amounts of exchangeable and soluble ammonium ions, and by the end of May, NO_3^- concentrations were high. If data for May and early June 2003 are taken into account, together with data from April, 2004, this late winter/early spring seasonal effect of high amounts of NH_4^+ and NO_3^- ions was highly significant (NH_4^+: $F_{31,84} = 8.91$; $P < 0.0001$, NO_3^-: $F_{31,84} = 4.63$; $P < 0.0001$) (Tables 9 and 10). Within the supratidal marsh, the degraded soils had higher amounts of exchangeable and soluble ammonium ions than corresponding values in soils beneath intact swards per unit dry weight of soil.

Soil soluble NH_4^+ and NO_3^- were measured in intact and degraded soil water, in both marshes. The NH_4^+ and the NO_3^- in the soil solution averaged 0.13 and 0.05 μg N per gram of dry weight soil, respectively. Thus the soluble NH_4^+ and NO_3^- represents, on average, 5.0 % and 8.3 % of the extractable and exchangeable NH_4^+ and NO_3^-.

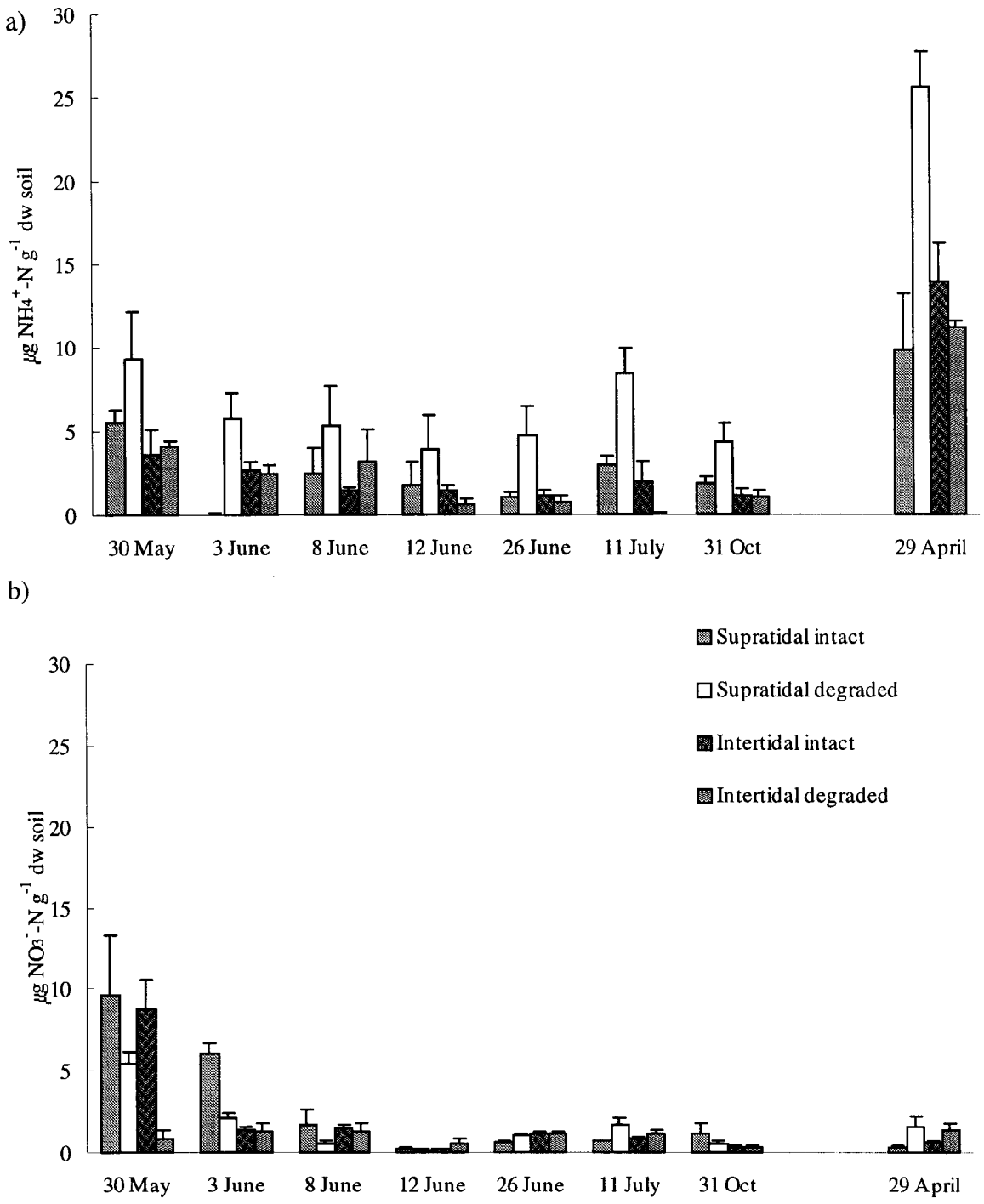

FIG. 7 Soil exchangeable and soluble (2 *M* KCl-extractable) **(a)** ammonium and **(b)** nitrate concentrations per unit dry soil weight by date, within intact and degraded plots, collected from the supratidal and intertidal marshes at La Pérouse Bay, MB. Samples were collected from the May 2003 to April 2004. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

TABLE 9. Three-way factorial ANOVA of soil inorganic NH_4^+-N in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on eight dates between 30 May 2003 to 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{31,84} = 8.91$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,84	38.59	<.0001
Treatment	1,84	8.06	0.01
Date	7,84	23.47	<.0001
Site*Date	7,84	1.59	0.15
Site*Treatment	1,84	38.92	<.0001
Treatment*Date	7,84	0.28	0.96
Site*Treatment*Date	7,84	1.68	0.12

TABLE 10. Three-way factorial ANOVA of soil inorganic NO_3^--N in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on eight dates between 30 May 2003 to 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{31,84} = 4.63$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,84	0.07	0.79
Treatment	1,84	0.39	0.53
Date	7,84	15.17	<.0001
Site*Date	7,84	2.94	0.01
Site*Treatment	1,84	0.04	0.83
Treatment*Date	7,84	1.79	0.10
Site*Treatment*Date	7,84	0.53	0.81

Soil C:N ratios (Fig. 8), based on *total* amounts of carbon and nitrogen in the soil, did not follow a common seasonal pattern. However, there was a pattern of increasing soil C:N ratio within the intertidal site, which may indicate a summer decline in N availability. Ratios for all sites ranged between 17 and 40. Intertidal soils had an overall higher C:N ratio than supratidal soils, and degraded soils in the supratidal marsh had a higher C:N ratio than values for supratidal soils with vegetative cover. ($F_{23,68} = 9.69$; $P < 0.0001$) (Table 11). Total soil percent C and N are presented in Table 12. The supratidal marsh has a greater mean total soil C and N across all dates measured.

TABLE 11. Three-way factorial ANOVA of soil C:N ratio in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on six dates between 30 May and 31 October 2003. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{23,68} = 9.69$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,68	138.70	<.0001
Treatment	1,68	14.02	0.0004
Date	5,68	3.33	0.01
Site*Date	5,68	2.87	0.02
Site*Treatment	1,68	1.76	0.19
Treatment*Date	5,68	3.49	0.007
Site*Treatment*Date	5,68	1.23	0.31

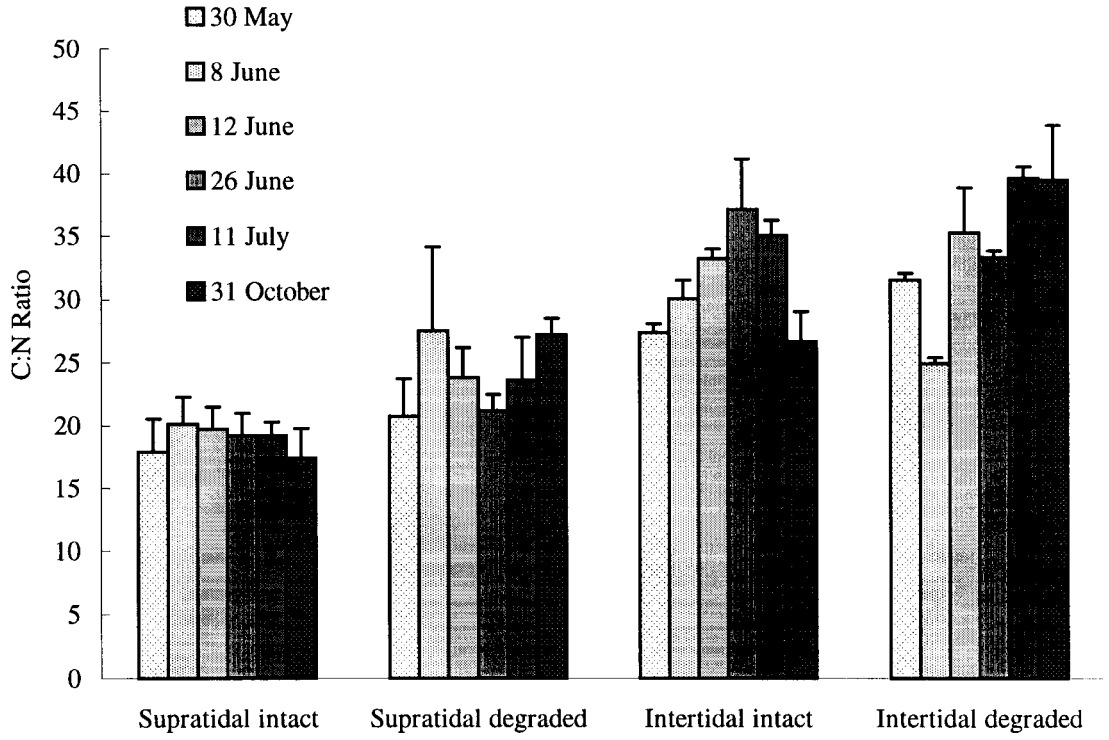

FIG. 8 Seasonal changes in soil C:N ratio for the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, from the end of May to the end of October 2003. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

TABLE 12. Soil C and N as a percent by weight of total soil. Data are mean values and standard errors for 30 May, 8 June, 12 June, 26 July, 11 July and 31 October, 2003. Soils were collected from the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB

	Total C	SE	Total N	SE
Intertidal degraded	9.53	0.14	0.29	0.01
Intertidal intact	9.81	0.27	0.33	0.02
Supratidal degraded	11.38	0.30	0.52	0.04
Supratidal intact	14.07	0.47	0.85	0.07

Seasonal controls on microbial activity

Microbial biomass carbon (MBC) in soil (Fig. 9) differs between the two marshes and between soils receiving the two treatments per unit dry weight of soil ($F_{19,56} = 3.31$, $P = 0.0003$) (Table 13). If the results are placed on a unit volume basis they are not significant for site differences ($F_{19,56} = 3.17$, $P = 0.0004$) (Table 14). The supratidal marsh soils have twice to three times the amount of microbial carbon per unit weight compared with similar values for the intertidal marsh soils, but the variance of the results at a given sampling date in the supratidal marsh soils is much greater. After an initial flush on 8 June, 2003 in the supratidal marsh, there is a decline in microbial carbon as the season progresses. In contrast, the intertidal marsh soils have relatively stable values for microbial carbon, with a slight increase in vegetated sites and an increase in the degraded sites, per unit dry weight, from spring to winter (2 June to 31 October, 2003).

Values of microbial biomass nitrogen (MBN) (Fig. 10) differ between the two marshes when expressed per unit dry weight of soil ($F_{27,76} = 6.77$, $P<0.0001$) (Table 15), but not per unit volume ($F_{27,76} = 5.04$, $P<0.0001$) (Table 16), thus this difference is a function of the greater soil bulk density in the intertidal marsh. The difference between intact and degraded soils is significant per unit dry weight, and per unit volume. Values

of MBN are less seasonally variable than those of MBC in the supratidal marsh soils, but not in the intertidal marsh soils. The estimates of MBN in late winter (April 2004) in the degraded soils contribute substantially to the seasonal differences, within site and treatment, based on both per unit dry weight and per unit volume.

TABLE 13. Three-way factorial ANOVA of soil microbial biomass carbon per unit dry weight soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 2 June and 31 October 2003. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 3.31$, P = 0.0003, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,56	9.79	0.003
Treatment	1,56	7.05	0.01
Date	4,56	3.67	0.01
Site*Date	4,56	6.34	0.0003
Site*Treatment	1,56	0.37	0.54
Treatment*Date	4,56	0.79	0.54
Site*Treatment*Date	4,56	0.90	0.47

TABLE 14. Three-way factorial ANOVA of soil microbial biomass carbon per unit volume of soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 2 June and 31 October 2003. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 3.17$, P = 0.0004, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,56	0.36	0.55
Treatment	1,56	4.32	0.04
Date	4,56	4.13	0.005
Site*Date	4,56	7.18	<.0001
Site*Treatment	1,56	0.04	0.84
Treatment*Date	4,56	0.86	0.49
Site*Treatment*Date	4,56	1.41	0.24

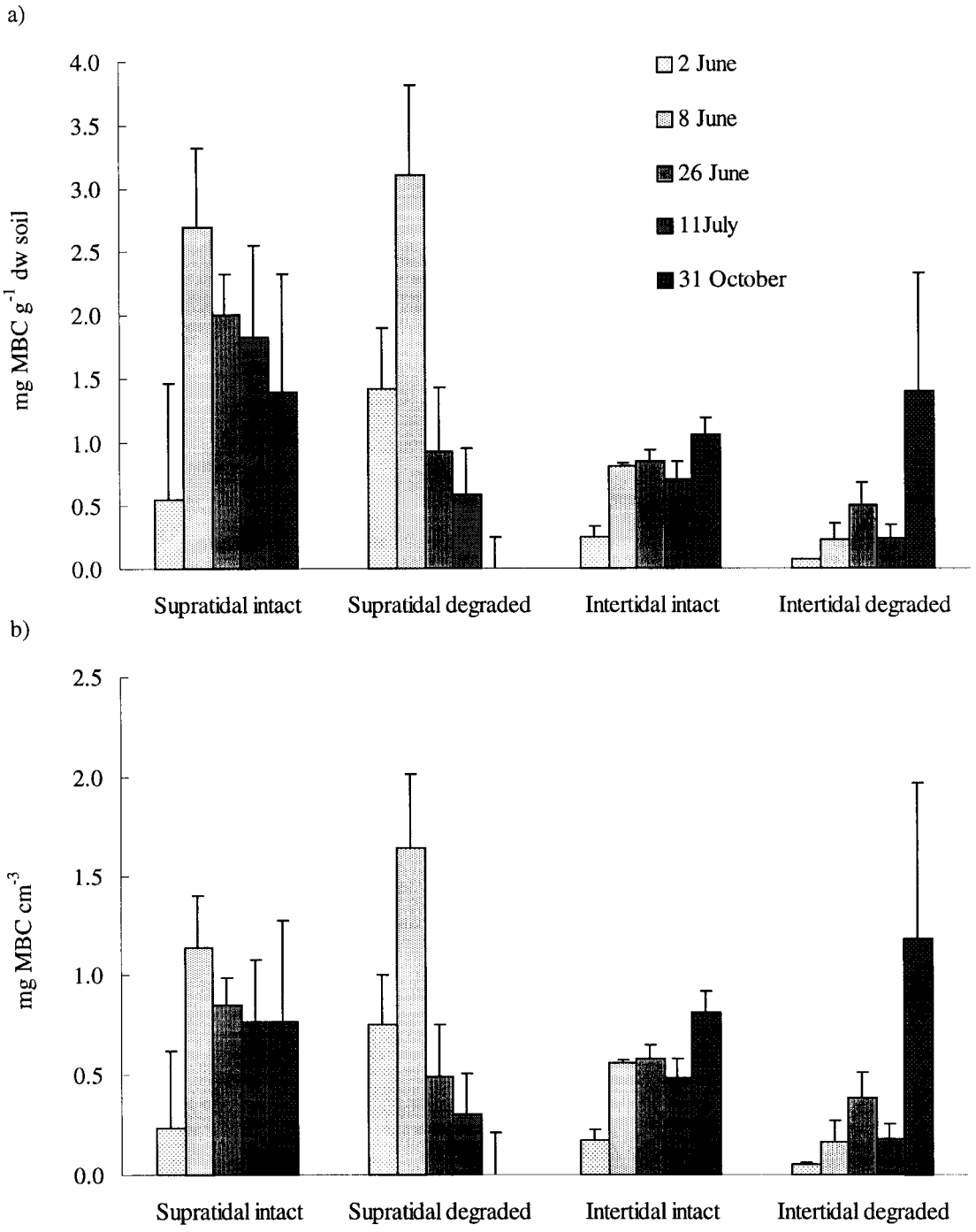

FIG. 9 Seasonal changes in microbial biomass carbon **a)** per unit dry weight (mg C g^{-1} dw soil) and **b)** per unit volume (mg C cm^{-3}) at the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, from June to October 2003. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

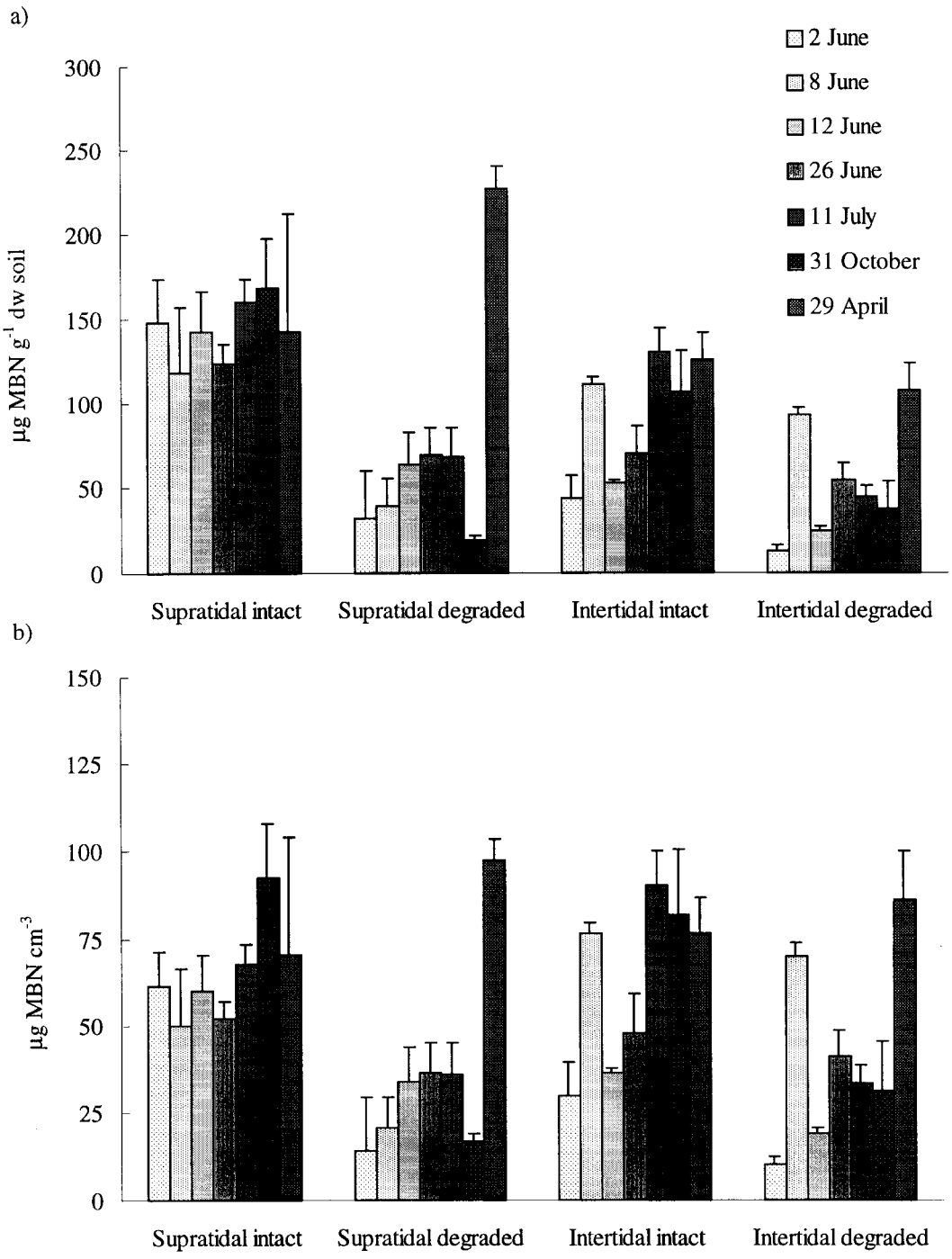

FIG. 10 Seasonal changes in microbial biomass nitrogen **a)** per unit dry weight (µg N g^{-1} dw soil) and **b)** per unit volume (µg N cm^{-3}) at the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, from June to October 2003. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

TABLE 15. Three-way factorial ANOVA of soil microbial biomass nitrogen per unit dry weight soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on seven dates between 2 June 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{27,76} = 6.77$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,76	20.72	<.0001
Treatment	1,76	47.61	<.0001
Date	6,76	7.13	<.0001
Site*Date	6,76	2.18	0.05
Site*Treatment	6,76	4.12	0.05
Treatment*Date	1,76	4.86	0.0003
Site*Treatment*Date	6,76	2.18	0.05

TABLE 16. Three-way factorial ANOVA of soil microbial biomass nitrogen per unit volume of soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on seven dates between 2 June 2003 and 29 April 2004. Site (marsh), treatment and date are fixed effects. Whole model $F_{27,76} = 5.04$, $P<0.0001$, at $\alpha = 0.05$.

Source	DF	F Ratio	P
Site	1,76	0.12	0.73
Treatment	1,76	30.69	<.0001
Date	6,76	7.44	<.0001
Site*Date	6,76	2.38	0.04
Site*Treatment	6,76	0.65	0.42
Treatment*Date	1,76	5.05	0.0002
Site*Treatment*Date	6,76	0.78	0.58

The microbial biomass carbon:nitrogen ratio (MBC:N) (Fig. 11) is higher in the supratidal marsh ($F_{19,52} = 4.87$, $P<0.0001$) (Table 17) compared with the intertidal marsh. The statistical significance of this difference is reduced by the high variance of values in degraded treatments, and this variability is associated with the inconsistency of values of MBC across dates in degraded areas (Fig. 9). In general, the supratidal intact soils have a MBC:N ratio of approximately 15, with a low ratio in spring, and the intertidal sites have a MBC:N ratio of about 10, with a lower ratio in spring and late summer.

TABLE 17. Three-way factorial ANOVA of soil microbial biomass C:N ratio in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 2 June and 31 October 2003 (12 June 2003 not included). Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,52} = 4.87$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,52	9.85	0.003
Treatment	1,52	10.21	0.002
Date	4,52	6.17	0.0004
Site*Date	4,52	5.61	0.0008
Site*Treatment	1,52	3.06	0.09
Treatment*Date	4,52	2.79	0.04
Site*Treatment*Date	4,52	3.61	0.01

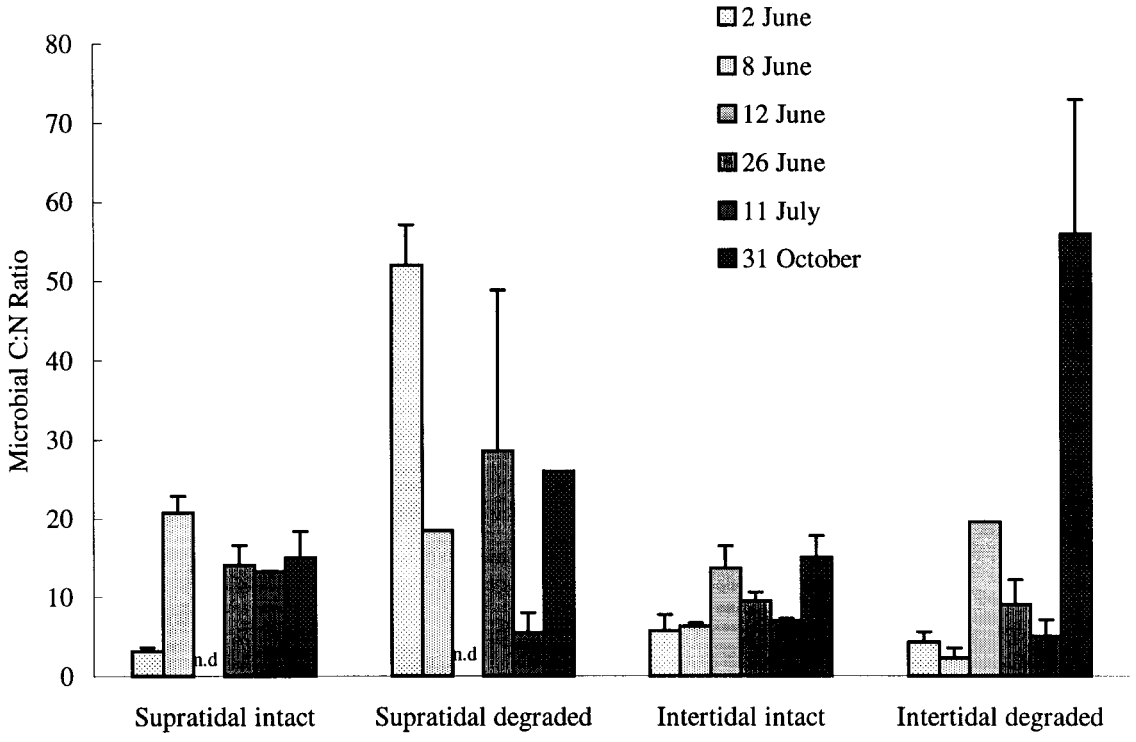

FIG. 11 Seasonal changes in the microbial biomass C:N ratio, at the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, from June to October 2003. Samples from the supratidal marsh on 12 June were lost during processing. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

The amount of label in the microbial biomass after 24 hours of incubation ($MB^{15}N$ excess) (Fig. 12) is negatively affected by the absence of plants and the associated characteristics, such as high salinity of degraded soils (per unit dry weight: $F_{19,56} = 8.24$, $P<0.0001$, per unit volume: $F_{19,56} = 4.74$, $P<0.0001$) (Tables 18 and 19). The seasonal pattern of microbial ^{15}N uptake within treatments and at sites is not significant except in interaction with a treatment effect. There is a summer and early winter peak in intact sites, with a decline in late winter. In degraded sites, there is increase in microbial activity in late winter.

There is no significant difference between marsh locations or treatments in the rate of gross mineralization ($F_{19,56} = 1.35$, $P = 0.19$) (Table 20), as measured by the use of the isotope dilution method (Fig. 13a & b). Despite the large amount of NH_4^+-N present in the marsh soils in late winter (April 2004) (Fig. 7a), there was little to no gross mineralization occurring in soils at that time. However, the seasonal trend of a summer peak and a winter decline is significant (Fig. 13a). Low gross mineralization and high exchangeable and soluble NH_4^+-N in late winter is supported by low gross microbial immobilization at this time (Fig. 13c). In general, microbial inorganic N immobilization is much lower at degraded sites ($F_{19,56} = 7.76$, $P<0.0001$) (Table 21), and there is a significant peak in summer and early winter followed by a late winter decline at vegetated sites in both marshes.

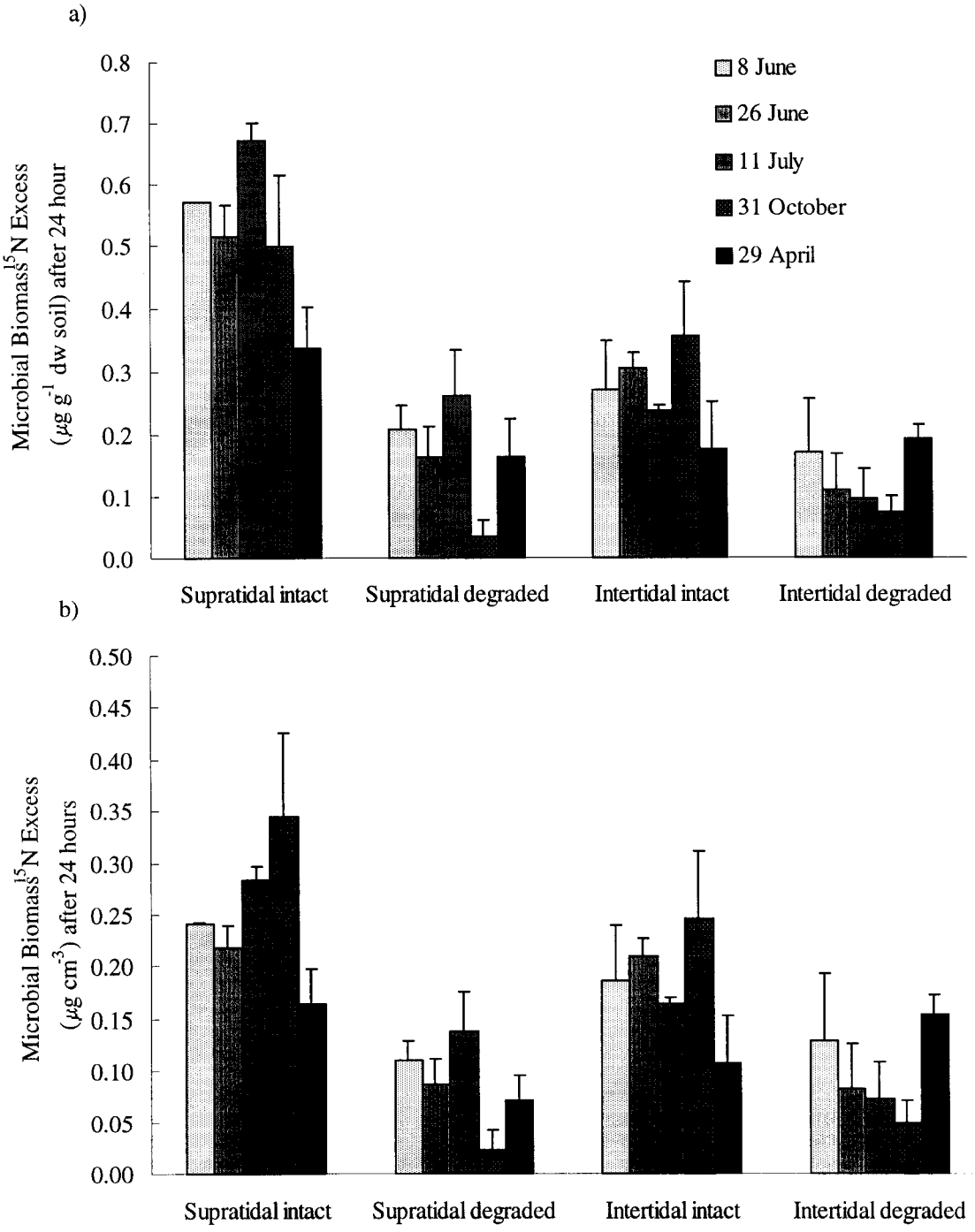

FIG. 12 Seasonal changes in ^{15}N incorporation after 24 hours into the microbial biomass nitrogen **a)** per unit dry weight (μg ^{15}N g^{-1} dw soil) and **b)** per unit volume (μg ^{15}N cm^{-3}), at the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, from June 2003 to April 2004. ^{15}N excess refers to the amount of ^{15}N measured in the corrected ($k_N = 0.4$) extractable microbial N, relative to the background natural abundance. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

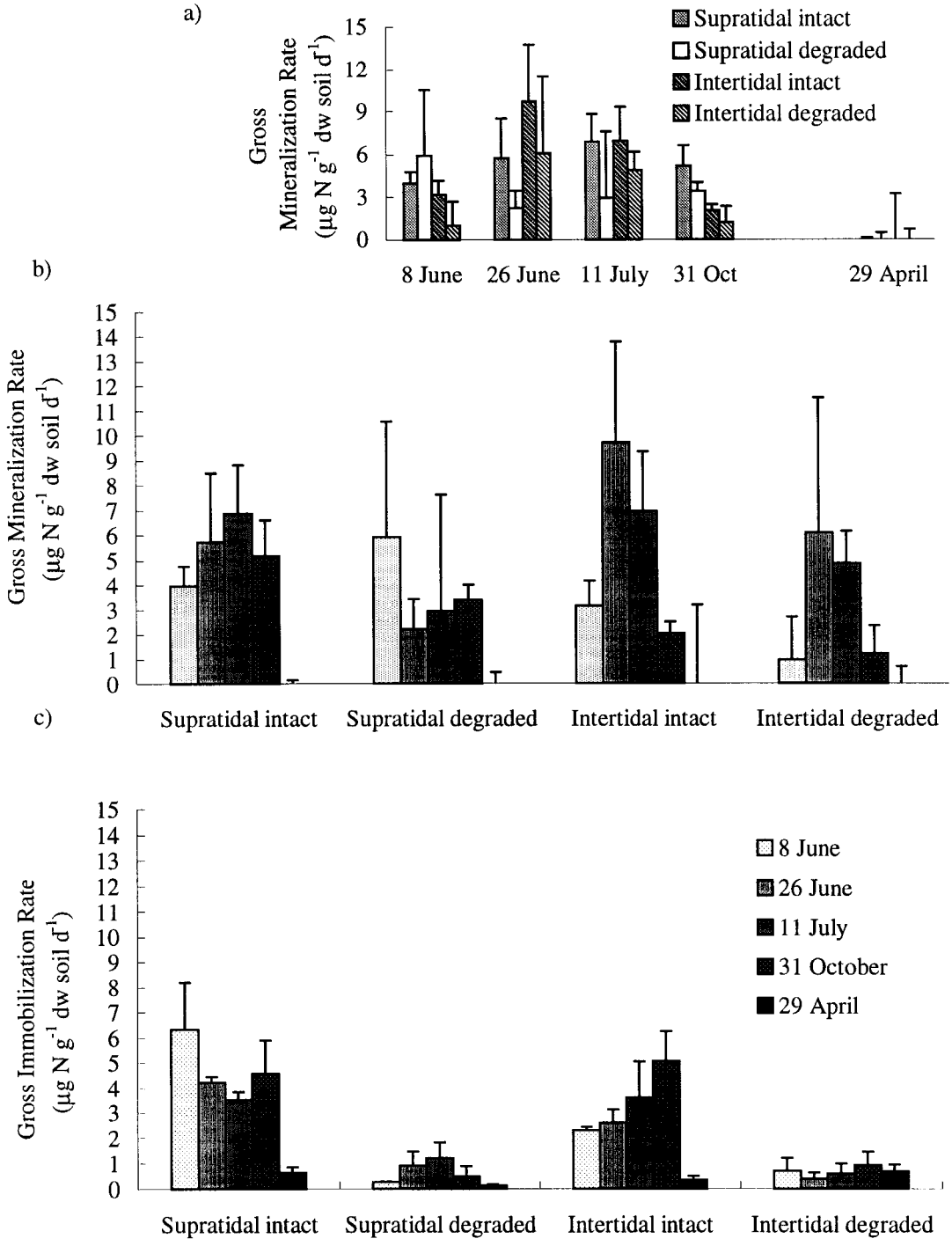

FIG. 13 Rates of **a)** gross mineralization by date **b)** gross mineralization by marsh site and **c)** gross immobilization by marsh site, per unit dry weight of soil per day, as calculated by the isotope dilution equation and assuming an exponential decline of the ^{15}N pool. Soils were collected from the supratidal and intertidal marshes within intact and degraded areas at La Pérouse Bay, MB, from June 2003 to April 2004. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

TABLE 18. Three-way factorial ANOVA of soil microbial biomass ^{15}N excess per unit dry weight soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 8.24$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	24.66	<.0001
Treatment	1,56	74.74	<.0001
Date	4,56	1.70	0.16
Site*Date	4,56	2.67	0.04
Site*Treatment	1,56	13.74	0.0005
Treatment*Date	4,56	2.83	0.03
Site*Treatment*Date	4,56	0.17	0.95

TABLE 19. Three-way factorial ANOVA of soil microbial biomass ^{15}N excess per unit volume in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 4.74$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	1.25	0.27
Treatment	1,56	44.27	<.0001
Date	4,56	0.68	0.60
Site*Date	4,56	1.00	0.41
Site*Treatment	1,56	4.51	0.04
Treatment*Date	4,56	4.62	0.003
Site*Treatment*Date	4,56	0.46	0.77

TABLE 20. Three-way factorial ANOVA of soil gross N mineralization rate per unit dry weight soil per day in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh), treatment and date are fixed effects. Whole model $F_{19,56} = 1.35$, $P = 0.19$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	0.16	0.69
Treatment	1,56	0.82	0.37
Date	4,56	4.47	0.003
Site*Date	4,56	1.11	0.36
Site*Treatment	1,56	0.44	0.51
Treatment*Date	4,56	0.27	0.90
Site*Treatment*Date	4,56	0.16	0.96

TABLE 21. Three-way factorial ANOVA of soil gross N immobilization rate per unit dry weight soil per day in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh), treatment and date are fixed effects. Whole model $F_{19,56} = 7.76$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	0.41	0.52
Treatment	1,56	83.83	<.0001
Date	4,56	6.05	0.0004
Site*Date	4,56	1.37	0.26
Site*Treatment	1,56	2.48	0.12
Treatment*Date	4,56	3.99	0.006
Site*Treatment*Date	4,56	0.99	0.42

The effect of absence of vegetation on soil nitrate-N and ammonium-N

The lack of vegetation in degraded marsh soils apparently has a significant positive effect on exchangeable and soluble amounts of label in the soil. This effect of treatment is evident for both the soluble and exchangeable $^{15}NH_4^+$-N pool (Fig. 14) (per unit dry weight: $F_{19,56} = 7.84$, $P<0.0001$, per unit volume: $F_{19,56} = 7.60$, $P<0.0001$) (Tables 22 and 23) as well as for the total soluble and exchangeable ^{15}N ($DT^{15}N$) (Fig. 15) (per unit dry weight: $F_{19,56} = 27.42$, $P<0.0001$, per unit volume: $F_{19,56} = 25.43$, $P<0.0001$) (Tables 24 and 25). Lower values for ^{15}N excess in soluble and exchangeable nitrogen $DT^{15}N$ versus $^{15}NH_4^+$-N are a product of the calculation for ^{15}N excess, which multiplies pool size by atom percent excess ($^{15}N/^{14}N$). This dilutes the ^{15}N excess signal in the $DT^{15}N$ pool, as most of the ^{15}N in this pool are probably from the original $^{15}NH_4Cl$ injection. At sites with intact vegetation in late winter, when plants are present but not active, there is a significant amount of label remaining in solution, comparable to that in degraded soils. This high amount of label remaining in the soil solution of cores from

degraded soils and winter soils may be analogous to a loss of N from the internal soil cycle in an open system without plants.

TABLE 22. Three-way factorial ANOVA of soil extractable and exchangeable $^{15}NH_4^+$ excess per unit dry weight soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 7.84$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	22.44	<.0001
Treatment	1,56	36.32	<.0001
Date	4,56	14.82	<.0001
Site*Date	4,56	4.34	0.004
Site*Treatment	1,56	0.31	0.58
Treatment*Date	4,56	2.79	0.03
Site*Treatment*Date	4,56	0.37	0.83

TABLE 23. Three-way factorial ANOVA of soil extractable and exchangeable $^{15}NH_4^+$ excess per unit volume in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 7.60$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	8.39	0.005
Treatment	1,56	47.09	<.0001
Date	4,56	12.17	<.0001
Site*Date	4,56	5.69	0.0007
Site*Treatment	1,56	0.54	0.47
Treatment*Date	4,56	2.84	0.03
Site*Treatment*Date	4,56	0.48	0.75

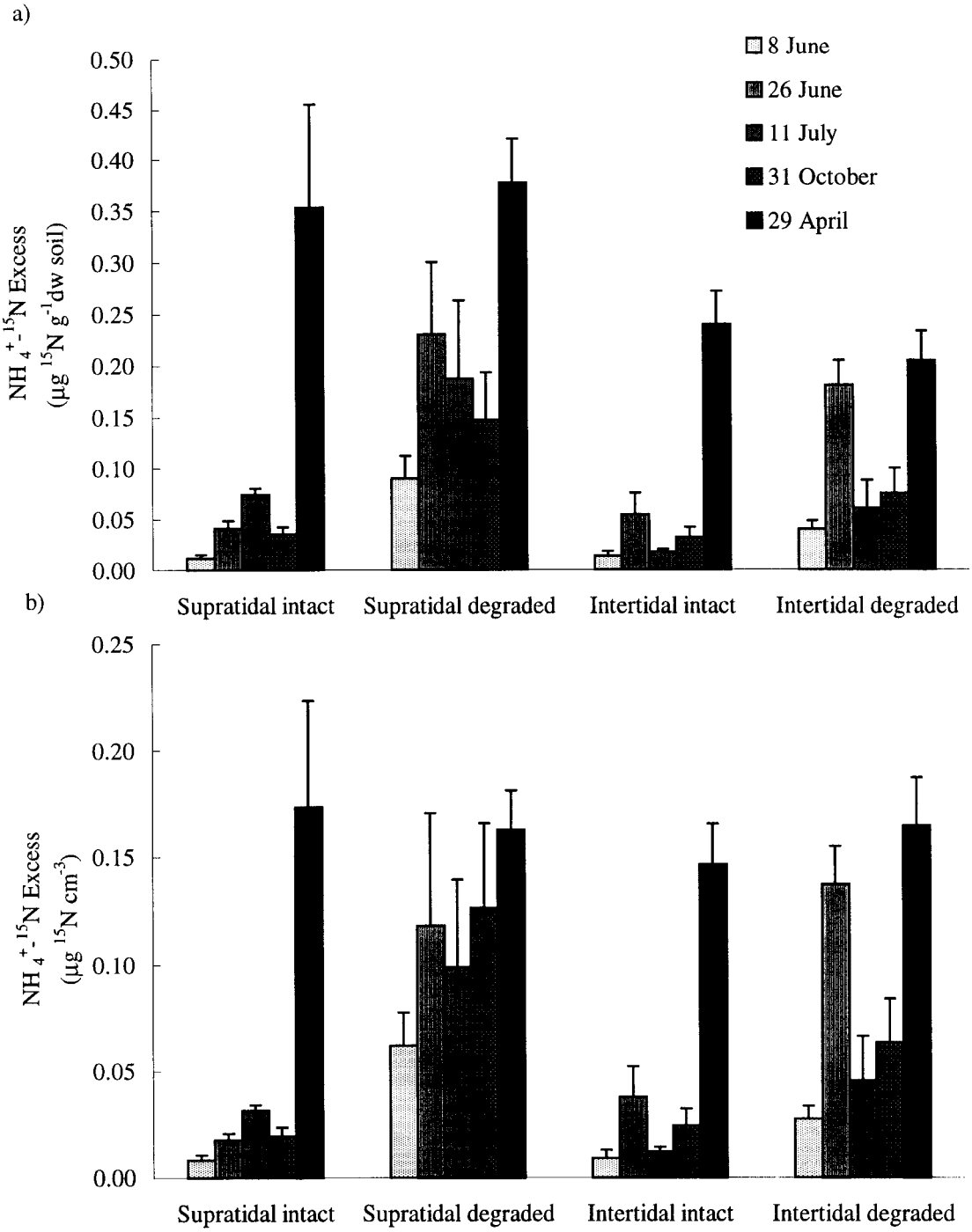

FIG. 14 Seasonal changes in ^{15}N incorporation after 24 hours into the exchangeable and soluble ammonium pool, **a)** per unit dry weight ($\mu g\ ^{15}N\ g^{-1}$ dw soil) and **b)** per unit volume ($\mu g\ ^{15}N\ cm^{-3}$), at the supratidal and intertidal marshes within intact and degraded areas at La Pérouse Bay, MB, from June 2003 to April 2004. ^{15}N excess refers to the amount of ^{15}N measured in the soil extracts, relative to the background natural abundance. Bars and lines represent mean and one standard error, respectively (n=3 to 5).

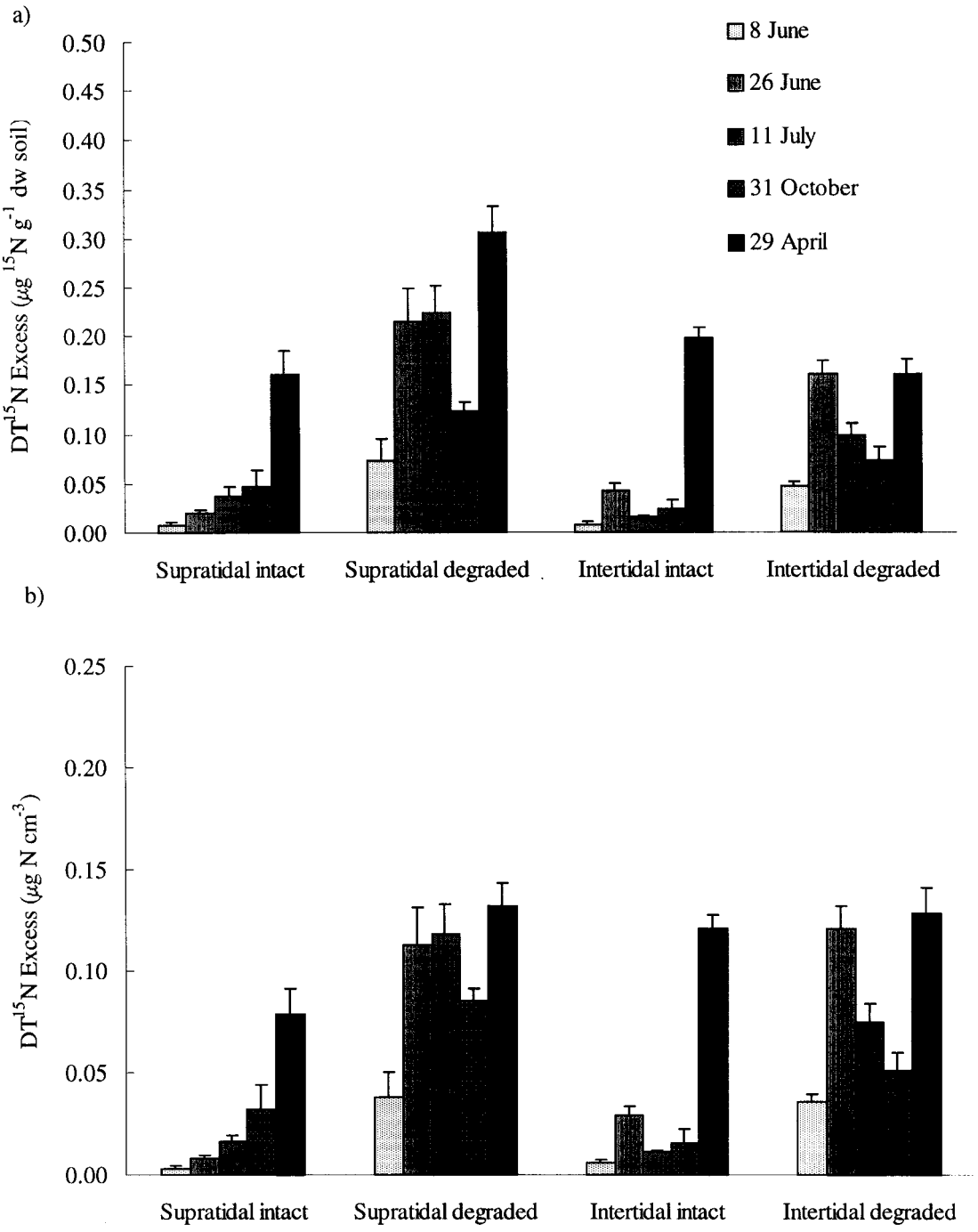

FIG. 15 Seasonal changes in ^{15}N incorporation after 24 hours into the dissolved total nitrogen pool **a)** per unit dry weight (μg ^{15}N g^{-1} dw soil) and **b)** per unit volume (μg ^{15}N cm^{-3}), at the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, from June 2003 to April 2004. ^{15}N excess refers to the amount of ^{15}N measured in the soil extracts, relative to the background natural abundance. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

TABLE 24. Three-way factorial ANOVA of soil extractable and exchangeable total ^{15}N excess per unit dry weight soil in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh) is a random effect, treatment and date are fixed effects. Whole model $F_{19,56} = 27.42$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	17.54	0.0001
Treatment	1,56	198.61	<.0001
Date	4,56	52.82	<.0001
Site*Date	4,56	3.00	0.02
Site*Treatment	1,56	17.31	0.0001
Treatment*Date	4,56	8.97	<.0001
Site*Treatment*Date	4,56	1.59	0.19

TABLE 25. Three-way factorial ANOVA of soil extractable and exchangeable total ^{15}N excess per unit volume in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh), treatment and date are fixed effects. Whole model $F_{19,56} = 25.43$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,56	0.15	0.70
Treatment	1,56	217.61	<.0001
Date	4,56	43.61	<.0001
Site*Date	4,56	5.56	0.0008
Site*Treatment	1,56	6.10	0.02
Treatment*Date	4,56	9.43	<.0001
Site*Treatment*Date	4,56	0.31	0.87

Nitrification of $^{15}NH_4^+$ did occur in these soils, especially in the early spring in soil microsites. Results were obtained for June, 2003 and April, 2004, and values were highly variable in spring within and between treatments, and low overall in late winter (Table 26). Summer values were not recovered, but this may be due to the very low amount of NO_3^- in soils combined with difficulties in recovering low amounts of NO_3^- with the diffusion procedure. As these soils were injected with $^{15}NH_4^+$, not $^{15}NO_3^-$, gross nitrification cannot be measured with pool dilution. However, in the early spring, it is evident that intact sites generally decrease in enrichment over 24 hours and degraded

sites increase. The greatest nitrate accumulation was in the supratidal degraded site, with slightly more $^{15}NO_3^-$ in the soil solution after 24 hours than $^{15}NH_4^+$. These results suggest that some nitrification is occurring in 24 hours, that plants or microbes are consuming this nitrate in intact sites, but that in degraded sites nitrate is accumulating in the soil solution.

TABLE 26. ^{15}N Excess ($\mu g\ ^{15}N\ g^{-1}$ dry weight soil) in NO_3^- recovered in soils at the supratidal and intertidal marshes within intact and degraded plots at La Pérouse Bay, MB, in June 2003 and April 2004 after 15 minutes and after 24 hours of incubation. Data are means and one standard error (n=3 to 4). NO_3^- was not recovered in the summer of 2003, but this may be due to methodological error. See text for discussion.

Date	Duration	Supratidal intact	Supratidal degraded	Intertidal intact	Intertidal degraded
8 June 2003	15 minutes	0.05 (0.03)	0.04 (0.04)	0.01 (0.00)	0.03 (0.00)
	24 hours	0.02 (0.01)	0.16 (0.10)	0.02 (0.01)	0.04 (0.01)
26 June 2003	15 minutes	n.d.	n.d.	0.19 (0.05)	0.12 (0.10)
	24 hours	n.d.	n.d.	0.08 (0.02)	0.18 (0.02)
29 April 2004	15 minutes	0.00 (0.00)	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)
	24 hours	0.03 (0.01)	0.03 (0.02)	0.01 (0.00)	0.00 (0.00)

N uptake and allocation of nitrogen between shoots and roots

The primary grass in the two marshes, *Puccinellia phryganodes*, differs in ^{15}N uptake over 24 hours between marshes, per unit area (Fig. 16) ($F_{9,30} = 6.43$, $P<0.0001$) (Table 27) and per unit dry weight (Fig. 17) ($F_{9,30} = 27.05$, $P<0.0001$) (Table 28). The summer increase in ^{15}N excess is significant in grass from both marshes, but is greater in the intertidal marsh. The roots of the intertidal grass have higher ^{15}N excess at both 15 minutes after label injection, and 24 hours later, suggesting continual uptake from a N-limited system (Fig. 17) (15 minutes: $F_{9,30} = 8.27$, $P<0.0001$, 24 hours: $F_{9,30} = 4.52$, $P = 0.0008$) (Tables 29 and 30).

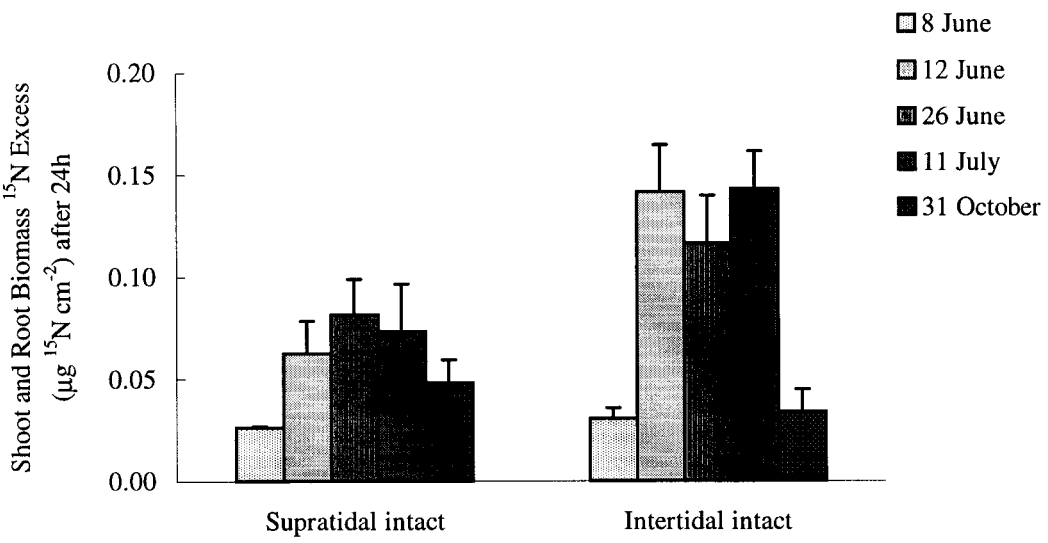

FIG. 16 Seasonal changes in ^{15}N incorporation after 24 hours into the roots and shoots of the primary salt marsh grass, *Puccinellia phryganodes*, per unit area, at the supratidal and intertidal marshes at La Pérouse Bay, MB, from June to October 2003. ^{15}N excess refers to the amount of ^{15}N measured in the plant biomass, relative to the background natural abundance. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

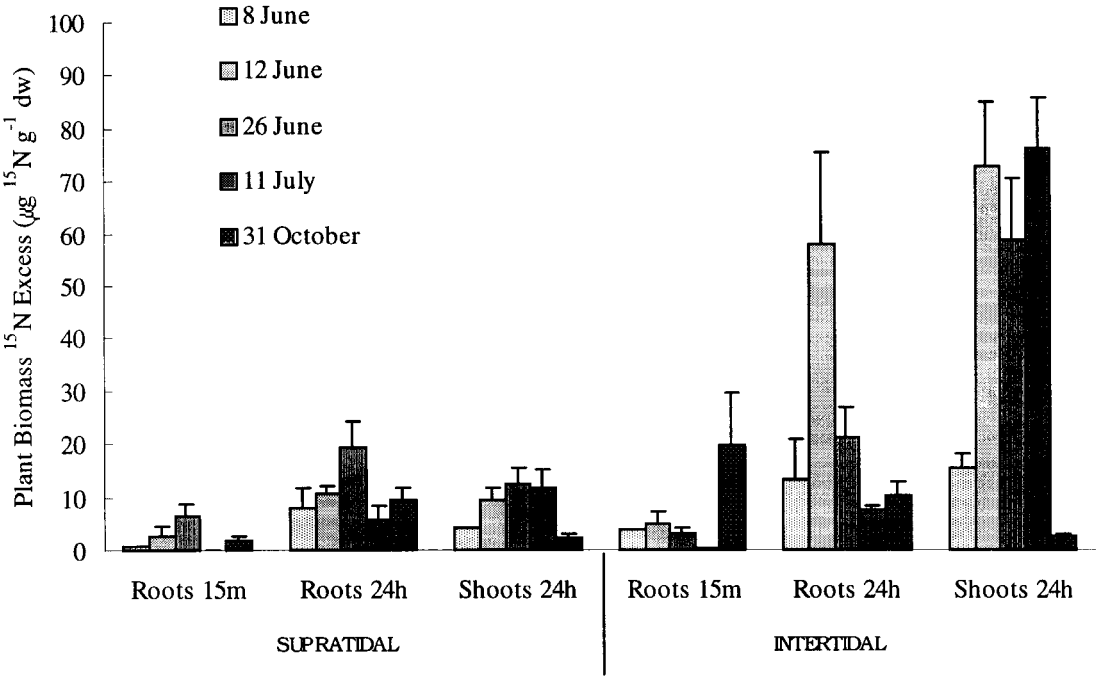

FIG. 17 Seasonal changes in ^{15}N incorporation after 15 minutes and 24 hours into the roots and shoots of the primary salt marsh grass, *Puccinellia phryganodes*, per unit dry biomass weight, at the supratidal and intertidal marshes at La Pérouse Bay, MB, from June to October 2003. ^{15}N excess refers to the amount of ^{15}N measured in the plant biomass, relative to the background natural abundance. Bars and lines represent mean and one standard error, respectively (n = 3 to 5).

TABLE 27. Two-way factorial ANOVA of tissue ^{15}N excess per unit area after 24 hours, in *P.phryganodes* shoots and roots from the supratidal and intertidal marshes at La Pérouse Bay, MB, on five dates between 8 June and 31 October 2003. Site (marsh) is a random effect and date is a fixed effect. Whole model $F_{9,30} = 6.43$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,30	8.91	0.006
Date	4,30	9.36	<.0001
Site*Date	4,30	2.92	0.04

TABLE 28. Two-way factorial ANOVA of tissue ^{15}N excess per unit dry weight after 24 hours, in *P.phryganodes* shoots from the supratidal and intertidal marshes at La Pérouse Bay, MB, on five dates between 8 June and 31 October 2003. Site (marsh) is a random effect and date is a fixed effect. Whole model $F_{9,30} = 27.05$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,30	64.15	<.0001
Date	4,30	40.56	<.0001
Site*Date	4,30	5.29	0.002

TABLE 29. Two-way factorial ANOVA of tissue ^{15}N excess per unit dry weight after 15 minutes, in *P.phryganodes* roots from the supratidal and intertidal marshes at La Pérouse Bay, MB, on five dates between 8 June and 31 October 2003. Site (marsh) is a random effect and date is a fixed effect. Whole model $F_{9,30} = 8.27$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,30	7.39	0.01
Date	4,30	13.96	<.0001
Site*Date	4,30	2.58	0.06

TABLE 30. Two-way factorial ANOVA of tissue ^{15}N excess per unit dry weight after 24 hours, in *P.phryganodes* roots from the supratidal and intertidal marshes at La Pérouse Bay, MB, on five dates between 8 June and 31 October 2003. Site (marsh) is a random effect and date is a fixed effect. Whole model $F_{9,30} = 4.52$, $P = 0.0008$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,30	10.72	0.003
Date	4,30	1.57	0.21
Site*Date	4,30	5.83	0.001

The effects of treatment on soil N allocation

The proportional distribution of added ^{15}N label within the soil (Fig. 18) indicates that both the marsh sites and treatments affect ^{15}N allocation to labile pools ($F_{59,168} = 10.90$, $P<0.0001$) (Table 31). In general, microbes dominate the ^{15}N uptake over 24 hours in soils under intact grass swards, although the proportion (c. 0.5-0.7) allocated to microbes is less than that measured in other Arctic N-cycling experiments (Grogan and Jonasson 2003, Grogan *et al.* 2004, Nordin *et al* 2004). Under intact swards in the supratidal and intertidal marshes, very little ^{15}N remains in the soil solution, except in late winter (April 2004). Plant ^{15}N uptake did not differ between sites, but was characterized by a seasonal trend, increasing from spring to summer, then decreasing by early winter, and this trend was significant in the intertidal marsh (Figs. 16, 17 & 18).

TABLE 31. Four-way full factorial ANOVA of proportion of original ^{15}N injection recovered in plant, microbial and soil solution pools in intact and degraded soils within the intertidal and supratidal marshes at La Pérouse Bay, MB, on five dates between 8 June 2003 and 29 April 2004. Site (marsh) is a random effect, and pool, treatment and date are fixed effects. Whole model $F_{59,168} = 10.90$, $P<0.0001$, at $\alpha = 0.05$

Source	DF	F Ratio	P
Site	1,168	5.09	0.03
Treatment	1,168	45.66	<.0001
Pool	2,168	127.80	<.0001
Date	4,168	1.36	0.25
Site*Treatment	1,168	1.11	0.29
Site*Pool	2,168	5.24	0.006
Site*Date	4,168	1.66	0.16
Treatment*Pool	2,168	65.01	<.0001
Treatment*Date	4,168	2.05	0.09
Pool*Date	8,168	5.72	<.0001
Site*Treatment*Pool	2,168	6.92	0.001
Site*Treatment*Date	4,168	0.69	0.60
Site*Pool*Date	8,168	1.00	0.44
Treatment*Pool*Date	8,168	5.53	<.0001
Site*Treatment*Pool*Date	8,168	0.89	0.53

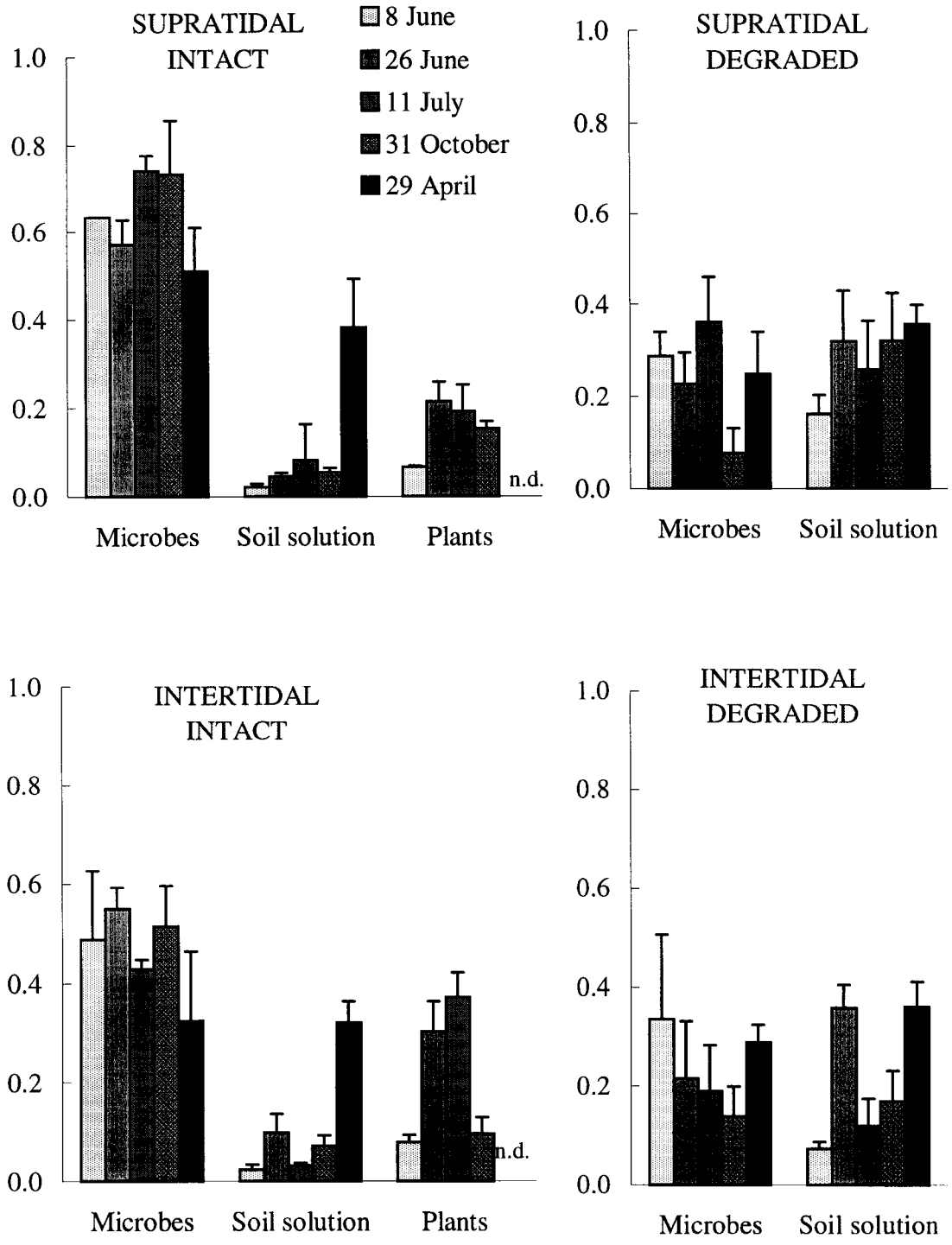

FIG. 17 Distribution of ^{15}N after 24 hours, as a proportion of the original amount of ^{15}N injected, into the microbial biomass, the plant biomass and the soil solution, at the supratidal and intertidal marshes, within intact and degraded plots at La Pérouse Bay, MB, from June 2003 to April 2004. Bars and lines represent mean and one standard error, respectively (n = 3 to 5). Plant biomass was not processed in April 2004.

In contrast to sites where the vegetation was intact, there was clearly less ^{15}N uptake by microbes in degraded plots, and more ^{15}N remained in the soil solution (Figs. 14, 15 & 18). Further, there was no significant difference between ^{15}N uptake to the microbial pool and the ^{15}N remaining in the soil exchangeable and soluble nitrogen pool (Fig. 18). Therefore, microbes are not compensating for lack of plant uptake and must be limited by some nutrient other than N. Alternatively, the soil microorganisms are negatively affected by the edaphic conditions of degraded soils, such as the very high salinity.

The means presented in Fig.18 are calculated as the amount of ^{15}N excess in an individual pool, per unit area, based upon the amount of ^{15}N injected per unit area. The total recovery from all pools, after 15 minutes and 24 hours (Table 32), illustrates that a large amount of label is lost over the 24-hour incubation period, and that much more is lost within the initial 15 minutes in degraded soils. On several dates, the means sum to a proportion greater than 1.00. This probably is the outcome of cumulative error arriving from the multiplicity of assumptions and calculations that are made to derive these values. For example, the recovery of the label in the total soil was examined on one sample date in late June (Table 33), and an average recovery for all soils shown in Table 33 is 97%. The total soil recovery should be closer to 80% in intact soils and 100% in degraded soils, based upon an expected 100% recovery minus the proportion allocated to plants. Furthermore, there was variation in this mean recovery, and the majority of this variation can be explained by the greater recovery calculated within the intertidal marsh soils than the supratidal marsh soils (Table 33). Examples of error include mean bulk density and biomass values used for placing extract concentrations on an area basis, and

fumigation correction factors, which do not account for variability in soil communities between sites and treatments. There is a greater amount recovered in supratidal soils in the summed pools than the total pools, and the opposite in the intertidal soils. In the situation such as the intertidal marsh, where there is greater recovery in the total soil than the summed pools, the difference between these figures is assumed to be due to rapid abiotic immobilization of label within the lattice structure of clay particles or humic material (Kaye *et al.* 2003), as discussed later. This fraction is not easily exchangeable using potassium chloride or potassium sulfate as extractants.

TABLE 32 Recovery of the proportion of initial label injected (mean and one standard error) (n = 3 to 5) across summed microbial, plant and soil pools, at time 0 (after ~15 minutes) and after 24 hours, from 8 June 2003 to 29 April 2004 at La Pérouse Bay, MB. Shoots were not analyzed at time 0 and no plants were analyzed on 29 April 2004. Proportion data should not exceed 1.00, please see text for discussion.

15 minutes (no shoots)				2003	2004
	8 June	26 June	11 July	31 October	29 April*
Supratidal intact	2.14 (0.88)	1.13 (0.19)	1.20 (0.13)	1.48 (0.10)	0.90 (0.19)
Supratidal degraded	0.77 (0.29)	0.95 (0.19)	0.90 (0.13)	0.87 (0.08)	0.26 (0.81)
Intertidal intact	2.23 (0.32)	1.24 (0.06)	1.20 (0.13)	1.05 (0.11)	0.80 (0.20)
Intertidal degraded	1.19 (0.45)	0.97 (0.11)	0.59 (0.20)	0.56 (0.15)	0.54 (0.60)
24 hours				2003	2004
	8 June	26 June	11 July	31 October	29 April*
Supratidal intact	0.72 (0.01)	0.83 (0.03)	1.01 (0.03)	1.10 (0.19)	0.89 (0.21)
Supratidal degraded	0.45 (0.05)	0.55 (0.11)	0.62 (0.11)	0.40 (0.07)	0.61 (0.10)
Intertidal intact	0.59 (0.15)	0.95 (0.06)	0.83 (0.05)	0.81 (0.13)	0.54 (0.17)
Intertidal degraded	0.41 (0.15)	0.57 (0.11)	0.31 (0.10)	0.30 (0.06)	0.65 (0.02)
					*no roots or shoots

TABLE 33 Recovery of the proportion of initial label injected (mean and one standard error) (n = 3) in total soil, at time 0 (after ~15 minutes) and after 24 hours, on 26 June 2003 at La Pérouse Bay, MB. Proportion data should not exceed 1.00, please see text for discussion.

15 minutes	26 June
Supratidal intact	0.96 (0.13)
Supratidal degraded	0.72 (0.11)
Intertidal intact	1.42 (0.09)
Intertidal degraded	1.31 (0.17)
24 hours	
Supratidal intact	0.53 (0.07)
Supratidal degraded	0.62 (0.08)
Intertidal intact	0.92 (0.15)
Intertidal degraded	1.25 (0.23)

DISCUSSION

In this study, intact cores of salt-marsh soils were monitored *in situ* for 24 hours, to determine the allocation of inorganic nitrogen between soil, microbial and plant pools. Only small amounts of highly enriched stable isotope were used for these short-term incubations, which enabled the ^{15}N label to be traced between the labile pools. Any fertilization effect of the addition of the isotope is minimal, and it is assumed that there is little microbial recycling of nutrients over the short incubation period.

Addressing the first research question, results from this study indicate that there are differences in the allocation of $^{15}NH_4^+$ between the intertidal and supratidal marshes at La Pérouse Bay. The principal difference is the greater uptake of $^{15}NH_4^+$ by *P. phryganodes* in the intertidal marsh. In addition, within each marsh, there are large differences in edaphic conditions between soils with grazed vegetation and degraded soils, which have little or no vegetation as a consequence of grubbing by lesser snow geese. The second research question examined differences between these intact and degraded marsh soils in the allocation of $^{15}NH_4^+$ to labile soil pools. Here, the main difference is the amount of label remaining in the soil solution of the degraded soils and greater microbial immobilization in the intact soils.

Generally, the upper layers of the soils of the older, supratidal marsh are rich in organic matter (Wilson and Jefferies 1996), they become hypersaline in summer and there is considerable temporal variability in the amounts of microbial carbon in soils. The third research question examined seasonal variability in microbial activity. Seasonal patterns of the proportion of N allocated to the different pools were similar between marshes, with allocations to plant and microbial biomass peaking at a ratio of

approximately 2 to 1 respectively, in summer, at which time little label remained in the soil solution. Microbial and plant uptake of label continued in the early winter, but by late winter, a large proportion of the added isotope remained in the soil solution. In degraded soils, devoid or nearly devoid of vegetation, an equal proportion of label was distributed between the microbial biomass and the soil solution in both marshes, suggesting that the uptake of the labelled ammonium ions by microbes was limited by something other than a shortage of N. Addressing the final research question, the shared use of a single resource, $^{15}NH_4^+$, suggests that plants and microbes compete for inorganic nitrogen in these marsh soils, at least from early spring until early winter.

Some of the methodological assumptions were also tested, particularly assumptions associated with the use of the chloroform-fumigation-extraction method for assessing microbial biomass as discussed below.

Testing of assumptions based upon the use of the chloroform-fumigation extraction

The measurement of amounts of carbon and nitrogen in microbial biomass is an essential requirement for quantifying many biogeochemical soil processes, yet it is plagued with procedural difficulties. An early method used to estimate microbial biomass of a soil required fumigation of a soil sample with chloroform, and then incubation of fumigated and non-fumigated soils. The flushes of CO_2-C and inorganic N, which were measured during incubation in the fumigated soil and result from the decomposition of the cell contents of the fumigated microbes by recolonizing microbial populations, were assumed to be proportional to the carbon and nitrogen in the microbial biomass before fumigation. The non-fumigated soil acted as the control (Jenkinson 1976, Jenkinson and

Powlson 1976). Several difficulties arose with the use of the method, including choosing an appropriate control based upon the assumption that the post-fumigant population would be the same as the previous population (Voroney and Paul 1984). Other difficulties included the appropriate incubation time, the proportion of microbes killed by chloroform fumigation (expressed as correction factors, k_C and k_N, which are the reciprocals of the proportions of organisms killed), and the method used to prepare the sample. In addition, the interpretation of the quantity of N mineralized by the incubated population was confounded by denitrification and immobilization that occurred during the incubation period. Many of the difficulties were overcome in the "direct extraction" method proposed by Brookes *et al.* 1985a,b, a method subsequently used by many researchers, as it did not require a post-fumigation incubation period. Microbial C and N are calculated as the difference between chloroform-labile and non-chloroform-labile C and N in a soil. However, this method still requires the fumigation of soils with chloroform in a desiccator under vacuum, a difficult procedure when using water-saturated soils (c.f. Inubushi *et al.* 1991), especially in an Arctic field camp. In a recent experimental study the effect of fumigation with chloroform on flooded rice soils at atmospheric pressure was investigated (Witt *et al.* 2000). This study found that soil-extractable C and N in fumigated samples were, on average, 6-7% greater with chloroform vapour under vacuum (CFE) than with chloroform vapour at atmospheric pressure (CFAP). Contrary to that study, the fraction of the soil microbial biomass targeted by CFE and CFAP was not significantly different in soils from the supratidal marsh at La Pérouse Bay (Fig. 6). In addition, soils subjected to the two fumigation methods were labelled with ^{15}N, and both methods produced an identical amount of label (~ 50% of the amount originally injected)

in the microbial biomass after one week. This is an important result because when ^{15}N is injected into the soil, the soil microbial community will immobilize a portion of the label, however, it will not be incorporated into the soil N *uniformly* (Brookes *et al.* 1985a). For instance, as a large proportion of $^{15}NH_4$ may attach to soil humic material or be taken up by different microbial populations within the soil community, it is important to clarify that the two fumigation methods (CFE, CFAP) are not targeting different soil or microbial fractions. As the two methods have produced the same amount of microbial N and the same amount of label has been incorporated into the microbial fraction, there is a very high probability that both methods of fumigation are targeting the same microbial fraction in the soil N (c.f. Brookes *et al.* 1985a).

As with the above modification of the fumigation procedure, a fumigation period of one day is a widely-used modification of the original incubation period of five days (Brookes *et al.* 1985b). Here the assumption is that the maximum flush is reached in five days, but that a constant, well-correlated proportion of the release occurs after one day. Several researchers have investigated the effect of time on the release of C and N from microbial biomass (Brookes *et al.* 1985a, Davidson *et al.* 1989, Ross and Tate 1993, Witt *et al.* 2000, Haubensak *et al.* 2002), based on incubation periods of different soils ranging from four hours to ten days. It is generally accepted that the proportion of the flush recovered after one day relative to five days varies between a proportion of ~0.5 to 1.0. Much of the range of values corresponds to variation in soil type, and that over half of the soils tested required only one day of fumigation (Haubensak *et al.* 2002). As the assumption that one day was a suitable fumigation period had not been tested in sub-Arctic wetland soils, the effect of fumigation time on release of microbial C and N was

examined. There was no significant difference in microbial C and N, when soils were incubated either for 18, 24, 30 or 36 hours of fumigation (Fig. 5). The assumptions were only tested on the supratidal soils, and similar studies of intertidal soils are needed. However, relative to the range of soils often examined by other researchers, the two marsh soils studied at La Pérouse Bay are very similar. The final assumption tested was that frequent manual shaking and continuous mechanical shaking of 0.5 M K_2SO_4 soil extracts did not differ significantly in amounts of exchangeable and soluble inorganic N extracted. The assumption was found to be correct (Fig. 5), and soil extracts could be prepared in the field laboratory without the need for a heavy mechanical shaker.

Another assumption inherent in this method to estimate microbial biomass is that the factors that correct for incomplete fumigation (coefficients k_C and k_N) are constant across soil types and seasons. Many of the correction factors used by researchers are compiled using the original fumigation-incubation method, by adding known organisms and measuring the flush of N produced upon their decomposition. The organisms used are typically a wide range of culturable yeasts, fungi, actinomycetes and bacteria. These microbes often are not native to the soil fumigated and may not even be typically found in the soil environment (Jenkinson 1976). A large amount of literature has been produced in response to this method, and the studies have led to some important changes to the correction factors (Cheng and Virginia 1993, Davidson *et al.* 1989, Brookes *et al.* 1985b, Voroney and Paul 1984). However, a method of assessing the appropriate correction factor at a site, within a reasonable time, is required. Furthermore, with evidence that microbial populations change with season (Schadt *et al.* 2003), the application of a single factor for both summer and winter is highly assumptive. As winter microbial activity

becomes recognized as an important factor in the annual cycling of nutrients, microbial ecologists will likely respond to this uncertainty. Unfortunately, addressing this assumption with the design of suitable experiments is beyond the scope of the present study.

Controls on nitrogen availability for microbial and plant growth

A comparison of the characteristics of the two marsh soils indicates that the older supratidal marsh has a greater amount of total soil C and N per unit mass or volume than the intertidal marsh soil (Table 12), resulting in a lower bulk density and wetter soils (Table 3). However, the C:N ratio of the more organic, supratidal marsh soils falls below a theoretical dividing line between the N limitation and C limitation of heterotrophic microorganisms, with a ratio of less than 30:1 (Fig. 8) (Kaye and Hart 1997). This 30:1 ratio, below which C limitation may be expected to occur, reflects the C:N ratio of the organisms themselves and their rates of respiration and assimilation. As decomposition proceeds, the C:N ratio of plant litter increases, and thus its quality decreases with respect to the release of inorganic N from net mineralization (Paul and Clark 1996, Kaye and Hart 1997). It is important to answer the question of which one of these two elements limits the growth of soil microorganisms, in order to determine the outcome of competition between plants and microorganisms and to understand the controls on N availability to plants, which are known to be N-limited at La Pérouse Bay (Cargill and Jefferies 1984; Ngai and Jefferies 2004). In grazed sites in the supratidal marsh, net mineralization of N is in the low range of 0.5 g N m^{-2} during the snow-free season, but it is even lower in the intertidal marsh at 0.1 g N m^{-2} (Wilson and Jefferies 1996). Although

there are more exchangeable soil NH_4^+ in the supratidal sites (Fig. 7a), the allocation of label to different soil compartments, and small amount remaining in the soil solution (Fig. 18), are indicative that soils of both vegetated marshes are N-limited. Likewise, $^{15}NH_4^+$ uptake to the soil-labile pools over 24 hours indicates soil microbial N limitation in soils beneath vegetated areas at both marsh sites (Fig. 18).

As expected, degraded soils, stripped of vegetation and exhibiting associated soil characteristics, had a lower overall amount of ^{15}N recovered in the soil compartments (Table 3). On some dates, more than half the label was not recovered, and approximately half of what was recovered remained in the soil solution (Figs 14 and 15). However, whereas it was anticipated that the microbes could not compensate completely for the absence of plants and that some N would remain in the soil solution, it was not expected that other limitations on growth in degraded soils would result in a microbial $^{15}NH_4^+$ uptake lower than that in sites with grazed vegetation (Fig. 12). These results suggest that, unlike the microbial community in vegetated soils, the microbes in degraded soils are limited by something other than N. Redox potentials below 300 mV (E_h) were recorded in all marsh soils on occasions, but they were more prevalent in degraded soils, and this suggests that microbial growth may be slowed due to facultative anaerobic respiration (Paul and Clark 1996). In this situation, decomposition of soil organic matter proceeds with an alternate electron acceptor in place of oxygen. In the damp depressions from ephemeral pools within both marshes, soils are anaerobic (-200 to -100 mV), and sulfate reduction occurs. In the raised plateaus between pools, where samples for this study were taken, and where *P. phryganodes* grows, redox potentials suggest that occasionally iron reduction occurs (-100 to 100 mV), and more commonly, dissimilatory

nitrate reduction takes place (100 to 300 mV) (Paul and Clark 1996) (Table 3). The implications of this are that nitrate accumulation is short-lived and ammonium ions produced via mineralization, and dissimilatory nitrate reduction, are the main form of inorganic N in the soil (Tate 2000) (Fig.7a & b). The development of hypersaline soils in summer in the degraded supratidal marsh also may account for the decline in microbial biomass carbon (Fig. 9), and it has been shown to slow nitrification and urea hydrolysis in these soils (Wilson *et al.* 1999).

The faecal input from grazing geese is an important source of nitrogen to plants in the marsh, which is made available by the hydrolysis of urea (Wilson *et al.* 1999). In addition, the faecal pellets are a source of soluble NH_4^+ and amino acids that can be taken up directly by plants and microorganisms (Ruess *et al.* 1989, Henry and Jefferies 2002). The extreme edaphic conditions may therefore explain how the soil solution is a sink for ^{15}N, especially in degraded marsh soils, where mineralization of N may be restricted and nitrogen inputs via goose faeces represent an important source of this element in the upper layers of the soil.

Seasonal controls on nitrogen cycling

Although it has long been known that the seasons exert considerable control on Arctic soil processes, the recognition that microbial respiration and nutrient cycling still occur over the long winter are more recent discoveries (Hobbie and Chapin 1996, Oechel *et al.* 1997). There has been a flurry of studies in Arctic and alpine locations, in an attempt to clarify the seasonal patterns of nutrient cycling. A common seasonal phenomenon in alpine studies is the temporal displacement of plant and microbial N

uptake, with plants acquiring N early in the season, coincident with the start of the growth period, and microbes acquiring N later in the season, after plant senescence (Brooks *et al.* 1998, Jaeger *et al.* 1999). This trend has also been seen in a sub-Arctic forest (Grogan and Jonasson 2003). At La Pérouse Bay, there was a general pattern of high activity by both plants and microbes during the plant-growing season (Fig.18). However, in both vegetated marsh sites, there was an increase in microbial excess of ^{15}N in the early winter, after 3 weeks of frozen ground (31 October) (Fig. 12). This was coincident with a decline in the translocation of N to the shoots of plants (Fig. 17), and a lower overall plant uptake of N (Fig. 16). There was therefore a late-season displacement of N uptake at La Pérouse Bay.

The present model of seasonal microbial activity in cold soils is based on alpine studies (Schmidt and Lipson 2004). It is predicted that microbial C and N will be highly variable during the plant-growing season and then increase in the fall, finally peaking in the spring only to crash as the snowpack retreats (Lipson *et al.* 1999). These large changes in microbial growth are predicted as a response to C availability, whereby in summer, plant inputs to the soil drive microbial community dynamics through the exudation of organic compounds from the roots and sloughing-off of root cells. In autumn and winter the decomposition of dead plant material and the release of soluble nutrients from cells feeds microbial population growth. However as winter reaches a close, microbes become C-limited, and when spring warming begins and metabolism of cryogenic microbes increases, C reserves in cells are insufficient to maintain the microbial community (Schmidt and Lipson 2004). This, together with rising temperatures, led to the decline of cryogenic microbes.

At La Pérouse Bay, ^{15}N excess in microbial biomass in late spring and summer is relatively stable (Fig. 12), peaking in mid-summer in the supratidal marsh, but earlier in the intertidal marsh. Autumn measurements were not taken, but the prediction of low C reserves in late winter is seen in the soils at La Pérouse Bay, as is a low microbial C:N ratio in spring in all but the supratidal degraded sites (Fig. 11). In these soils, microbial communities may be responding more to edaphic conditions than to early season C-limitation, as soil salinity on 2 June was already higher than sea water at 40.54 g dissolved solids L^{-1} (Table 3), and microbial N was low in both degraded sites at this time of year (Fig. 10). Alternatively, the microbial C:N ratio on 2 June may not be a reflection of early spring conditions. In 2003, the spring thaw occurred earlier and faster than expected, prior to the start of sampling. The tail-end of the nutrient flush resulting from freeze-thaw cycles can be seen in the exponential decline in soil-exchangeable nitrate from 30 May to 8 June, 2003 (Fig. 7b). Presumably this decline is coincident with an increased uptake by plants and microbes, as nitrification still occurred on 8 and 26 June (Table 26), but did not accumulate in the soil solution (Fig 7b). This early spring nitrate flush is likely a by-product of microbial turnover, as there was little nitrate recovered over the summer, and none present in the soil solution in late winter of the following year after seven months of frozen ground (April 2004) (Fig. 7b) (Table 26). There was, however, a large amount of NH_4^+-N present in the soils in April 2004 (Fig. 7a). Typically, as NH_4^+ increases, chemoautotrophic nitrifiers begin to operate. However, no $^{15}NO_3^-$-N signal was detected in the soil solution after 24 hours on the April sampling date (Table 26), suggesting any nitrogen that was mineralized to NH_4^+-N was not oxidized to NO_3^--N within the 24 hours. In April, it is not likely that chemoautotrophic

nitrifiers are operating in frozen soil. Other types of microbes have been found active at temperatures as low as -20°C in soil (Rivkina *et al.* 2000), and are believed to exist in the thin water-solute film (~50 nm at -10°C) that surrounds ice-covered soil particles. However, these are predominantly psychrotolerant mesophiles (Carpenter *et al.* 2000). The nitrifying bacteria can operate at laboratory temperatures between freezing and 40°C and utilize an obligatory aerobic process (Tate 2000). Microsites for nitrification may occur in frozen, saturated soil, but these sites will be minimal. Although the activity of other microbial groups is evident in the soils processed in April, as seen from the ^{15}N excess of the microbes at that time (Fig. 9), this may be a by-product of laboratory processing and therefore does not represent true *in situ* response for that time of year. A better indicator of winter activity is the accumulation of NH_4^+-N in the soil solution (Fig. 7 a) on 29 April, 2003, a product of seven months of slow N mineralization. By late May soil temperatures are sufficiently high for nitrifying bacteria to oxidize this NH_4^+ to NO_3^- (Fig. 7 b).

The primary forage grass, *Puccinellia phryganodes* had a predictable seasonal ^{15}N-uptake pattern. The amount of nitrogen allocation to shoots was low in spring, when relatively little growth was underway, but by mid-June, shoot growth had begun and growth was maintained in the summer for the dates investigated (Fig. 16). Interestingly, active root uptake of isotope occured in soils processed in October, although shoots had senesced and little translocation was evident (Fig. 17). The high uptake in the grass roots after 15 minutes in October likely represents the demand by the active fine roots at the onset of the thaw of the cores during processing. Some plant species end the dormant

growth phase under the melting snowpack (Mullen and Schmidt 1993, Chapin 1974 a,b), and *P. phryganodes* is known to start shoot growth under snow cover in early spring.

Nitrogen allocation to soils, microorganisms and plants

The proportional allocation of ^{15}N to plants in soil cores with intact vegetation is high relative to other similar investigations of Arctic soils (Grogan and Jonasson 2003, Grogan *et al.* 2004, Nordin *et al.* 2004), and this result is consistent with the finding of previous studies at La Pérouse Bay (Henry and Jefferies 2003). During the growing season of plants the average microbe:plant ^{15}N uptake ratios are approximately 3.5:1 and 1.5:1 for the supratidal and intertidal marsh, respectively (Fig. 18). This is comparable to ^{15}N uptake ratios of approximately 7:1, 35:1 and 50:1 in other Arctic studies (Grogan and Jonasson 2003, Grogan *et al.* 2004, Nordin *et al.* 2004, respectively, based upon estimates from figures in these cited works). In the studies listed above many of the tundra plants examined were slow-growing Ericaceous species and not species with a high intrinsic growth rate, such as *P. phryganodes.*

The higher proportion of isotope allocated to plants in these soil cores may also be a grazing effect of the geese, as it has been shown that a common feedback associated with herbivore grazing is an increase in N allocation to shoots in many ecosystems (Green and Detling 2000, Guitian and Bardgett 2000), and specifically in the salt-marsh grass, *P. phryganodes,* at La Pérouse Bay (Hik and Jefferies 1990). The mechanisms accounting for this high allocation are typically linked to an increase in labile C in the rhizosphere derived from an elevated translocation of C to the roots and an increase in root exudates (Bardgett *et al.* 1998). This in turn increases microbial turnover and N

mineralization. Furthermore, increased faunal feeding on soil microbes (Guitian and Bardgett 2000) will increase this microbial turnover. However, the plant response that increases the supply of photosynthate to roots varies with species. Many graminoids are tolerant to grazing but show little or no change in N allocation with defoliation (Guitian and Bardgett 2000) suggesting that increased microbial turnover is not taking place in response to C release from root exudates. At La Pérouse Bay, the positive feedback leading to increased net primary production and new shoots that are rich in nitrogen is dependent upon the input of goose faeces, and in the absence of the addition of goose faeces following defoliation, net annual primary productivity does not increase in grazed swards (Bazely and Jefferies 1986, Hik and Jefferies 1990). The system at La Pérouse Bay is, therefore, not responding to any alleviation of C limitation via defoliation, as predicted in temperate grasslands (Bardgett *et al.* 1998), but to the direct N-fertilizer effect of the faeces. The increased allocation of N to plants in the intertidal versus the supratidal site may also be connected to grazing, as the intertidal meadow typically supports a higher density of lesser snow geese. However, the intertidal marsh has been shown to have a higher nitrogen turnover rate, independent of grazing effects (Wilson and Jefferies 1996), likely due to regular allochthonous inputs with tidal and stream flooding, of seaweed, sediment, and dissolved nutrients.

Although plants removed a substantial amount of label from the inorganic soil pool, it is clear that microbes removed more (Fig. 18). The ability of microbes to access N before plants can take up the element has long been speculated upon, because of the advantageous microbial surface-to-volume ratio and their potentially rapid growth rates in response to environmental change. However, many recent studies have shown that

plants access nutrients despite microbial competition for both organic and inorganic N (Grogan and Jonasson 2003, Henry and Jefferies 2003, Nordin *et al.* 2004). It is clear that in many ecosystems, both plants and microorganisms are limited by N, and that the relative success of either group of organisms at acquiring N is confounded by rapidly changing environmental variables (Schimel and Bennett 2004, Verchot *et al.* 2002). In the marsh soils investigated here, microbial nutrient limitations are affected by season, goose grazing and edaphic conditions.

Isotope dilution and gross nitrogen processing rates

The use of ^{15}N in intact soil cores, for short-term *in situ* incubations has been hailed as the "best way to assess plant-microbe competition events" (Kaye and Hart 1997), primarily because researchers can assess the use of the same resource by these two groups of organisms. This procedure can be combined with isotope dilution calculations, first introduced by Kirkham and Bartholomew (1954), to assess gross rates of mineralization and immobilization. Gross N mineralization is the N flux from microbes, gross N immobilization is the amount of N consumed by microbes for cell growth, and net mineralization is the difference between these two. In N-limited environments, net measurements usually record net immobilization (negative net mineralization), or very low rates of mineralization, because measurements of N accumulation over time confound the two processes. In the Arctic, net mineralization is usually a cold-season phenomenon (Hobbie and Chapin 1996, Schimel *et al.* 2004), and at La Pérouse Bay, there are very low net mineralization rates in summer (Wilson and Jefferies 1996). Gross mineralization, measured as the influx of $^{14}NH_4^+$-N into an $^{15}NH_4^+$-N pool of a known

size (Appendix 5), shows a seasonal trend opposite to net mineralization, with a strong peak in late spring, a decline in the rate in early winter to very low values in late winter at La Pérouse Bay (Fig. 13a). This late-spring/early-summer peak is higher in the intertidal site with vegetation, as indirectly indicated by higher primary production and heavy grazing (Fig. 13b). The summer peak in the intertidal degraded site may be a result of the downstream location of the intertidal marsh relative to losses of inorganic N and dissolved organic N from the more upstream degraded marsh areas. Gross microbial immobilization, measured as the exponential decline in soil $^{15}NH_4^+$-N and concomitant appearance as microbial biomass ^{15}N (Appendix 5), follows a seasonal trend opposite to gross mineralization (Fig. 13c). Thus, lower early-spring and early-winter gross mineralization rates reflect higher respective rates of gross immobilization at these times. The much lower rates of immobilization in degraded sites and late winter soils, relative to sites with vegetative cover and relative to gross mineralization rates in degraded soils, suggest that microbes in degraded sites and winter soils are utilizing N more for maintenance than for growth (Schimel and Weintraub 2003). This is further support that the growth of these microbes is limited by a factor other than N.

Gross measurements calculated by isotope dilution (Appendix 5) are useful for clarifying N dynamics, but the power of these measurements is challenged by the principal assumptions. There are three initial assumptions: (1) that there is no isotopic fractionation, (2) that injected label, once mineralized, will not then be immobilized and re-mineralized, and (3) that rates of immobilization and mineralization are constant (Kirkham and Bartholomew 1954). Most experiments will violate all of these assumptions (Davidson *et al.* 1991). However, the impact of violating these assumptions

is minimized if the length of incubation is kept short. The largest error is probably due to microbial recycling of ^{15}N, and the chance of this occurring increases with time, and as the ratio of labelled N species to unlabelled N species increases (Fierer *et al.* 2001, Kirkham and Bartholomew 1954). An extension of this microbial recycling problem is the likelihood of creating very enriched microsites if the label is not well dispersed throughout the core. A uniformly injected solution will never distribute homogeneously, and so averaged microbial "bloom" reactions at enriched microsites may not be representative of the mean microbial activity per core volume. The problem of heterogeneously labelled microsites has been well investigated, and studies show that error, while minimal, increases with net immobilization (Luxhoi *et al.* 2004, Monaghan 1995, Davidson *et al.* 1991). Another difficulty with this method is that the substrates of the process are added to estimate their rates (Hart *et al.* 1994, Davidson *et al.* 1991). Although pool dilution is not affected by this addition of substrates, even adding very small amounts of highly enriched N will probably lead to increased microbial activity and thus estimates of N immobilization.

Other important assumptions occur when calculating gross rates of immobilization (Appendix 5). The rate at which the inorganic N pool declines, in this study, is assumed to be exponential, based upon the research of Davidson *et al.* (1991), which predicts inorganic turnover within 1 day. Although the model constructed by Davidson and colleagues is a refinement over assuming a linear rate of decline, the exponential decline is based upon a single compartment system and an infinite sink for the label (Sheppard 1962, Davidson *et al.* 1991). A more appropriate model would

consider at least two compartments, and the potential for label saturation with alternate limitations, such as P or C, or salinity toxicity, as is probable in these degraded soils.

An interesting and not well-understood topic that has arisen from a number of ^{15}N studies is the abiotic loss of N in short-term soil core incubations. In several studies, the ^{15}N returned in labile pools has been less than 100%, with the remainder of the label recovered in analysis of the total soil (Davidson *et al.* 1991, Perakis and Hedin 2001, Barrett and Burke 2002, Fitzhugh *et al.* 2003, Kaye *et al.* 2003). Originally, it was assumed that this loss was due to experimental "noise", but is now credited to abiotic immobilization (Davidson *et al.* 2003). Relatively little is understood about the pathways for abiotic versus biotic N immobilization of the isotope, but recent research has suggested that it may be as important as biotic immobilization for preventing N loss from watersheds (Perakis and Hedin 2001, Davidson *et al.* 2003). In this study, the total amount recovered was often beyond a realistic proportion (>1.00) in intact sites, but regardless of this inherent error, there is clearly less label recovered in plants, microbes and exchangeable and soluble N in many sites and on many dates (Table 32). The total amount of label recovered was as low as 30% in degraded soils and 54% in intact soils after 24 hours. This abiotic loss, especially in the degraded soils, was very sudden, with a substantial amount of label missing in labile pools after only 15 minutes of incubation. These cores were incubated without allowing for leaching, so it may be presumed that any label not recovered was abiotically immobilized. The amounts not recovered in the soil exchangeable, microbial and plant pools are consistent with other studies (Barrett and Burke 2002, Davidson *et al.* 1991). Major pathways proposed for abiotic immobilization of NH_4^+-N are fixation in clay lattices, chemical interactions between NH_4^+-N and clay

surfaces and direct reaction with soil organic C to form humus (Kaye *et al.* 2003). There is slightly higher clay content in the degraded soils at each marsh site (mean = 13% degraded, 10% intact (Handa and Jefferies 2000)) but a lower organic C content. Conventional theory associates higher N retention with high net ecosystem productivity and organic matter accumulation, as inorganic N inputs are typically stored in soil organic pools (Kaye *et al.* 2003). However, this does not apply in the degraded marsh soils with little or no vegetation. Abiotic mechanisms of N immobilization may involve reactions of N with more recalcitrant C compounds, of which there would presumably be more, relative to labile C, in degraded soils. Increased abiotic immobilization in degraded sites may also be due to increased opportunity for this to occur, because as there is more label present in the soil solution due to lack of plant or microbial uptake, mass flow of solution would increase the chances for inorganic N to react with recalcitrant C-compounds.

Although abiotic immobilization of label may be happening in these soils, it is difficult to quantify this phenomenon due to the inherent inaccuracies in the methodology. Total soil analysis records an average recovery of 97% (Table 33), however there is variation in this mean that is biased towards greater recovery in the intertidal marsh than supratidal marsh. Furthermore, in the intertidal marsh, the recovery of label in the total soil is greater than that in summed pools, but in the supratidal marsh, the greater recovery is in the summed pools. It is possible that the difference in the intertidal marsh could be explained by abiotic immobilization of label, and that in the supratidal marsh by calculation error, however these contributions are probably not that clearly teased apart, and both marsh soils may be affected by these complications. The

most likely cause of calculation error is from inaccurate bulk density measurements, which are used to estimate proportion recovered from the core volume. The procedure used to collect soil cores may compress the two marsh soils differentially. It is recommended for future investigations that bulk density measurements be taken from a sub-sample of the ^{15}N procedural core volume.

Aside from abiotic mechanisms of label consumption and difficulties in measuring proportional recovery, ^{15}N excess in the soil solution, especially in degraded sites (Figs 14 and 15), is not likely to be immobilized, and may therefore be lost to stream waters. Although these degraded soils represent an alternate stable state (Jefferies *et al.* 2003), the tight N cycling inherent in an undisturbed soil is not part of their equilibration process. Loss of N throughout the season from these degraded soils contributes to the persistence of a nutrient-limited ecosystem.

CONCLUSIONS

This study has demonstrated, based on the results of the shared distribution of a single resource, that plants and soil microbes may be competing for inorganic N in an N-limited system. At La Pérouse Bay, soil microorganisms remove more label from the soils solution than plants, but their competitive advantage is dampened relative to microbes in other Arctic ecosystems by the effect of goose grazing. Herbivore grazing and faecal fertilization leads to an increase in plant primary productivity, and this may be responsible for the greater uptake of $^{15}NH_4^+$ by *P. phryganodes* in the more heavily grazed, intertidal marsh. Although both plants and microbes exhibit active N-uptake in the plant-growing season, there is a seasonal displacement, as microbes continue to mineralize and slowly immobilize nitrogen in the winter. Loss of vegetation as a result of goose grubbing has led to changes in soil characteristics, including high salinity and low redox potentials. These edaphic conditions may be dampening N uptake by soil microorganisms, as microbes are not N-limited in degraded soils. Seasonal and treatment conditions interact to contribute to a large potential N loss from these soils, although this is minimized by the abiotic fixation of inorganic N.

Unlike long-term tracer experiments of months to years, this study provides insight into the short-term N dynamics in salt-marsh soils. Short-term processes are important for clarifying the mechanisms that control nutrient availability to plants and soil microorganisms in this grazed ecosystem.

Future research

An investigation of the microbial community composition associated with these salt-marsh soils would benefit this research. Clarifying how microbial diversity is related to function is important for understanding the underlying mechanisms of soil processes. This question could be addressed with the use of bacterial/fungal ratios, phospholipid fatty acid analysis (PLFA) and DNA sequence methods to characterize soil microbial communities. If such an endeavour were extended to include soils affected by goose grubbing, functional restrictions on microorganisms inhabiting saturated, saline soil could be clarified. To further tease apart the effects of nutrient limitation and sodium (Na) toxicity, factorial CNP and Na additions in degraded plots should be applied, as well as a measure of respiration with ^{13}C and ^{15}N tracer, to confirm microbial growth or maintenance from N uptake. Furthermore, substrate-induced respiration measurements provide a confirmation of the microbial biomass estimated by the chloroform-fumigation-extraction method.

Although intact soil cores and tracer provide a good environment to approximate short-term plant and microbial dynamics, a more realistic hydrological regime for a marsh may be achieved with *in situ* mesocosms. Together with tracer and a lysimeter, this experimental set-up could be used to follow seasonal N loss from intact and degraded soils, and thus clarify the contribution of soil degradation to the persistence of N limitation in this ecosystem.

Finally, a comparative assay of root exudates from the marsh grass *P. phryganodes*, in the intertidal and supratidal marshes, is important to understand the

mechanistic basis of higher ^{15}N uptake and higher primary productivity in the intertidal marsh.

APPENDIX 1

Chloroform-fumigation-extraction for estimation of microbial C and N

Reagents:

1. Prepare ethanol-free $CHCl_3$ (in a separatory funnel, wash 100 mL of chloroform with 100 mL of 5% H_2SO_4, then rinse chloroform with 3 x 100 mL of deionized water)

2. 0.5 M K_2SO_4

Procedure:

1. Place 70 g of root- and stone-free fresh soil in a 250 ml Erlenmeyer flask, add 140 mL of 0.5 M K_2SO_4, close tightly and shake well by hand or on a mechanical shaker for 1 hour.

2. Filter through Whatman GF/A filter paper. Freeze extracts (c.20 ml) for future analysis.

3. Place a second 70 g root- and stone-free fresh soil sample in a 250 mL Schott bottle, add 2 mL ethanol-free chloroform, close tightly and shake well.

4. Incubate Schott bottle in the dark (cover with foil) at room temperature for 24 hours.

5. Open Schott bottle in the fume hood, and leave open for 30 min.

6. Extract contents of Schott bottle with 140 mL 0.5 M K_2SO_4, close tightly and shake and filter as above

7. For microbial C analysis, include chloroform blank (no soil) in incubation process to assess contamination by chloroform C, if any. Include extractant blanks for final analysis.

(modified from Witt *et al.* 2000)

Microbial C = (C_{fum} - C_{unfum}) x (1/0.35) Second term is the correction factor (see text)

where C_{fum} and C_{unfum} are the organic C measured (Appendix 3) in the fumigated and non-fumigated samples, respectively.

Microbial N = (N_{fum} - N_{unfum}) x (1/0.4)

where N_{fum} and N_{unfum} are the total N measured (Appendix 2) in the fumigated and non-fumigated samples, respectively. (Second term is correction factor)

APPENDIX 2

Total N determination using alkaline persulfate oxidation

Reagents:

1. Dissolve 25 g of low N-$K_2S_2O_8$ and 15 g of H_3BO_3 in 50 ml 3.75 M NaOH, make up volume to 500 mL with deionized water.

Procedure:

1. Pipette 2 mL of soil extract and 2 mL of oxidizing reagent into a glass tube, and seal immediately with screw caps containing Teflon liners. Maintain a consistent headspace with all sample tubes.

2. Weigh tubes and place in autoclave for 30 min at 120°C. Reweigh tubes to determine water loss, and use this loss to correct the final NO_3^- concentration in the solutions.

3. Analyze the nitrate concentration in the solution.

(modified from Cabrera and Beare 1993)

Total N in a sample:

$N\ (\mu g\ mL^{-1}) = (A \times ((mL_{total} - mL_{loss}) / mL_{sample}) - blank)$

where A = NO_3^- concentration returned from auto analysis in ppm ($\mu g\ mL^{-1}$)
mL_{total} = mL in glass tube before autoclaving
mL_{loss} = mL lost in autoclaving
mL_{sample} = mL of soil extract used before autoclaving
blank = field blank N concentration ($\mu g\ mL^{-1}$)

$N\ (\mu g\ g^{-1} dw\ soil) = N\ (\mu g\ mL^{-1}) / (DW / mL_{extract})$

where DW = dry weight of soil used for extraction (g)
$mL_{extract}$ = amount of soil extractant used (mL)

APPENDIX 3

Organic C estimation by dichromate digestion

Reagents:

1. 4 mM glucose standard (0.3605 g of $C_6H_{12}O_6$ in 500 mL of 0.5 M K_2SO_4)

2. 0.5 M K_2SO_4

2. 0.07 M $K_2Cr_2O_7$

3. 98% H_2SO_4

4. 88% H_3PO_4

5. 0.01 *N* $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 0.4M H_2SO_4 titrate (dissolve 1.96 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 100 mL deionized water, add 11.1 mL 98% H_2SO_4, make up volume to 500 mL with deionized water), store in dark bottle (wrap in foil) in cold (~2°C) up to 3 days

6. 4.7 mM N-phenylanthranilic acid in 0.01 M Na_2CO_3 indicator (dissolve 106 mg of Na_2CO_3 in just under 100 mL of deionized water, add 100.2 mg of N-phenylanthranilic acid and make up volume to 100 mL with deionized water), store in dark bottle in cold (~2°C) up to 3 days

Procedure:

1. Pipette 1 mL of sample extract, 1 mL of 0.07 M $K_2Cr_2O_7$, 2 mL of 98% H_2SO_4 and 1 mL of 88% H_3PO_4 into a test tube with 2 anti-bumping granules and a reflux marble.

2. Prepare hot blanks and standards as above, but with 1 mL of 0.5 M K_2SO_4 or 1 mL of standard in place of sample extract.

3. Digest for 30 min at 150°C, include hot blanks and glucose standards, and allow to cool. Samples that are green after digestion must be digested again with a more dilute sample.

4. Rinse reflux and sample into beaker with 10 mL of deionized water, add 120 µL of indicator and magnetic stir bar, and then titrate digested sample with the 0.01 *N* $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 0.4 M H_2SO_4 until the sample flashes green. Record mL of titrate consumed.

5. Analyse standards, hot blanks and cold blanks (1 mL undigested 0.07 M $K_2Cr_2O_7$) with every preparation of titrate.

(modified from Nelson and Sommers 1996)

Organic C concentration in a sample:

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

$(((B_h\text{-}t) \times ((B_c\text{-}B_h)/B_c) + (B_h\text{-}t) - B_f) \times 0.01 \times (3/1000) \times 100) / (DW/extract)) \times 10$

where B_h = mean hot blank titrate value (mL)
t = sample titrate value (mL)
B_c = mean cold blank titrate value (mL)
B_f = field blank titrate value (mL)
DW = soil DW used in K_2SO_4 extraction (g)
extract = volume of K_2SO_4 extract (mL)

APPENDIX 4

Diffusion technique for preparing 2M KCl solutions and persulfate digests for ^{15}N analysis (modified from Hart *et al.* 1994)

Materials:

1. 250 mL glass jars (e.g., Mason jars, Bernardin, Rye, NY, for persulfate) or 120 mL plastic containers (e.g., urine sample containers, VWR, West Chester, PA, catalogue no. 25384-148, for KCl) with tight-fitting lids

2. Whatman no.1 paper filters: 7 mm diameter discs cut with a clean paper punch from pre-leached (2 M KCl and 3 x deionized water) filter paper, pierce hole in center of disc with steel wire

3. Stainless steel wire, new paper clips, tweezers, latex gloves

4. 12.5 mm wide PTFE (Teflon) tape (Scienceware, from VWR, West Chester, PA, catalogue no. 60490-100), cut into 8 cm strips

Reagents:

1. 2.5 M $KHSO_4$ (add 7 mL of concentrated H_2SO_4 to 50 mL of deionized water, add 22 g of K_2SO_4, add more deionized water, mix until salt is dissolved, make up volume to 100 mL)

2. MgO

3. Devarda's alloy

4. 0.14 M NH_4Cl in 2M KCl

5. 10 M NaOH

6. 0.7143 M $K^{14}NO_3$

7. Persulfate digest blank (50% 0.5 M K_2SO_4, 50% alkaline persulfate oxidizing reagent (Appendix 2), autoclaved 30 min at 120°C)

8. 1 mM $^{15}NH_4Cl$ and 1 mM $K^{15}NO_3$

Procedure: sequential diffusion of NH_4^+-N and NO_3^--N in KCl solution

1. Wearing clean gloves, construct acid traps by placing two filter paper discs ~4mm apart on one half (4 cm) of the PTFE strip. Pipette 5 µL of 2.5M $KHSO_4$ onto

each disc, fold over the other half of the strip and seal PTFE tape around the discs with the open end of an 11 mm diameter glass culture tube.

2. For each sample, 0.2 g of MgO and one acid trap are added to 8 mL of extract and 20 mL of 0.14M NH_4Cl / 2M KCl carrier solution in 120 mL specimen containers.

3. Close lids tightly and immediately, seal containers with Parafilm, and diffuse with containers upside-down for 6 days, mixing daily.

4. Remove traps and dip into deionized water. Open traps with tweezers, slide traps onto stainless wire with a new paperclip, and dry by inserting wire into a Styrofoam sheet that is placed in a desiccator over H_2SO_4.

5. Place pairs of dried discs in a 5 x 9 mm tin cup, weigh, and submit in microtiter plate for $^{14}N/^{15}N$ analysis by mass spectrometry.

6. Leave samples open for 6 days, weigh to monitor water loss.

7. For each sample, add replacement-deionized water, 0.2 g of MgO, 0.4 g of Devarda's alloy, 5 µL of 0.7143 M $K^{14}NO_3$ as carrier, and an acid trap.

8. Repeat steps 3 to 5.

Procedure: diffusion of NO_3^--N in persulfate digests

1. Construct acid traps as above

2. For each sample, add 2 mL 10 M NaOH, 20 mL of alkaline persulfate blank and 4 mL of persulfate-oxidized sample to 250 mL glass jars. Leave open for 3 days and weigh to monitor water loss.

3. Add replacement-deionized water, 0.4 g of Devarda's alloy, 0.3 mL of 10 M NaOH, 5 µL of 0.7143 M $K^{14}NO_3$ as carrier, and an acid trap.

4. Close lids tightly and immediately, diffuse for 6 days, mixing daily.

5. Repeat steps 4 and 5 from the KCl procedure above.

NB: All diffusion procedures require blanks (extract or digest with alkaline and reduction reagent, no N), N standards (extract or digest with carrier and alkaline and reduction reagent, no sample), diffused ^{15}N standards (prepared as N standard, but with 5 µL of 1 mM ^{15}N solution), and non-diffused ^{15}N standards (5 µL of 1 mM ^{15}N in 0.714 M ^{14}N solution pipetted directly to a paper disc).

APPENDIX 5

^{15}N calculations

1. Convert to atom percent enrichment (E) (Shearer and Kohl 1993)

Because $\delta 15N = ((E_{sample}-E_{standard})/E_{standard}) * 1000$,

therefore $E_{sample}= ((\delta^{15}N \text{ sample}/1000) * 0.3663) + 0.3663$

2. Correct E_{sample} for carrier dilution (Powlson and Barraclough 1993)

Because $E_{sample} = (M_s * E_s + M_{car} * E_{car})/ (M_s + M_{car})$

where M_s= quantity of N in sample (μg, colourimetrically determined)
E_s= true enrichment of sample
M_{car}= quantity of N in carrier (μg, colourimetrically determined)
E_{car}= true enrichment of carrier (from carrier blank)

therefore, solve for $E_s= (E_{sample} * (M_s + M_{car}) - (M_{car} * E_{car})) / M_s$

Colourimetrically determined quantities converted from ppm to μg N $ml_{extract}^{-1}$
then content in sample calculated as ug N $ml_{extract}^{-1}$ * ml sample diffused

3. Blank correction of ^{15}N enrichments using a calculated blank (Stark and Hart 1996)

Mass of N in blank is calculated by comparing a diffused and non-diffused isotope standard to determine the amount of dilution that occurred in the diffused standard:

$M_b = (M_{std} (E_{dstd} - E_{ndstd})) / (E_b - E_{dstsd})$

where M_b = mass of N in blank (μg)
M_{std} = mass of N in the standard (μg)
E_{dstd} = enrichment of diffused standard
E_{ndstd} = enrichment of non-diffused standard
E_b = enrichment of blank (0.3663%)

Then the calculated blank is used to blank-correct the ^{15}N enrichment of the sample:

$E = E_m + (M_b * (E_m - E_b) / M_s)$

where E = corrected ^{15}N enrichment of sample
E_m = dilution-corrected but not blank-corrected enrichment of sample
M_s = mass of N in sample, colourimetrically determined

4. ^{15}N excess is calculated as:

Pool enrichment = $(E - E_{background}) * N$ pool size

E is blank-corrected and carrier dilution-corrected ^{15}N enrichment of sample
$E_{background}$ is determined by enrichment of non-injected soil, plants and microbial biomass samples
N pool size is the amount of N per volume of soil, measured independently of mass spectrometry.

5. Gross rates of mineralization are determined by the isotope pool dilution method whereby reduction in the ratio of $^{15}NH_4$:$^{14}NH_4$ assumes input of newly mineralized $^{14}NH_4$-N into the extractable pool. (Kirkham and Bartholomew 1954, Davidson *et al.* 1991, Hart *et al.* 1994)

$$m = (([NH_4^+]_0 - [NH_4^+]_t) / t) * (\log(APE_0/APE_t) / \log([NH_4^+]_0/[NH_4^+]_t))$$

where m = gross N mineralization rate (μg of N g^{-1} soil day^{-1})
t = time (days, here this is 1)
APE_0 = atom % excess of NH_4^+ pool at time-0
APE_t = atom % excess of NH_4^+ pool at time-t
$[NH_4^+]_0$ = total NH_4^+ concentration at time-0
$[NH_4^+]_t$ = total NH_4^+ concentration at time-t

6. Gross rates of immobilization are determined from recovery of ^{15}N in the microbial biomass pool. The calculations assume that ^{15}N enrichment of the NH_4^+ pool declines exponentially as organic ^{14}N is mineralized to $^{14}NH_4^+$, and that the microbial biomass is an infinite sink for immobilized ^{15}N (Davidson *et al.* 1991)

Gross immobilization rate constant:

Because $(APE_t/[NH_4^+]_t) = (APE_0/[NH_4^+]_0) * e^{-kt}$

Therefore $k = -\ln (APE_t * [NH_4^+]_0 / APE_0 * [NH_4^+]_t) / t$

Gross immobilization rate (i) (μg N g^{-1} soil d^{-1}):

$$i = v_t / (APE_0 / [NH_4^+]_0) * (1-e^{-k} / k)$$

where v = microbial biomass ^{15}N excess (μg N g^{-1} soil) after 24h
APE_0 = atom % excess of NH_4^+ pool at time-0
APE_t = atom % excess of NH_4^+ pool at time-t
$[NH_4^+]_0$ = total NH_4^+ concentration at time-0
$[NH_4^+]_t$ = total NH_4^+ concentration at time-t

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