Soil Microbial and Nutrient Dynamics During Late Winter and Early Spring in Low Arctic Sedge Meadows

by

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Graduate Department of Ecology and Evolutionary Biology
University of Toronto

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Abstract

Microbial activity occurs year-round in Arctic soils, including during the winter when soils are frozen. From 2004 to 2008 I monitored soil microbial and nutrient dynamics in low Arctic wet and dry sedge meadows near Churchill, Manitoba. I documented a consistent annual pattern in which soil microbial biomass (MB) and soil nutrients peak in late winter, and decrease during the early stages of spring thaw, remaining in low abundance during the summer. Based on a series of experiments, resource shortages do not appear to be the cause of the microbial decline, as has been hypothesized. Observations and theoretical considerations regarding soil physical properties indicate that this decrease is driven by the influx of liquid water at thaw that brings about a rapid change in the chemical potential of water, leading to cell lysis. I have used ^{15}N isotope tracing to show that inorganic nitrogen is taken up very quickly at thaw by the roots of the dominant plant, *Carex aquatilis*. This represents a critical window of opportunity for these plants, as nitrogen remains abundant only for a short time.

The described annual pattern was pronounced in wet sedge sites, but some inter-annual variation is evident, for example a post-thaw soil nitrogen pulse in 2006, and low winter MB in 2008. In the dry sedge meadow, fluctuations in MB and nutrients were dampened relative to wet sites, and

the annual pattern was variable, particularly after 2006. Over four years, peak winter values of soil MB and nutrient variables declined in both wet and dry sites, and this could be related to a drying trend.

This work improves our understanding of the controls on decomposition and primary productivity in a system that is experiencing climate warming and increased precipitation. Changes to hydrology, carbon and nitrogen cycling, and primary productivity will have further effects on vegetation communities and higher trophic levels, including several species of migratory birds.

Acknowledgments

My supervisory committee members are Ken Abraham, Nathan Basiliko, Peter Kotanen, and Vic Timmer. All have been exceptionally encouraging throughout my time as a graduate student, providing a rich learning experience and instilling me with confidence. Locke Rowe selflessly supervised the end of my program and I am grateful for the thoughtful attention that he has given to me during this process. I thank Paul Grogan and Hugh Henry, and their lab members from recent years, for annual symposia that helped shape my research, and for their continued involvement and advice. Rocky Rockwell has also played an important role in my development as a northern scientist and I thank him for his ongoing unconditional support, both personally and professionally. Kate Buckeridge has been a wealth of inspiration and knowledgeable insights. I have enjoyed every opportunity to spend time with her and hope that there will be many more good discussions to come.

The Churchill Northern Studies Centre was my home in the field and is run by an exceptional group of individuals that I am thankful to know as friends. LeeAnn Fishback has provided unparalleled research support and mentorship and continues to encourage my scientific and personal growth. Jenn McCullogh, Carley Basler, Katrina Jensen, and Krista Hanis provided excellent field help, and many other staff members at the CNSC have been integral to making each stay enjoyable and productive. More generally, I am honored to be a member of the CNSC research community, which has provided many important learning opportunities, personal connections, and friendships.

I thank Deborah Tam for maintaining and operating laboratory equipment through many years of sample analyses. I have great confidence in her work, and much of my research has depended on her patience, expertise, and diligence.

Many assistants have worked alongside me on this project and each one of them was a pleasure to work with. I thank Anna Simonsen, Blake Turner, Yue (Claire) Jiang, Shannon Refvik, Geoff Legault, Sarah Dungan, and Freyja Forsythe for joining me in Churchill, and Adrian Xu and Caroline Jeon for helping with lab work in Toronto. Emma Horrigan was also a supurb field assistant who quickly became lab-mate and friend. I thank Emma especially for her positive

attitude and determination, and for her loyal friendship and commitment to our collective research goals. It has been a real joy to share so many experiences together in recent years.

Finally, this work began essentially as an apprenticeship with my supervisor Bob Jefferies, but had evolved into a true partnership before his passing in July 2009. Bob treated me with all the compassion of a parent, helping me to push the limits of my capabilities and to discover my own research path, while ensuring scientific success. His fine character, curiosity, grace, and gumption were all infectious. These, and so many other admirable qualities that he demonstrated, will guide me through the rest of my life.

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Contributions of other Investigators

Chapter 2 was published in 2006 in *Soil Biology and Biochemistry*. Soil temperature data were supplied by G.P Kershaw and much of the field sampling was conducted by J. McCulloch. The manuscript was co-written by my supervisor R.L. Jefferies and myself.

Citation: Edwards, K. A., McCulloch, J., Kershaw, G. P., Jefferies, R. L. (2006) Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. *Soil Biology and Biochemistry* 38, 2843-2852.

Chapter 3 has been accepted by *Journal of Ecology* and is currently in press. The project was designed and executed with input from my supervisor and co-author R.L. Jefferies.

Chapter 4 is unpublished. The experiments were designed and executed with input from my supervisor R.L. Jefferies.

Chapter 5 was published in 2010 as a review paper in *Soil Biology and Biochemistry*. It is based on field observations and discussions between R.L. Jefferies and myself, and on a series of discussions between R.L. Jefferies, A. Walker, and J. Dainty. The sections of the paper related to soil physics were written primarily by A. Walker, while R.L. Jefferies and myself co-wrote the microbiological sections. My contribution was therefore in the literature review, writing, and editing of the microbiological component, and I provided the data that was re-published and discussed in the paper. This chapter has been included in the thesis because it discusses the chemical-physical environment of winter microbial habitat and also provides a physiologically based explanation for the observed late-winter microbial decline. This hypothesis was developed because of the lack of experimental support for the dominant hypothesis already in the literature (Chapter 4) and provides a plausible explanation for both the pattern that I observed (Chapters 2, 6) and for the lack of microbial response to resource treatments (Chapter 4).

Citation: Jefferies, R. L., Walker, N. A., Edwards, K. A., Dainty, J. (2010) Is the decline of soil microbial biomass in late winter coupled to changes in the physical state of cold soils? *Soil Biology and Biochemistry* 42, 129-135.

Chapter 6 is unpublished. The project was carried out with input from my supervisor R.L. Jefferies.

Chapter 1
General Introduction

Arctic ecosystems and climate change

Climate change threatens to alter Arctic ecosystem processes at a time when a comprehensive understanding of ecosystem functioning in these regions is lacking. Warming temperatures and increased precipitation are likely to lead to changes in permafrost and landscape hydrology, plant biomass and community structure, soil microbial functioning, and elemental cycling. These in turn will have significant impacts on higher trophic levels, and will alter the global carbon cycle, eventually feeding back on the climate system. The work presented in this thesis contributes to an understanding of ecosystem functioning in cold regions by integrating soil, microbial, and plant processes both within and outside of the plant growing season, and at multiple sites and over several years. A general review is provided here of the relevant characteristics of northern systems, anticipated future climatic patterns, and how some ecosystem functions could be affected by the changing climate.

Northern ecosystems are typically characterized by short growing seasons, cold temperatures, and limited drainage due to the presence of permafrost, resulting in slow rates of decomposition, and restricted nutrient availability (Nadelhoffer et al. 1992, Hinzman et al. 2005). These conditions support organic matter accumulation, and peaty organic soils of wet and moist tundra sites store an estimated 43 Pg (43×10^{15} g) of carbon (C), amounting to about 2 % of the earth's total terrestrial carbon stocks (Shaver et al. 1992). Plant productivity is generally limited by available nutrients, but growth rates are high despite relatively low temperatures, and summer carbon sequestration by plants can be substantial, particularly in low and sub-Arctic regions (Shaver et al. 1992). Carbon losses from the ecosystem occur through respiration, methane efflux, which occurs primarily in waterlogged soils (Le Mer and Roger, 2001), and hydraulic leaching of dissolved organic carbon (Davidson and Janssens 2006). Environmental disturbances such as N-deposition, climate warming, altered hydrology, and species range expansion (including invasive species) can disrupt the coupling of production and respiration, leading to conditions where tundra systems may become significant sinks or sources within the global system. The interactions of these disturbances with biotic processes, and the effect across various

spatial and temporal scales, are not well understood, and are not generally incorporated into global carbon cycling models (Chapin et al. 2009).

Current climate models project general warming across the Arctic, with increases in precipitation also anticipated, particularly during the winter. Regional differences are substantial, with the western North American Arctic experiencing higher rates of warming than the northeastern part of the continent (IPCC 2007). The work presented here was conducted near Churchill, Manitoba, on the western coast of Hudson Bay, Canada. Regional forecasts estimate winter temperature increases of 7 -10 °C and summer increases of 3 – 3.5 °C between 2000 and 2099 (20 year averages, between 1980-1999 and 2080-2099). Over the same time period, precipitation is expected to increase by more than 30% in winter, resulting in a 10-25% increase in March snowpack, while summer increases of 5 – 10% are forecast (IPCC 2007). As a result, the growing season for Arctic flora will be longer in duration, and will increasingly include periods of relatively short daylength at both the start and the end of the warm season, However this could be mediated to some extent by extended periods of snowmelt due to deeper winter snowpack.

The impacts of these warmer and wetter conditions on Arctic systems include changes to watershed dynamics, permafrost degradation, and generally drier landscapes due to increased rates of evapotranspiration (Hinzman, 2005). Climate affects plant growth directly and indirectly through changes to temperature and the availability of water and nutrient resources. The consequences of these environmental shifts on terrestrial primary producers may include species range and population changes, phenological changes, and increased productivity (Chapin et al. 2009). Plant community changes include increasing shrub cover, decreasing lichen and moss cover, and decreased diversity and evenness (Walker et al. 2006). Changes in plant communities and in climate patterns in turn affect insect, bird, and mammal populations, potentially changing habitat and landscape use, foraging strategies, and the timing of life history events such as insect emergence, animal breeding, and migration.

Below-ground biological effects of climate change are likely to be driven by changes in temperature and moisture, mediated by altered permafrost and hydrology conditions, including altered snowpack dynamics. Plant community change can also be expected to impact the soil microbial community composition and functioning. Increases in average temperatures will accelerate rates of decomposition through increased activity rates, including higher rates of

enzyme activity and nutrient mineralization (Davidson and Janssens 2006). Higher air temperatures coupled with increased winter snowpack will contribute to higher soil temperatures in winter, increasing rates of litter and soil organic matter (SOM) decomposition. This could increase nitrogen supplies, in turn supporting elevated primary productivity (Chapin et al. 2009). Further, increased SOM decomposition may contribute to the breakdown of recalcitrant carbon from soils, increasing C efflux to the atmosphere through higher rates of microbial respiration. Degrading permafrost may also reduce springtime water-logging in wet sites, changing the height of the water-table and shifting the oxygen balance in the upper peat layer, leading to changes in the relative efflux of CO_2 and CH_4. Soil microbial communities will likely shift taxonomically and genetically as cryo-tolerance becomes less critical to survival and organisms that thrive under higher temperatures are favored. Whether increasing soil temperatures will lead to a positive feedback on the climate system (dominance of increased respiration) or a negative feedback (dominance of increased sequestration) remains unknown, both for northern systems (Loya and Grogan, 2004, Chapin et al. 2009) and also on the global scale (Davidson and Janssens 2006).

Historically, ecological work conducted in the Arctic has focused on patterns and processes during the growing season. More recently, attention has also been given to the colder months of the year, but a great deal of basic knowledge about ecosystem processes outside of the growing season is still lacking. Below-ground biogeochemical processes that continue throughout the cold seasons represent crucial components of the annual turnover of carbon and nutrient resources. Understanding the effect that climate change is having on Arctic ecosystems requires an integrated knowledge of biogeochemical processes with climate predictions on a seasonal basis, incorporating results that span the entire annual cycle. It is also essential that multi-year studies are conducted that can reveal the year-to-year integrity of seasonally-driven ecosystem patterns and processes. This will contribute to our ability to understand and predict the effects of environmental perturbations such as climate warming and changes in precipitation patterns, land-use change, and nitrogen deposition, on northern ecosystems, and the potential large-scale feedbacks to the global climate system that will result.

Study sites

Research was carried out at sites near Churchill, Manitoba. The area is characterized by an ecological transition from boreal forest to coastal tundra, with the proximity of Hudson Bay conferring a large climatic influence on sites close to the coast. Near-coastal sites in the Churchill area are climatically and vegetatively representative of low Arctic ecosystems, where some trees and tall shrubs may be present in sheltered and low-lying sites, but upland sites are typically low-shrub, dwarf-shrub, or mire tundra (Bliss and Matveyeva 1992). The region lies at the intersection of three terrestrial ecozones, namely the Hudson Plains (to the southeast), the Taiga Shield (to the west), and the Southern Arctic (to the north) (Natural Resources Canada website). The Hudson Plains ecozone contains large amounts of stored (below-ground) carbon in its vast wetlands, typically over 75 kg / m^2. The wet sites utilized for the research presented here (described below) are representative of these Hudson Plains wetlands (International Boreal Conservation Campaign website).

The research presented in Chapters 2, 3, and 4 was conducted primarily in a wet sedge meadow approximately 2 km east of the Churchill Northern Studies Centre (Figs. 1.1, 1.2, Table 1.1). The site is nearly always waterlogged, and surface water flows slowly northward toward the coast. The vegetation is dominated by *Carex aquatilis* and several species of mosses, and the organic soil is near-neutral pH and is typically 30 cm deep, underlain by calcareous gravel. This is a shallow layer of peat relative to most boreal and low Arctic fens that are often 1 - 5 m peat depth (Bliss and Matveyeva 1992). However, I seldom observed plant roots reaching the mineral layer, and so I consider the site to be a fen (perhaps a young one), and also refer to it as a mire, in the European tradition of my supervisor (Keddy 2000).

In Chapter 6, I present data both from wet and dry sedge meadows (Figs. 1.1, 1.2, Table 1.1). The dry sedge meadow sites are located in a large area of low-shrub tundra that is wet in spring but dries considerably during summer. The organic layer is between 5 and 15 cm deep, underlain by sand. The site was previously a beach ridge but is now nearly 1 km from the shoreline, presumably the result of isostatic uplift.

Table 1.1. Locations of research sites near Churchill, Manitoba and which thesis chapters contain data collected from these areas. Sites are listed from most westerly to most easterly.

Site code	GPS coordinates	wet/dry	relevant chapter(s)
B	N58° 44.850 W93° 53.800	dry	6
A	N58° 44.767 W93° 53.533	dry	6
C	N58° 44.767 W93° 53.333	dry	6
S	N58° 44.783 W93° 53.100	wet	2, 5, 6
T	N58° 44.050 W93° 48.300	wet	2, 3, 4, 5, 6
R	N58° 44.033 W93° 48.433	wet	2, 3, 4, 5, 6

Figure 1.1. Map of study sites including dry (A, B, C) and wet (S, R, T) meadows. Soil temperature data presented in Chapters 2 and 6 were collected from the marked location south of the "R" site. This image was created using Google Earth 5.1.

a)

b)

Figure 1.2. Photographs of a) wet and b) dry sedge meadows used as study sites. Wet sites are characterized by larger Carex aquatilis biomass and lower plant species diversity relative to dry sites.

Outline of Thesis

This thesis begins with an exploration of some below-ground biological variables during the winter-spring seasonal transition in wet sedge meadows. Chapter 2 describes the pattern of microbial and nutrient variables from soil collections made in 2006. From the pattern that was established, several mechanistic questions arose, and two of these in particular addressed in Chapters 3, 4, and 5. Finally, Chapter 6 reports measurements taken for four years from two different ecotypes, providing a temporal and spatial extension of Chapter 1.

Chapter 3 asks whether high nitrogen levels that were observed in late winter can be exploited by *Carex aquatilis*, the dominant vascular plant in the sites of interest, despite very cold temperatures. Several authors have speculated about early nitrogen uptake by graminoids but few studies have been conducted to address the issue. We used isotopic tracing to demonstrate significant uptake of ammonium-N during soil thaw, revealing an important N flux in spring and N retention in the system that could be altered under different thaw scenarios and is thus vulnerable to the effects of climate change.

Chapters 4 and 5 address the question of what drives the reduction in microbial biomass during the soil thaw. This question has been discussed by several authors but has not been satisfactorily explored or resolved. The microbial decline is the single most dramatic event in the annual microbial biomass pattern, and results in both reduced potential for SOM decomposition by these organisms and also the release of large quantities of compounds into the soil due to microbial cell lysis. It is also accompanied by a number of other changes to the microbial community and soil nutrient pools. A common explanation for this decline is that microbes exhaust the soil resources, particularly available forms of carbon, but possibly also nitrogen. This hypothesis was tested through a series of laboratory and field experiments and no supporting evidence was found. These experiments have been compiled to form Chapter 4.

Several insights were gained from conducting the experiments described in Chapter 4, namely that the presence of liquid water seems to be a key element of the microbial biomass decline. Chapter 5 explores theoretically the idea that microbial biomass may be susceptible to osmotic changes at thaw that result from the large increase in liquid water. This is a review/synthesis paper.

Finally, Chapter 6 reports year-round soil nutrient and microbial biomass data from wet and dry sedge meadows sampled over 4 years. The differences between the two sites as well as between years provide insights about the consistency and possible reasons for variation in the annual patterns first described in Chapter 2. Some environmental data is discussed and the impacts of a putative landscape-wide drying trend, which may be driving changes in the annual pattern of microbial and nutrient dynamics, are explored.

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Chapter 2
Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring

Abstract

Microbial activity is known to continue during the winter months in cold alpine and Arctic soils often resulting in high microbial biomass. Complex soil nutrient dynamics characterize the transition when soil temperatures approach and exceed 0°C in spring. At the time of this transition microbial biomass declines dramatically together with soil pools of available nutrients. This pattern of change characterizes alpine soils at the winter-spring transition but whether a similar pattern occurs in Arctic soils, which are colder, is unclear. In this study amounts of microbial biomass and the availability of carbon (C), nitrogen (N) and phosphorus (P) for microbial and plant growth in wet peaty soils of an Arctic sedge meadow have been determined across the winter-spring boundary. The objective was to determine the likely causes of the decline in microbial biomass in relation to temperature change and nutrient availability. The pattern of soil temperature at depths of 5-15 cm can be divided into three phases: below -10°C in late winter, from -7°C to 0°C for 7 weeks during a period of freeze-thaw cycles and above 0°C in early spring. Peak microbial biomass and nutrient availability occurred early in the freeze-thaw phase. Subsequently, a steady decrease in inorganic N occurred, so that when soil temperatures rose above 0°C, pools of inorganic nutrients in soils were very low. In contrast, amounts of microbial C and soluble organic C and N remained high until the end of the period of freeze-thaw cycles, when a sudden collapse occurred in soluble organic C and N and in phosphatase activity, followed by a crash in microbial biomass just prior to soil temperatures rising consistently above 0°C. Following this, there was no large pulse of available nutrients, implying that competition for nutrients from roots results in the collapse of the microbial pool.

Introduction

Strong seasonal dynamics shape northern ecosystems; growing seasons are short, winters are cold, and soils remain frozen for most of the year, limiting decomposition and nutrient turnover (Shaver and Chapin, 1980; Chapin and Shaver, 1985; Giblin et al., 1991; Nadelhoffer et al., 1991; Jonasson, 1992). Although air and soil temperatures in the Arctic in mid-winter are well below -10°C, where a snowpack is present in early and late winter, the soil is insulated so that temperatures beneath the snow rarely fall below -10°C and freeze-thaw events are frequent at these times. Although bulk soil water freezes just below 0°C, liquid water films remain around soil particles, at least down to temperatures of -8°C (Romanovsky and Osterkamp, 2000; Price and Sowers, 2004). In spite of these sub-zero temperatures, microorganisms can remain physiologically active if unfrozen water is available (Coxson and Parkinson, 1987; Rivkina et al., 2000; Mikan et al., 2002). Substantial microbial activity has been recorded in alpine and Arctic soils in winter, even at temperatures of -5°C or lower (Clein and Schimel, 1995; Zimov et al., 1996; Hobbie and Chapin, 1996; Brooks et al., 1996, 1998; Oechel et al., 1997; Fahnestock et al., 1998; Jones et al., 1999; Welker et al., 2000). If soil temperatures fall below -10°C, as occurs in mid-winter, microbial biomass declines rapidly (Mikan et al., 2002). Hence, microbes can remain active and contribute significantly to the annual nutrient budget during early and late winter in an Arctic setting and throughout much of the winter in alpine regions where temperatures are not as extreme as those in the Arctic (Schimel et al., 2004; Schmidt and Lipson, 2004).

This current paradigm for soil microbial activity in winter comes largely from studies in the Colorado alpine tundra zone (Brooks et al., 1998; Lipson et al., 2000; Lipson et al., 2002; Schadt et al., 2003; Schmidt and Lipson, 2004). As described by Schmidt and Lipson (2004), plant and microbial growth during summer often deplete soils of available nitrogen. Decomposition of plant litter in the fall and early winter results in a release of available N and C substrates in soil (Robinson et al., 1997), which supports microbial growth during the cold season. The fungal/bacterial ratio is apparently high in winter but in summer the ratio decreases, at least in alpine sites (Lipson et al 2002; Schadt et al., 2003). Microbial biomass reaches an annual peak toward the end of the cold season, followed by a decline at the winter-spring transition, in part, caused by a shortage of C substrates (Lipson et al., 2000; 2002). Co-incident with this decline in

microbial biomass is a release of available soil N which peaks in late winter and decreases in early spring (Brooks et al., 1998). Much of this pulse of available N is presumed to come from the lysis of cells of the late winter microbial flora that are nutrient-limited and intolerant of freeze-thaw events, although some nutrients may be released from melting snow and plant and animal necromass. In experimental studies, a single freeze-thaw event kills up to 50% of the viable soil microbes. However, Lipson and Monson (1998) and Lipson et al. (2000) noted that microbial biomass was unaffected by single or multiple moderate freeze-thaw events in alpine meadows. Likewise, Grogan et al. (2004) reported that experimental periods of moderate freeze-thaw fluctuations appeared to have only a minor influence on the annual pattern of C and nutrient dynamics in seasonally cold sub-Arctic soils. Nutrients released from the cells of the senescing microbes can be utilized by surviving microbial flora, but additional freeze-thaw cycles can lead to a steep decline of the cold-adapted microbial population (Soulides and Allison, 1961; Skogland et al., 1988; DeLuca et al., 1992). This release of nutrients represents the largest single annual input of available N into these cold soils and is mostly absorbed by plants for summer growth (Jaeger et al., 1999; Lipson et al., 1999; 2000). Although it is known that some roots can take up N under the snowpack in Arctic sites (Bilbrough et al., 2000), it is unclear whether similar complex nutrient dynamics occur in Arctic soils as in alpine soils (Schimel and Mikan, 2005).

The availability of exchangeable N and P is low in Arctic organic wetland soils (Shaver and Chapin, 1980; Ngai and Jefferies, 2004). The combination of a short growing season, low soil temperatures and nutrient-poor environments limits nutrient availability and constrains microbial and plant growth. Longer, colder winters, or shallower snow cover in the Arctic could leave soils too cold for significant microbial activity to take place (Bilbrough et al., 2000). In contrast, the higher winter temperatures, which are predicted to occur in the Arctic in response to climate change (Serreze et al., 2000), are likely to result in increased freeze-thaw cycles that will alter seasonal patterns of microbial biomass and biogeochemical processes controlling the availability of soil C, N and P. Given these scenarios and the lack of detailed studies of changes in microbial activity and resource availability at the winter-spring transition in Arctic soils, it is important to establish the sequence of soil events at this seasonal boundary and whether the alpine tundra model is fully applicable to Arctic soils.

The transitional period occurring before and during the final thaw when microbial biomass declines and nutrients are released is recognized as a critical time of year for understanding annual nutrient cycles and plant-nutrient acquisition. This study was conducted in order to describe the sequence and timing of soil microbial and nutrient dynamics prior to the onset of the Arctic growing season. Elucidating the precise chronology of events at the winter-spring transition also should provide information on processes that control both the decline in microbial biomass and nutrient release ahead of spring allowing comparison of results with those from alpine regions.

Materials and methods

Site description

The study was conducted in calcareous fen mires (wet sedge meadows) within two kilometres of the Churchill Northern Studies Centre, Churchill, Manitoba, Canada (58° 44' 16" N and 93° 49' 09" W) located on the boundary between the sub-Arctic and the Arctic. The mires are dominated by extensive stands of *Carex aquatilis*, which is the most common type of plant community present in the coastal regions of the Hudson Bay Lowlands. Other vascular species present at a low frequency include *Carex glareosa, C. gynnocrates, C. rariflora, Eriophorum angustifolium* and *Triglochin palustris*. Nomenclature of vascular plants follows Porsild and Cody (1980). The bryophyte flora is limited in abundance, but includes *Tomentypnum nitens* (Hedw.) Loeske, *Drepanocladus revolvens* (Sw.) Warnst. and *Blepharostoma trichophyllum* (L.) Dum.

The layer of organic-rich soil (pH 6.5-7.5, C, 37% and N, 2.5% of dry weight) within stands of *Carex aquatilis* is about 25-30 cm deep and it overlies a mineral soil of sand, silt and fragmented limestone. The soil is a fibrous sedge peat which is highly humified. The entire coastal plain is typically wet and is characterised by the water table close to the surface for much of the summer (typically 4-8 g of water g^{-1} dry soil at 5-15 cm depth), as a result of the presence of permafrost and the small change in elevation across the coastal zone of the Hudson Bay Lowland (c. 1 m change in elevation every 3-4 km). The bulk soils are usually anaerobic (E_h -50 to +150 mv at thaw and +100 to +200 mv in summer at 5-15 cm depth) and cold in the immediate post-thaw period. The presence of oxidized iron deposits in the rhizosphere in summer strongly suggests

that oxygen movement via root aerenchyma of the sedges is occurring. The base-rich water drains in shallow vernal streams from the sedge meadows during and after thaw.

The average summer temperature (1932 -1995) for July and August, which represent the frost-free months, is 11.8°C (Scott, 1996). Long-term weather data can be accessed from the Environment Canada National Climate Data and Information Archive (Environment Canada website). Snow can fall in any month but continuous snow cover usually does not occur until late October or early November. Spring thaw takes place in late May or early June in most years. Minimum winter air temperatures drop below -40°C on occasions.

Soil temperatures

A Campbell Scientific International CR10X was used to monitor air and soil temperatures. Type T thermocouples were used in conjunction with reference temperature thermistors on the logger wiring panel to measure temperatures at 5, 10, and 15 cm depths at a site adjacent to the experimental site. Overall reference junction accuracy was < 0.1C° in the 33°C to +48°C range and ≤1.0C° in the 40°C to +56°C range (Campbell Scientific International, 1991). There were 288 readings each day (5 min scan interval) used to determine the mean daily, maximum and minimum values of soil temperatures within stands.

Sample collection and processing

In the snow-free season, six soil samples (15 cm x 15 cm x 15 cm) were collected between *Carex* shoots, two from each of three sites, which were separated from each other by a distance of at least 0.5 km. Within a site, samples were taken from locations that were 50 metres or more apart and the same location was not re-sampled during subsequent collections. In winter and during the transition period at the end of winter, frozen soil was removed with the use of an axe after removal of snow cover from designated locations within a site. It was not always possible to collect six samples in winter, and on occasions as few as 3 samples were collected, one from each site. The soils were placed in plastic bags and loaded into cooled containers with ice packs for transport to the laboratory. From May until August samples were processed on the same day as they were collected at the Churchill Northern Studies Centre, but on all other occasions (i.e. in autumn and winter) they were flown in a frozen state to Toronto, where they arrived the next day. The frozen soils were processed within four days of sampling but frequently sooner. As

samples were collected from micro-sites (c. 20 cm in diameter) between stands of shoots, root density was not high. The surface litter layer, small stones, roots and rhizomes were removed from soil before use, and fine fibrous roots also were extracted as far as possible.

Sub-samples used for analyses were representative of the entire soil sample, which was mixed by hand (gloved) before use. Analyses included pore-water nutrient content (gloved-hand squeezing; dissolved organic N, C, Henry and Jefferies, 2002), exchangeable N (10 g f.w. of soil extracted in 50 ml of 1M KCl, Wilson and Jefferies, 1996) and microbial C, N, P (2 x 25 g. f.w. of soil extracted in 50 ml of 0.5 M K_2SO_4 using a modified chloroform-fumigation extraction (Witt et al., 2000; Henry and Jefferies, 2003). The K_2SO_4 extraction of unfumigated soils also provided an extract of exchangeable P. Frozen samples were not thawed prior to KCl and K_2SO4 extractions so were cut into small pieces (< $1cm^3$) and allowed 15 extra minutes of extraction time to permit thawing in solution. Samples for pore-water nutrient content were allowed to thaw at room temperature so that soil could be squeezed. All extracts were frozen in a freezer at -20°C at the Churchill Northern Studies Centre and remained frozen during the flight to Toronto and until further analysis.

Sample analyses

The exchangeable inorganic N (NH_4^+, NO_3^-) was measured in filtered 1 M KCl soil extracts with the use of an auto-analyser (Technicon AAII, Tarrytown, NY). Nitrate was reduced to NO_2^- with cadmium before the colorimetric estimation. To estimate microbial N, the total extractable N in filtered O.5 M K_2SO_4 fumigated and unfumigated soil extracts was oxidized with alkaline persulphate (Cabrera and Beare, 1993) and the nitrate was measured with the use of an auto-analyser after reduction to nitrite on cadmium columns. The difference in N between oxidized extracts of fumigated and unfumigated soils gave an estimate of microbial N. Soluble organic N (DON) was measured from pore-water solution using the alkaline persulphate oxidation method described above. Microbial carbon in the oxidized extracts was determined as the difference between unfumigated and fumigated samples using the dichromate volumetric method to measure carbon (Nelson and Sommers, 1996). Interference with the C estimation from soil Fe^{2+} in local soils of this type has been shown to be insignificant (Henry and Jefferies, 2003). Exchangeable phosphate was measured from unfumigated 0.5 M K_2SO_4 extracts using the ammonium molybdate method (Ngai and Jefferies, 2004). The difference in phosphate between

fumigated and unfumigated 0.5 M K_2SO_4 extracts gave an estimate of soil microbial P. Acid and alkaline phosphatase activities were measured following Tabatabai and Dick (2002), using wet soil (1g fresh weight) and 1 h incubation at 37°C in 4 ml of buffer and 1 ml of p-nitrophenyl-phosphate solution. This temperature was chosen in order to standardise conditions with other studies, although it may have led to denaturation of cold-adapted enzymes.

Meltwater collection and analysis

At one of the selected sites, a braided vernal stream carrying meltwater from a sedge meadow at a higher elevation flowed into the sedge meadow under study. The vegetation of the upper sedge meadow was very similar to that of the study site and it was dominated by *Carex aquatilis*. Inflows of N and P in the stream water may contribute to the bulk soil nutrient pool during the winter-spring transition. In order to establish the magnitude of the flux, water was collected daily at midday from the first day of flow to the last day of sampling (12 May to 2 June). The concentrations of soluble organic carbon and nitrogen, together with inorganic N and P in the stream water were analyzed as described above for soil water and exchangeable P, in order to establish if changes in nutrient availability were occurring over the thaw period.

Statistical analysis of the data

We tested for differences between sampling dates using a univariate repeated measures ANOVA because there is insufficient replication within levels (sampling dates) to use a repeated measures MANOVA. The three sites were treated as subjects, and for sampling dates when more than one sample was taken from a site, values were averaged to ensure equal sample sizes. The Huynh-Feldt epsilon was used to adjust the degrees of freedom, thus correcting for sphericity in the data where necessary (von Ende, 2001). Multiple comparisons between sampling dates were performed using the Difference (reverse Helmert) test, in which each category is compared to the mean effect from all previous categories. Linear regression was used to test for trends over time in meltwater nutrient variables. All analyses were done in SPSS version 10 (SPSS Inc., 1999).

Results

Soil temperatures during the winter-spring transition

Soil temperature changes during the winter-spring transition can be partitioned into three phases (Fig. 2.1). During March, 2005, which represented the first phase, soil temperatures were below -10°C for the entire month at all soil depths (5, 10, 15 cm). The second phase started on 1 April and continued until about 19 May. This period was characterized by successive freeze-thaw cycles, during which time the maximum daily soil temperature at a depth of 5 cm (the fibrous rooting zone) in the adjacent site reached or exceeded 0°C on 10 separate occasions in April and on two days it was above 5°C. On 13 May it exceeded 10°C. During the seven weeks, soil temperatures at a depth of 5 cm ranged between 0°C and -5°C for most of the time. Soil temperatures at depths of 10 and 15 cm showed similar trends, although the diurnal range of values was dampened. Over the seven weeks, much of the snow disappeared and the remainder was present as slush at the end of the period. An abrupt change to the third phase occurred immediately after 20 May when air and soil temperatures rose above 0°C, particularly at a depth of 5 cm, and sites were snow-free by 23 May. Minimum air temperatures in early June were just below the freezing point for several days, but soils did not freeze (Fig. 2.1). Hence, dates at the winter-spring boundary marking transitions in the soil temperature regimes were 1 April and about 19 May.

Microbial carbon, nitrogen and phosphorus

Values of microbial biomass based on estimates of microbial carbon showed large significant seasonal changes (Fig. 2.2a, Table 2.1). During the snow-free season in June and July, 2005, microbial carbon was a quarter to one fifth of peak values in late winter and the summer values were similar to the corresponding values in the summer of 2004. (Microbial biomass rose in October, 2004 to one third to one half of the late winter peak; data for 2004 not shown). The annual peak occurred in April when soil temperatures were between 0°C and -5°C. Thereafter, there was a decline in biomass, particularly during 12-29 May. An abrupt change in soil temperatures to values above 0°C occurred about 19 May when microbial biomass was 54% of maximum values (20 May). Values of microbial N and P in soils (Fig. 2.2b,c; Table 2.1) also showed significant peaks in mid-April, coincident with maximum values of microbial carbon. Subsequently, N and P values declined steadily and by 20 May estimates were 50% and 38% of

peak values respectively. There was a steep decline in biomass in the last 10 days of the month coincident with warmer soil temperatures, and by early June levels of microbial C, N and P were similar to summer values.

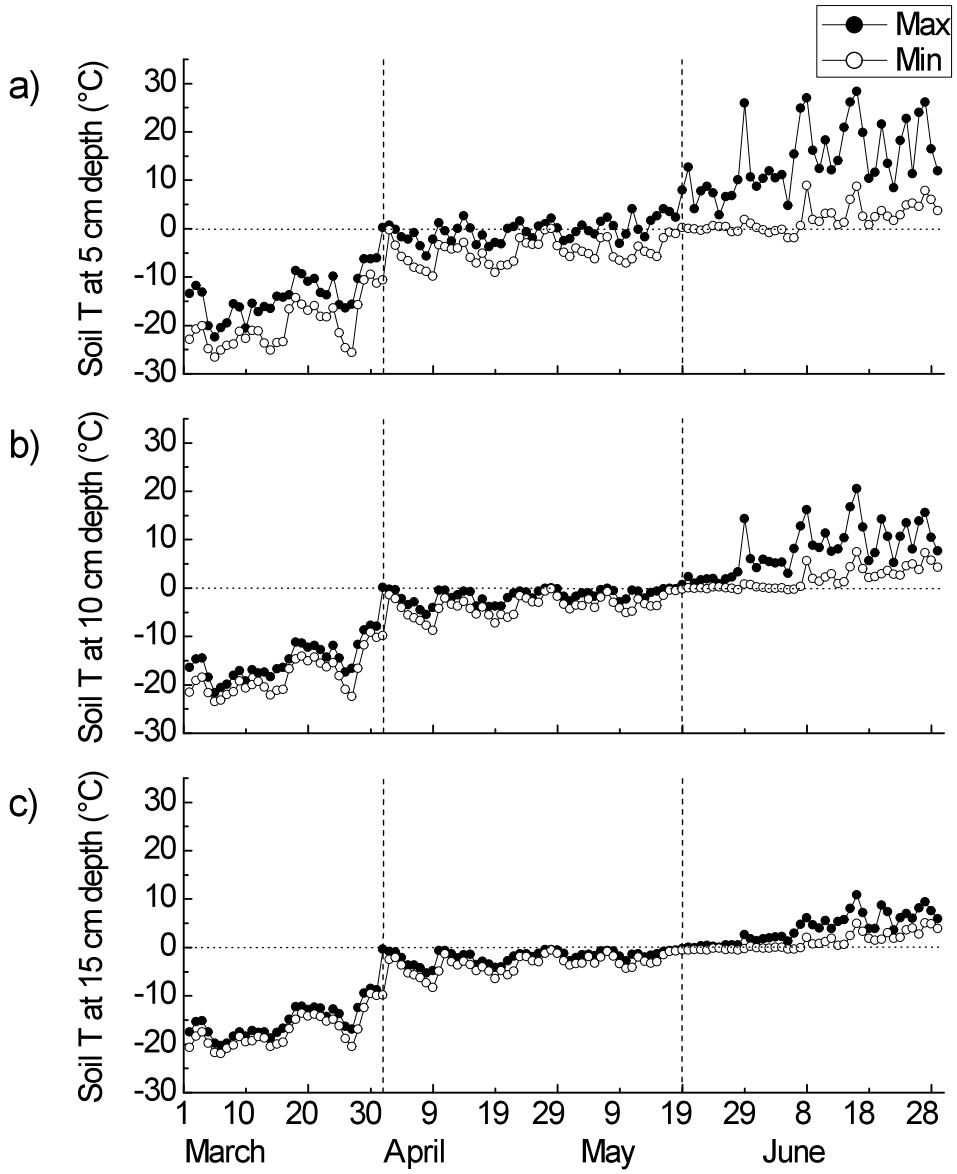

Fig. 2.1. Daily maximum and minimum soil temperature changes at depths of 5 cm (a), 10 cm (b), and 15 cm (c) during the winter-spring transition. Thermistor probes were connected to an automatic Campbell data logger to give readings every 15 minutes. Probes were located within 500 m of two of the three sampling sites, near Churchill, Manitoba. Key transitions in temperature profiles on 1 April and 19 May are indicated with dotted lines.

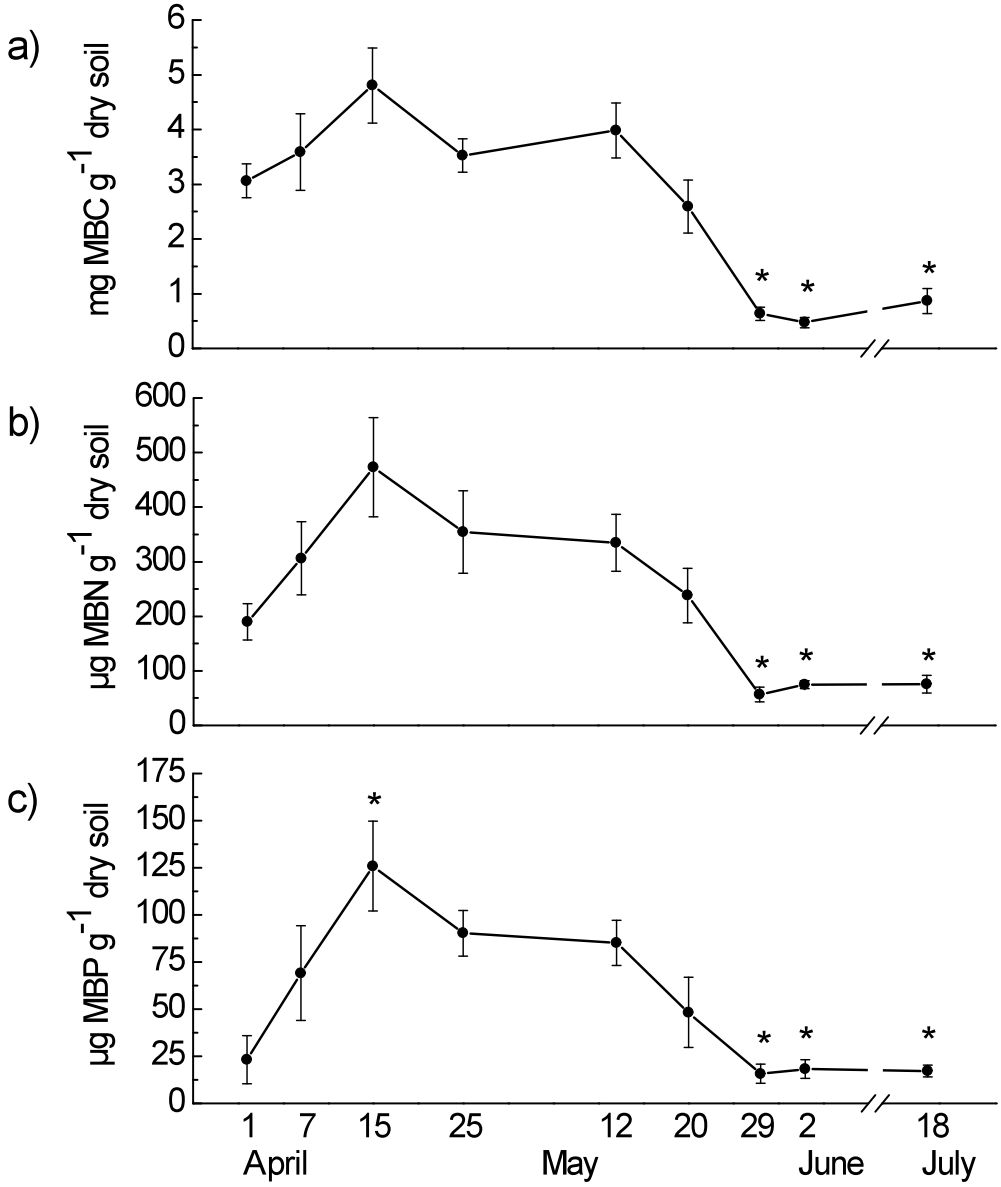

Fig. 2.2. Changes in the carbon (a), nitrogen (b), and phosphorus (c) of microbial biomass in calcareous mire soils at a depth of 5-15 cm dominated by stands of *Carex aquatilis*, near Churchill, Manitoba during the winter-spring transition. Means and standard errors are shown (n=3-6), and values that are significantly different (*P*=.05) from the mean of all previous measurements are indicated with an asterisk, as determined by the Difference (reverse Helmert) test.

Table 2.1. Univariate repeated measures ANOVAs of microbial carbon (MBC), nitrogen (MBN) and phosphorus (MBP) and exchangeable ammonium, nitrate and phosphate ions, dissolved organic carbon (DOC) and nitrogen (DON), and acid and alkaline phosphatase (AcidPase and AlkPase) in organic calcareous mire soils near Churchill, Manitoba for different sample dates between 1 April and 18 July, 2005. The Huyn-Feldt epsilon was used to adjust the degrees of freedom.

Measure	Huyn-Feldt epsilon	Adjusted df	MS[1]	F[1]	P[1]
MBC	1.000	8.00	62.563	10.338	.000*
Error		16.00	6.052		
MBN	.525	4.20	817038.016	5.682	.016*
Error		8.39	143805.807		
MBP	.475	3.80	64728.005	8.317	.007*
Error		7.60	7782.455		
Exch NH_4^+	1.000	8.00	1961.764	3.188	.023*
Error		16.00	615.395		
Exch NO_3^-	1.000	8.00	3.963	1.768	.158
Error		16.00	2.242		
DON	.233	1.63	100.847	7.599	.061
Error		3.26	13.272		
DOC	1.000	8.00	11.277	14.780	.000*
Error		16.00	.763		
Exch P	.251	2.01	.448	5.458	.071
Error		4.02	.08215		
AcidPase	.718	5.75	70.853	4.047	.021*
Error		11.49	17.508		
AlkPase	1.000	8.00	43.066	4.003	.009*
Error		16.00	10.759		

[1]MS = mean squared (treatment and error); F = F ratio for treatment; values labeled with '*' are significant at $P<0.05$.

Exchangeable and/or soluble amounts of carbon, nitrogen and phosphorus in soils

Amounts of soluble organic C and N in the soil solution of these wet sedge meadows increased steadily from early April until the end of the month, after which time values changed little until mid-May when there was a steep and significant decline in soluble C and N (Fig. 2.3a, Table 2.1). By early June, values of soluble C and N were about one-quarter to one-third of peak values in April. In contrast, although amounts of exchangeable ammonium ions rose in early April, the rise was not sustained and there was a progressive decline to the very low levels of early June (Fig. 2.3b, Table 2.1). Amounts of exchangeable ammonium resemble closely the observed patterns of microbial N over the same period. Concentrations of soluble nitrate were low throughout the entire period (<0.05 μg NO_3-N ml^{-1} soil water) (Fig. 2.3b, Table 2.1). Amounts of exchangeable orthophosphate also declined from a mid-May peak to very low values by the end of May that were less than 10% of the peak levels (Fig. 2.3c, Table 2.1).

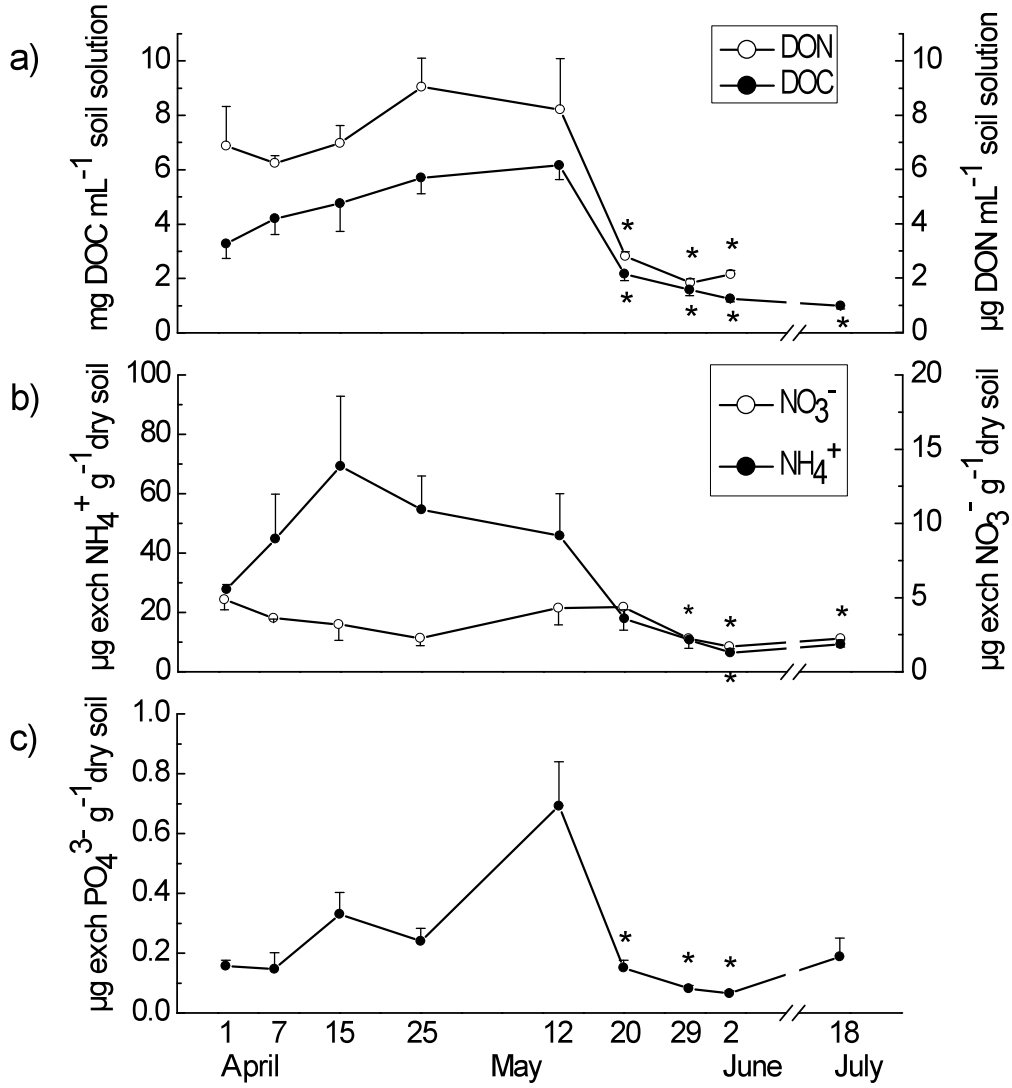

Fig. 2.3. Amounts of soluble organic carbon and nitrogen (a), exchangeable ammonium and nitrate ions (b) and phosphate ions (c) in calcareous mire soils at a depth of 5-15 cm dominated by stands of *Carex aquatilis*, near Churchill, Manitoba during the winter-spring transition. Means and standard errors are shown (n=3-6), and values that are significantly different (*P*=.05) from the mean of all previous measurements are indicated with an asterisk, as determined by the Difference (reverse Helmert) test.

Concentrations of C, N and P in vernal streams

Meltwater entering one of the sedge meadow sites was collected daily and analyzed for changes in soluble organic C and N, and for soluble inorganic N and P (Fig. 2.4a,b). In the case of N, organic N exceeded the combined inorganic fraction by an order of magnitude, but the absolute levels were low. Nitrate was very low at 0.01 μg ml^{-1} of water (data not shown). There was no crash in available N (organic and inorganic) or P concentrations at the end of May, although inorganic N declined slightly from mid-May to early June (R^2 = 0.26, p< 0.01). Soluble organic carbon also declined throughout May and early June to about half of the value at the start of sampling when water flow commenced (R^2=0.24, p=0.01).

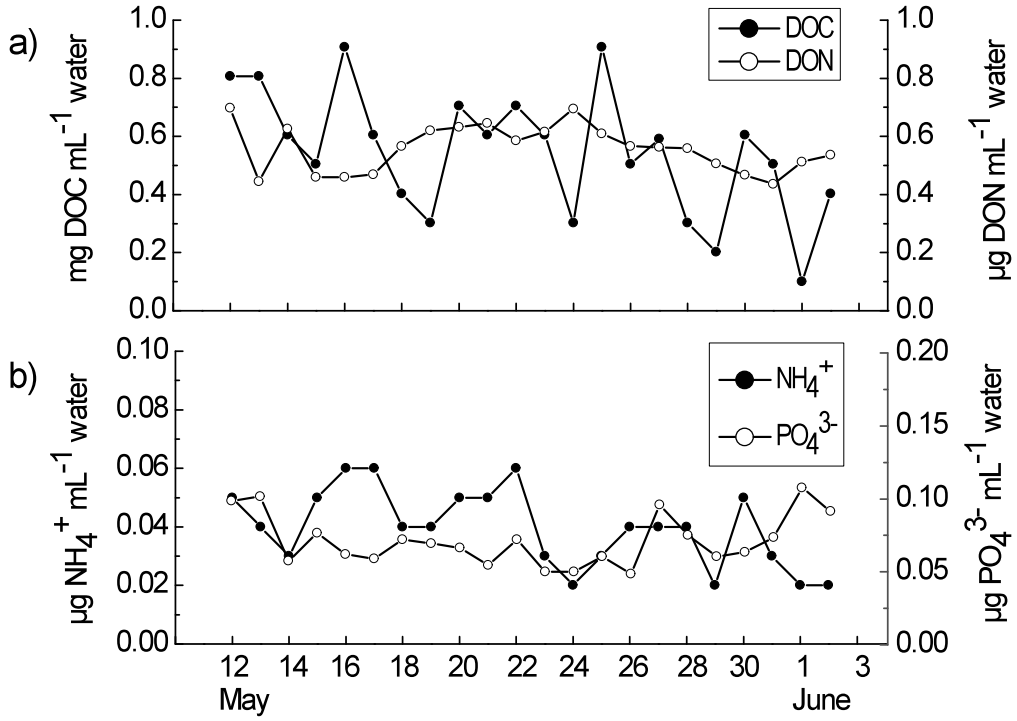

Fig. 2.4. The concentrations of soluble organic carbon and nitrogen (a) and soluble inorganic nitrogen and phosphorus as phosphate (b) in a small vernal run-off stream from a calcareous mire dominated by *Carex aquatilis*, near Churchill, Manitoba during the winter-spring transition.

Acid and alkaline phosphatase activities

Both acid and alkaline phosphatase activities, which were measured from early April until early June, had a similar pattern (Fig. 2.5a,b, Table 2.1). Activity increased from early April onwards and peak activities were maintained until mid-May when a steep decline occurred. At the end of May, acid and alkaline phosphatase activities were very low, which was characteristic of the pattern in the summer.

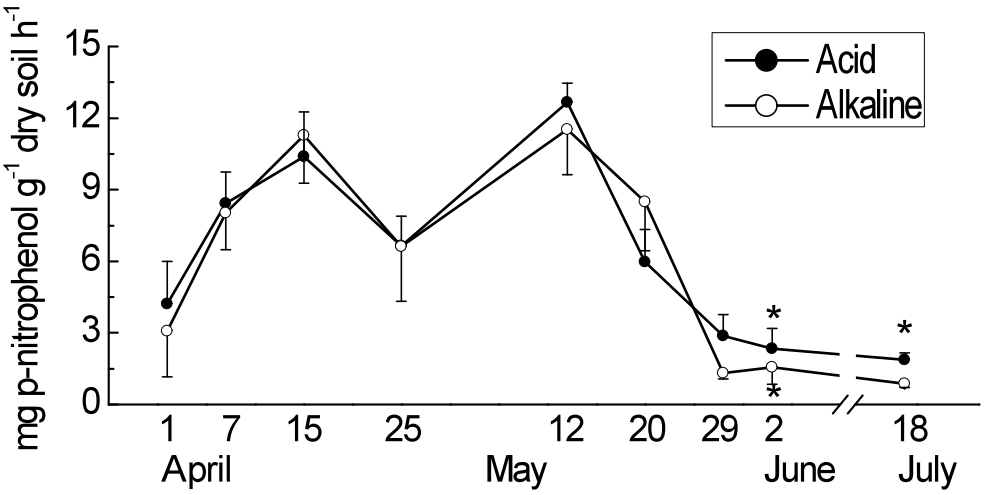

Fig. 2.5. Acid and alkaline phosphatase activities (measured at 37°C) in calcareous mire soils at a depth of 5-15 cm dominated by stands of *Carex aquatilis*, near Churchill, Manitoba during the winter-spring transition. Means and standard errors are shown (n=3-6), and values that are significantly different (*P*=.05) from the mean of all previous measurements are indicated with an asterisk, as determined by the Difference (reverse Helmert) test.

Discussion

Freeze thaw cycles and nutrient availability

The higher soil temperatures, which rose from mid-winter lows (below -10°C) to between –7°C and 0°C during April and most of May, 2005, allowed microbial growth to take place in this period characterized by freeze-thaw events. Temperature changes in soil at a depth of 5 cm were

more closely coupled to air temperatures and showed greater amplitude between day and night values compared with similar changes at depths of 10 and 15 cm. The freeze-thaw cycles are likely to lead to the downward percolation of melt water into the soil. Nutrient availability and microbial biomass increased during the first two weeks of April and peak biomass and availability of nutrients in soil occurred in mid-April, except for exchangeable P, which peaked in mid-May. The increase in resources probably represents the release of inorganic nutrients in free water in late winter and during spring thaw that had accumulated over winter, as a result of fragmentation of plant litter, cell lysis, leaching of nutrients from tissues and mineralization (Hobbie and Chapin, 1996). Taylor and Jones (1990) concluded that decomposition over the winter usually led to a loss of between 40% and 60% of the litter mass, based on the results from 17 studies. In addition to inorganic nutrients, pools of soluble organic C and N also rose in early April and much of this organic C and N was probably derived from the lysis of microbial necromass that had remained in a frozen state over the winter. In an experimental laboratory study of Alaskan tundra and taiga soils, the initial freeze-thaw cycle caused a flush of soil C and N, but subsequent cycles led to a reduction in the decomposition rate and the total amounts of C and N mineralised (Schimel and Clein, 1996). This implies that the readily mineralized fraction was quickly depleted in these soils and that as a result, microbial activity declined, as shown by the fall in soil respiration rates. At the Churchill site there also was a steady decline in exchangeable inorganic N from mid-April onwards and the concentration of soluble N in the vernal stream also declined in May. These trends are consistent with the experimental results of Schimel and Clein (1996).

In contrast to the decrease in the availability of inorganic N, concentrations of soluble organic carbon and nitrogen in the soils remained elevated until the crash which occurred after mid-May. In addition, acid and alkaline phosphatase activities were maintained until mid-May when a steep decline occurred. This suggests that the pool of soluble organic substrates included phosphate esters and that the demand for P by the microbial flora was at least partially met by the hydrolysis of these esters as the pool of inorganic P declined. However, amounts of soluble organic N relative to exchangeable ammonium ions were low, hence the supply of organic N appears to be insufficient to compensate completely for the decline in available inorganic N. In spite of the decline in microbial biomass N and soluble C and N during the final stages of snow melt, there was no subsequent flush of inorganic N, a similar finding to that of Brooks et al.

(1998) from alpine regions. In their studies, although net N mineralization occurred in soil beneath snow in early spring, it was quickly followed by net immobilization of N once snow melt took place (Brooks et al., 1996). As Schmidt and Lipson (2004) have pointed out, the microbial community acts first as a sink for nutrients in late winter when snow cover prevails and then as a source of nutrients in early spring at the time of snow melt, but it appears that this source is rapidly depleted in both the studies of Brooks et al., (1996) and our study.

Plant uptake of available nitrogen

The decline of biomass after snowmelt in alpine soils releases organic N that can be taken up plants (Lipson et al., 1999). A similar situation occurs on the Cape Churchill Peninsula where free amino acid concentrations relative to ammonium concentrations were highest during the period of rapid plant growth in late spring (Henry and Jefferies, 2002). In a short-term (24 hr) experimental field study using intact soil cores, the grass, *Puccinellia phryganodes*, incorporated ^{15}N labelled glycine, ammonium and nitrate ions in amounts which were 56%, 83%, and 68% respectively of the comparable incorporation of these sources of N by soil microorganisms (Henry and Jefferies, 2003). Application of $^{15}NH_4Cl$ to soil cores also indicated that plants are capable of incorporating the tracer very early in spring (Buckeridge, 2004). Uptake of different N sources by plants is known to be maximal at this stage of the season (Theodose et al., 1996; Jaeger et al., 1999), and Chapin and Bloom (1976) showed that the new roots of *Eriophorum* are capable of following the ice edge as the active layer develops and can absorb phosphate at very low temperatures (1-2°C).

Microbial biomass

The results indicate that dramatic changes occurred both in microbial biomass and resources over a period as little as one week to 10 days in the second half of May, just prior to the sustained rise in soil temperatures to above the freezing point in late May. This is similar to the decline in microbial biomass that occurred in dry alpine meadows where a decrease in microbial biomass took place before the snow had fully melted and soil temperatures were around 0°C (Brooks et al., 1998). Hence, the decline in microbial biomass and in resources precedes the sustained temperature rise above the freezing point and the results strongly suggest that the lack of resource availability contributes to the crash. The sequence of nutrient availability over time,

therefore, is initially a decline in exchangeable inorganic N from mid-April onwards, followed by a very large drop in soluble soil carbon and exchangeable P from mid-May onwards that precedes a steep decline in microbial biomass at the end of May. However, the steepness of the declines implies that other drivers are forcing the changes. The most likely causes of the declines are: a) competition for organic C and N from plant roots, which are known to take up amino acids directly from soil at low temperatures in Arctic systems (Chapin et al., 1993; Kielland, 1994; 1995; Henry and Jefferies 2003), b) the lysis of microbial cells in response to transient temperatures above 0°C and c) depletion of soil N and P. In laboratory experiments, Lipson et al. (2000) found that although soil microbial biomass was resistant to repeated fluctuations around 0°C, the winter microbial community was sensitive to soil temperatures that remained above the freezing point. In our study the microbial biomass crashed just prior to the soil temperatures remaining above 0°C, hence ^{15}N studies are required to examine in detail the competitive ability of plants to accumulate the tracer just prior to the bulk soils remaining above the freezing point. In addition, carbon and nitrogen sources need to be added to plots in the absence and presence of vegetation, in order to establish whether the microbial peak can be sustained in early spring at soil temperatures just above 0°C.

Although values of microbial biomass peaked in mid April, microbial activity at that time is likely to be low. Recent studies in coastal marshes on the Cape Churchill Peninsula of activity based on the residence time of ^{15}N in the microbial biomass indicated that the residence time was about 100 days at the end of April, 2003. This in sharp contrast to residence times of a few days in summer and early winter (October) when amounts of microbial biomass are substantially lower than values in late winter (Buckeridge, 2004). Similar low amounts of microbial biomass have been recorded in these wet sedge meadows in summer, but it is not known whether the turnover rates are faster than those in late winter. It is likely that the high microbial biomass values in late winter are linked to low microbial activity and that the reverse pattern occurs in summer. The microbial biomass values for C, N, and P continue to decline up to 29 May together and the C:N ratio declines from about 13:1 in mid-April and May to 7:1 in early June. Whether this relates to a shift from a fungal-dominated microbial community feeding on necromass in late winter to a bacterial–dominated community in summer, dependent on root exudation, as appears to occur in alpine communities (Lipson et al., 2002; Schadt et al., 2003), is unknown.

Conclusion

The transitional period occurring before and during the final thaw, when microbial biomass and inorganic and organic pools of nutrients decline and plant growth commences, is recognized as a critical time of year for understanding annual nutrient cycles and plant-nutrient acquisition (Bardgett et al., 2005). This study indicates that large changes occurred in soil microbial and nutrient dynamics prior to the onset of the Arctic growing season. The results show close similarities to trends observed in alpine soils. However, the pattern does not appear to be identical to that from alpine soils, based on limited data. The more extreme mid-winter temperatures, the absence of a nutrient pulse in spring and the presence of the sedge community may account for the differences between the Arctic and alpine sites.

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Chapter 3
Nitrogen uptake by *Carex aquatilis* during the winter-spring transition in a low Arctic wet meadow

Preface

In the previous chapter a seasonal pattern was presented in which soil inorganic nitrogen peaked during late winter and declined to low amounts during thaw. Inorganic nitrogen pools remained small throughout the plant growing season. In this chapter I ask whether *Carex aquatilis* can take advantage of relatively abundant supplies of inorganic nitrogen during a short window of time at spring thaw. In answering this I am also able to explain where at least some of the winter pool of inorganic nitrogen ends up after the completion of thaw, identifying an important nitrogen flux during the thaw seasonal transition.

Abstract

1. Processes of decomposition occur year-round in tundra ecosystems and respond quickly to seasonal changes. Characterizing the phenology of plant nutrient uptake in relation to these processes is essential to understanding the current and future productivity of Arctic ecosystems.

2. In wet sedge meadows located near Churchill, Manitoba, Canada, soil microbial biomass as well as inorganic and organic nutrient pools fluctuate seasonally, with late-winter peaks followed by declines of these variables during the early stages of soil thaw; however, it is unknown if the dominant plant in this community takes up nitrogen when levels of this nutrient are high but soil temperatures are 0 °C or below.

3. Stable isotope tracing was utilized by injecting $^{15}NH_4Cl$ into soil cores and incubating for 1 day or 8 days during spring thaw to determine the short-term capacity for uptake and transport of inorganic nitrogen into *Carex aquatilis* (roots, shoots and rhizomes), moss and soil microorganisms during this transitional time of year.

4. During three 8-day experimental trials in April and May 2007, *C. aquatilis* roots accumulated a substantial amount of the added nitrogen (33.5% increasing to 63.4%), when inorganic nitrogen was readily available in the soil, but declining. A smaller proportion of injected nitrogen was recovered from soil microbes (30% decreasing to 7%), and only trace amounts of injected ^{15}N were measured in plant shoots, shoot bases, rhizomes and mosses (2% or less).

5. *Synthesis.* Shifting seasonal patterns in northern ecosystems resulting from climate change are likely to alter the progression of events that lead up to the summer growing season. A substantial pool of inorganic nitrogen resides temporarily in the soil at the end of winter, and we have shown here that plants are able to take up nitrogen at this time. Increases in the frequency and temperature highs of late winter warming events are likely to trigger early episodes of soil thaw, potentially reducing the capacity of plants to take up this large ephemeral supply of nitrogen in early spring.

Introduction

The growth of Arctic vegetation is constrained by short summer seasons, cold temperatures, and low levels of available nutrients (Shaver and Chapin 1980; Nadelhoffer *et al.* 1991). However, while plant growth is generally restricted to summertime, soil microbial activity continues throughout the cold seasons, and nutrient transformations prior to the plant growing season contribute to a large pool of inorganic nitrogen (N) that is available for absorption by plants (Lipson *et al.* 1999; Grogan and Jonasson 2003; Schmidt and Lipson 2004, Kielland *et al.* 2006). The timing of the onset of plant nutrient uptake and seasonal patterns of uptake are not well described for northern ecosystems, which are particularly vulnerable to changing seasonal patterns as winters in high-latitude environments become warmer and temperatures become more variable (IPCC 2007).

Several studies conducted in Arctic and alpine tundra sites have found that soil microbes reach their annual peak biomass in late winter (Chapter 2, Brooks *et al.* 1998; Lipson *et al.* 1999; Grogan and Jonasson 2003; Schmidt and Lipson 2004; Larsen *et al.* 2007). High levels of nutrients (N, phosphorus [P]) have also been observed during the winter–spring transition, either concurrently with (Chapter 2) or shortly after the microbial biomass (MB) decline (Schmidt and Lipson 2004), although Brooks (1998) did not observe a nutrient flush at either time. Springtime

is known to be a crucial time for N uptake by plants in Arctic and alpine systems (Jaeger *et al.* 1999; Bilbrough and Caldwell 1997; Bilbrough *et al.* 2000; Grogan and Jonasson 2003; Schmidt *et al.* 2007); however, it is less well known whether vascular plant roots can take up N during the late winter and very early spring. Bilbrough *et al.* (2000) demonstrated, using a ^{15}N tracer applied to the snowpack, that some plants in an alpine moist meadow took up large amounts of snowpack-N during snowmelt (over 12% of annual uptake for *Deschampsia caespitosa*), but in an Arctic site less than 1% of annual uptake in *Eriophorum vaginatum* happened during snowmelt. Tye *et al.* (2005) applied ^{15}N tracer to high-Arctic tundra sites immediately after snowmelt in order to simulate a snowpack-derived N pulse and found significant uptake by bryophytes, MB and vascular plants after an incubation period of 19 days or more, with a large proportion (~40%) of tracer lost presumably due to leaching. This incubation extended well into the growing season, so it is difficult to know from these data whether vascular plants were taking up N early in the spring.

Wet sedge meadow soils near Churchill, Manitoba, Canada, are characterized by large MB and nutrient pools in late winter, which then decline during soil thaw and remain in low abundance throughout spring and summer (Chapter 2). We injected ^{15}N-ammonium into soil cores and then incubated the cores for 1 and 8 days, in order to determine whether *Carex aquatilis*, a non-mycorrhizal sedge and the dominant vascular plant in this system, would take up inorganic nutrients during the winter–spring transition, when soil temperatures are below 0 °C and inorganic N is relatively abundant for a short period of time. We observed the partitioning of labelled N throughout different tissues of the sedge, in mosses and in the soil MB. Given our observations in the field of fine white roots in the frozen soil and of over-wintering shoots embedded in ice and snow (Tieszen 1972; Bell and Bliss 1977; Jonasson and Chapin 1985), we hypothesized that *C. aquatilis* would be able to take up nutrients very early in spring, prior to the onset of rapid above-ground plant growth.

Materials and methods

Site description

We sampled cores from a wet sedge meadow (calcareous fen mire) located at 58° 44’ N 93° 48’ W, about 1 km east of the Churchill Northern Studies Centre, Churchill, Manitoba, Canada. The

region is at the boundary between sub-Arctic and Arctic biomes and near to both boreal forest and the Hudson Bay shoreline. The vascular plant community is dominated by *Carex aquatilis* Wahlenb. The soil is highly organic and about 30 cm deep, underlain by a mineral layer of silt and fragmented limestone, and permafrost at a depth of less than 1 m. Soils are water- saturated throughout most of the year, except in late summer. Soil pH varies between 6.2 and 7.2, and oxidation-reduction potential (*E*h) is typically between -50 to +150 mV at thaw (10 cm depth; platinum electrode with silver chloride reference, calibrated using Zobell's solution). Total carbon (C) and N values are approximately 37% and 2.5% of soil dry weight, respectively.

The depth of standing water varies across the site. Experimental cores were sampled from an area of approximately 20 m^2, along the margin of a wet depression, so that soil cores were water-saturated, but not submerged after thaw. This specific location differs somewhat from the wetter microsites normally utilized for long-term monitoring (Chapter 2), by having soil fresh-weight to dry-weight ratios (FW:DW) that are approximately 25% higher, a shallower moss layer (< 2 cm rather than 2–10 cm) and a c. 50% reduction in the maximum height of *C. aquatilis*.

Core sampling and processing

Core processing was modified from Hart *et al.* (1994). Cores with intact *C. aquatilis* shoots (2 or 3 shoots between 3 and 10 cm tall) were sampled using a custom made CRREL (U.S. Army Cold Regions Research and Engineering Laboratory) permafrost drill on 26 April 2007, and these frozen samples were thawed gradually by keeping them in a refrigerator (4 °C) for approximately 40 h. Small amounts of ice were still present in some cores, indicating that internal core temperatures did not rise significantly above 0 °C. On 8 May and 21 May 2007 we sampled thawed soils using a polyethylene tube and bread knife. These cores were stored at 4 °C for 20 h before injection.

Cores from 26 April were sorted into triplicate groups based on similarity in *C. aquatilis* size, presence of moss, amount of litter, and colour and texture of soil. Each triplicate group, of which there were 5 in total, contained cores for 24-h and 8-day incubations, and a core designated for immediate processing (to test the procedure for quantitative recovery, Buresh *et al.* 1982; herein referred to as a 'time-zero' core). Cores from subsequent sampling dates were sorted into matching pairs, one designated as the time-zero core and the other for 8-day incubation. Prior to

injection, the cores, which were 7.4 cm in diameter, were cut to 7.5 cm depth and were wrapped in plastic wrap (covering the sides and bottom). Cores were injected with 7 mL of 1mM $^{15}NH_4Cl$ (Isotech Laboratories Inc., Champaign, Illinois, USA, > 98 atom % ^{15}N) in a hexagonal + centre pattern, using a 15-cm double-sideport spinal syringe (Popper and Sons, Hyde Park, New York, USA). The cores were inverted, and the needle was inserted into the bottom of the core and then ejected gradually while spinning the needle and withdrawing it. The total amount of N injected into each core was 105 μg, theoretically increasing NH_4^+-N levels by approximately 10%, which is well within the natural variability of NH_4^+-N for these soils. Time-zero cores were processed within 30 min of injection, while 24-h incubations were placed in the refrigerator (4 °C) and 8-day incubations were put in small (18 cm x 19 cm), clear plastic Ziploc bags (S.C. Johnson and Son Inc., Racine, WI, USA) and planted back into the ground at the sampling site. The tops of the bags extended above ground and bags were kept open but the opening faced horizontally such that cores in the field were exposed to the air but somewhat protected from surface runoff and precipitation. Core temperatures were checked periodically during the final incubation period when air temperatures rose above 20 °C. The cores were not more than 1°C warmer than the surrounding soil, even near the surface, so the Ziploc bags were effective at protecting the cores without causing significant soil warming through greenhouse-like conditions.

We processed cores by first removing and sorting live and dead shoot, shoot base, rhizome and root material, and collecting any living moss. Plant material was rinsed in calcium-rich water to remove any ^{15}N from root surfaces and was dried at 50 °C for at least 48 h before weighing for dry mass estimation. After removal of plant tissues, each soil core was broken up and hand-mixed in a large Ziploc bag and portions were weighed for MB extractions and for FW:DW measurements (~10 g fresh weight, dried at 50 °C for a minimum of 48 h).

Microbial biomass extractions followed a chloroform fumigation and extraction method modified for wet soils (Witt *et al.* 2000; Henry and Jefferies 2003) using 25 g fresh soil in 50 mL 0.5 M K_2SO_4, shaken frequently for 1 h, then filtered through pre-leached Whatman 1 filter papers (Whatman International Ltd., Kent, UK). For the chloroform-fumigated fraction, 2 mL ethanol-free $CHCl_3$ was applied directly to the soil and incubated in sealed jars for 24 h, followed by K_2SO_4 extraction. Extracts were kept frozen until further analysis. Microbial biomass C and N were determined as the difference between fumigated and unfumigated organic

C and organic N in K_2SO_4 extracts. Organic C was determined using the potassium dichromate volumetric method (Nelson and Sommers 1996; Henry and Jefferies 2003) and the same extracts were used to determine total N using chemiluminesence (TOC-TN autoanalyzer, Shimadzu, Kyoto, Japan).

Isotope sample preparation

Dried plant tissues and soil were ground using a mortar and pestle, and weighed into tin capsules (2 ± 0.25 g for plant tissue, 0.7 ± 0.1 g for soil) for analysis by mass spectrometry (EA-IRMS; University of Waterloo Environmental Isotope Lab, Waterloo, ON, Canada). Actual mass values accurate to 0.001 g were used for delta-^{15}N calculations of each sample. Total plant N per core was determined from total mass values of each sample and tissue N concentrations from mass spectrometry analysis.

K_2SO_4 extracts for microbial ^{15}N were oxidized by alkaline persulphate digestion (Cabrera and Beare 1993) followed by a modified diffusion technique for recovering ^{15}N (Stark and Hart 1996). Sample extracts were oxidized (autoclaved after mixing with alkaline persulphate solution) and then poured into a 250-mL glass jar along with 2 mL 10 M NaOH and 20 mL alkaline persulphate blank solution. Jars were left open with daily stirring for 3 days and replacement water was added to adjust for evaporative loss. A carrier solution of 0.7143 M KNO_3 (5 µL) was then added to jars, along with 0.3 mL 10 M NaOH, 0.4 g Devarda's alloy, and an acid trap that consisted of 2 pre-leached filter paper discs (7 mm diameter; Whatman no. 1) each containing 5 µL 2.5 M $KHSO_4$, sealed in a strip of polytetrafluroethylene (PTFE) tape. Jars were sealed and diffused for 6 days, mixed daily; then traps were removed, rinsed in water, and filter discs were dried in a dessicator. Discs were weighed and folded into tin capsules, and these were analysed for isotopic composition by mass spectrometry (EA-IRMS; University of California, Davis Stable Isotope Facility, Davis, CA, USA).

Isotope calculations

Delta-^{15}N values were converted to atom % excess (Shearer and Kohl 1993), which is the level of ^{15}N enrichment beyond the standard of 0.3663 (^{15}N natural abundance of atmospheric-N). Calculations of ^{15}N recovered in plant and soil pools were then calculated using the following equation (Powlson and Barraclough 1994), where F represents N recovered from the labelled

addition (μg N g^{-1} tissue), T is the total weight of N in the sample tissue (μg N g^{-1} tissue), A_s is the atom % excess ^{15}N in the treated sample, A_B is the atom % excess ^{15}N in the unlabelled control, and A_F is the atom % excess ^{15}N in the label:

$$F = T(A_s-A_B)/A_F$$

The percentage of label-N that was recovered in each pool was calculated by multiplying F by the mass of tissue in the core and dividing by the mass of N injected into the core.

To calculate microbial immobilization of added N, atom % excess values were corrected for carrier dilution (Powlson and Barraclough 1993) and then corrected using a calculated blank (Stark and Hart 1996) in which the difference between diffused and non-diffused isotope standards is used to account for incomplete nitrate recovery during diffusion. Finally, atom % excess of background samples (no added ^{15}N) was subtracted from corrected sample atom % excess values, and the resulting sample atom % excess was multiplied by the total microbial N pool size and the core volume, and then expressed as a percentage of the total injected label-N. Microbial N values were not corrected using a K_N factor that would account for the proportion of microbes unsusceptible to chloroform fumigation, so the values presented for microbial ^{15}N immobilization may be underestimates.

Seasonal measurements of soil microbial biomass and inorganic nitrogen

Soils were sampled periodically in 2007 (monthly to twice-weekly, throughout the year) from the sedge meadow described above, and from a similar wet sedge area about 5 km west as part of a long-term monitoring project (Edwards, unpublished, but see Chapter 2 for description of methods and sites). In most cases, six replicates were sampled per sampling date, using a CRREL permafrost drill or axe when frozen, or a bread knife when the soil was thawed. Depth of soils sampled and soil processing were the same as described above for ^{15}N experimental cores. Microsites differed slightly, as described previously, with long-term monitoring samples often taken from areas with considerable standing water. Soils collected in the frozen state were kept frozen and processed within one day of sampling. Extractions using frozen soils were done by chopping the sample into small pieces (1 cm^3 or smaller) and allowing 15 extra minutes of extraction time to account for thawing during extraction. Microbial C was quantified as

described above and results were not corrected using a K_C factor. Thus, the MB results reported here may underestimate the true microbial component of C in the soils.

To quantify soil exchangeable ammonium (NH_4^+), 10 g of fresh soil was mixed with 50 mL of 1 M KCl and shaken frequently for 2 h, followed by filtration through pre-leached Whatman No. 1 filter papers. Extracts were stored frozen and NH_4^+ levels were then determined using an autoanalyser (Technicon AAII, Tarrytown, NY). Microbial biomass and NH_4^+ levels are expressed on a per-volume basis, because the ^{15}N experiment was conducted with cores of consistent soil volume, and because we are most interested here in plant root uptake.

Seasonal measurements of soil temperatures

Soil temperature readings were taken in a nearby site that is similarly wet and sedge-dominated, but also contains some shrubs (G. P. Kershaw, pers. comm.; see Chapter 2 for details on temperature monitoring). Soil temperatures at this site correlate well with measurements recorded from October 2008 to June 2009, from sensors that are located within the ^{15}N sampling area, and integrate between 5 cm and 10 cm soil depth (K. A. Edwards, data not shown). Thus the soil daily minimum and maximum temperature data reported here are not specific to the site from which the samples came, but provide a reasonable proxy for the conditions at the experimental site.

Statistics

Root biomass, shoot biomass, MB and total plant N from experimental cores were fitted to simple linear and linear spline regression models. Normality was checked using the Shapiro–Wilks test, and variables were square-root transformed as necessary. The absence of curvilinearity and heteroscedasticity in residual plots was verified by visual inspection. Two spline regression models were evaluated which differed in the placement of single pre-determined knots (6 May and 9 May). Linear and spline models were compared based on adjusted R^2 values, error terms and *p*-values, and for each variable the model of best fit is presented.

Both 1-day and 8-day incubations were carried out for the first experimental trial, and a Student's t-test was used to detect differences in ^{15}N uptake between the two incubation times,

using $\alpha = 0.05$. One-tailed tests were utilized in order to detect increases in microbial and plant ^{15}N accumulation and a decrease in soil ^{15}N accumulation over time. Normality and homogeneity of variances were verified (Shapiro–Wilks test, and both Levene and Bartlett's tests, respectively) and variables were transformed as necessary.

Accumulation of label-N in microbial and plant pools after 8 days of field incubation was evaluated using simple linear regression. Assumptions for regression analysis were checked as outlined above and data was transformed when necessary. All analyses were computed with JMP 7.0 (SAS, 2007).

Results

Timing of the experiment in relation to soil thaw, microbial biomass and inorganic nitrogen

Within the larger study site, soil inorganic N levels declined over the period of the isotope experiment from 27 April through May 2007, during the time that soil temperatures reached and rose above 0 °C (Fig. 3.1a). Microbial C levels also declined over this time (Fig. 3.1b). By the end of the experiment in June 2007, NH_4^+ and MB levels had reached low levels that are typical of this system during the summer months (Chapter 2). This decline corresponds with the onset of above-zero soil temperatures and steep increases in the amount of liquid water in the system, which was observed in the field beginning on 2 May 2007, resulting in the presence of standing water at the site. Snow melt and soil thaw continued at the site as air temperatures warmed, but cold events occurred on two occasions in May, most notably beginning 16 May when fresh snow covered the site, which had previously been snow-free, and the surface ground refroze for a couple of days.

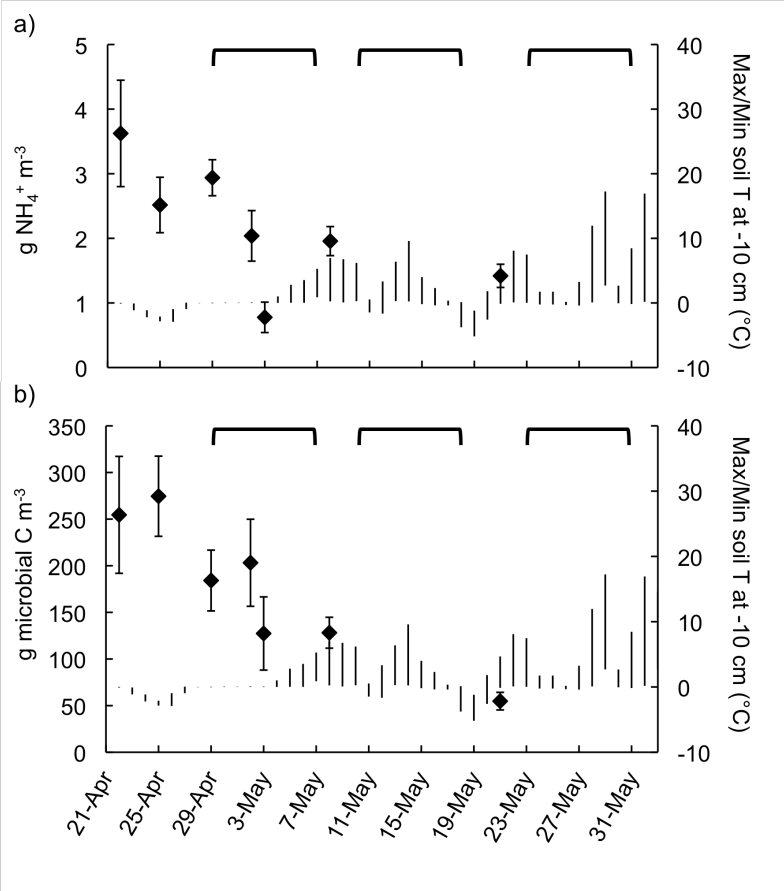

Fig. 3.1. Levels of a) ammonium and b) microbial biomass carbon (mean ± SE; n = 5) in wet sedge meadows near Churchill, Manitoba, Canada, during April and May 2007. Vertical lines are daily max-min soil temperature intervals as measured at 10 cm depth. The three periods of time shown by horizontal brackets indicate the timing of 3 experimental trials, each lasting 8 days.

Inorganic nitrogen uptake into microbial and plant pools

Time-zero cores were processed to verify the recovery of added ^{15}N in the soil prior to incubation (Buresh et al. 1982). Recovery of ^{15}N from the soil was usually between 70 and 100%, but some samples were outside of this range, probably reflecting inadequate mixing of soils before partitioning for extractions. Microbial recovery in time-zero cores tended to be unexpectedly high, close to 10% on average. This could also be the result of inadequate mixing, or rapid uptake by microbes may have taken place (within 30 minutes of injection), exacerbated by the disturbance of soil mixing, which could stimulate microbial activity. Significant levels of ^{15}N were not detected in plant and moss tissues from time-zero cores.

Recovery of injected-N was greatest in soil, root and microbial pools (Table 3.1). One-tailed t-tests did not detect differences between the 24-h and 8-day incubation periods (sampled on 21 April) of ^{15}N pools in microbes ($t_8 = 0.40$, $P = 0.351$) or roots ($t_8 = 1.09$, $P = 0.15$); however, soil ^{15}N recovery was lower after 8 days of incubation (mean = 25.5%) than after 24 h of incubation (mean = 76.2%; $t_8 = -1.90$, $P = 0.047$). This is likely an artefact of poor soil mixing and sampling for isotope measurement, since the two subsequent 8-day incubation trials resulted in soil ^{15}N recovery that was more similar to the 24-h incubation (means of 78.0% and 53.6%).

Root N-uptake appeared to increase over the entire experiment, with the mean label-N reaching 63% after the final 8-day incubation period (Fig. 3.2; $R^2 = 0.17$, $F_{1,13} = 3.78$, $P = 0.074$). Shoot ^{15}N was a smaller component of the total label-N recovery, reaching a mean of only 0.9% during the final 8-day trial, although this increase represents an upward linear trend ($R^2 = 0.71$, $F_{1,13} = 34.70$, $P < 0.001$). Recovery of label-N from the microbial N pool declined over the experiment from a mean of 30.4% in the first trial to 7.7% after the final trial (Fig. 3.2; $R^2 = 0.60$, $F_{1,13} = 22.26$, $P < 0.001$). Small amounts of tracer N were measured in rhizomes, shoot bases and shoots of *C. aquatilis*, while mosses took up only trace amounts of the N label (Table 3.1).

Table 3.1. Percentage recovery of added N into soil (excluding microbial), microbial, various *Carex aquatilis* and moss pools. Means and Min.-Max. intervals (n = 5) are shown for each incubation period. All cores were sampled from a wet sedge meadow near Churchill, Manitoba, Canada, in April and May 2007.

Sampling date	28-Apr	28-Apr	09-May	22-May
Incubation time	1 day	8 days	8 days	8 days
soil	76.2 (13.5-137.9)	25.5 (2.6-74.9)	78.0 (61.9-105.6)	53.6 (18.5-97.6)
microbes	25.7 (2.3-54.0)	30.4 (15.5-52.0)	15.6 (6.9-24.1)	7.7 (4.1-13.2)
roots	21.0 (11.3-30.0)	33.5 (13.3-65.6)	40.7 (15.8-69.1)	63.4 (15.8-37.3)
rhizomes	1.5 (1.0-2.7)	2.1 (0.8-4.2)	1.2 (0.0-4.3)	0.8 (0.0-0.3)
shoot bases	1.7 (0.0-7.3)	2.1 (0.0-3.8)	0.7 (0.3-1.4)	0.6 (0.3-0.1)
dead shoots	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
live shoots	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.2 (0.0-0.2)	0.9 (0.4-2.1)
mosses	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.0 (0.0-0.0)

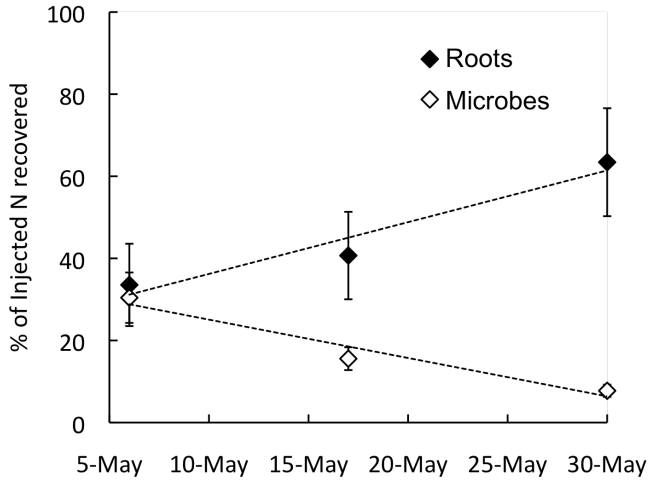

Fig. 3.2. Recovery of injected ^{15}N-ammonium in *Carex aquatilis* roots and soil microbes in experimental soil cores collected on three occasions in April and May 2007 from wet sedge meadows near Churchill, Manitoba, Canada, and incubated in the field for 8 days. The proportion of label-N recovered from microbes decreased over the three trials ($R^2 = 0.60$, $P < 0.001$) while root label-N uptake showed a marginally insignificant increasing trend ($R^2 = 0.17$, $P = 0.074$). Untransformed means and standard errors are plotted (n = 6) with linear regression lines.

Plant biomass, plant N and microbial biomass in experimental cores

The biomass of *C. aquatilis* roots in the experimental cores increased throughout the experiment (Fig. 3.3; linear regression $R^2 = 0.53$, $F_{1,40} = 47.43$, $P < 0.001$). Shoots from the same plants best fit a linear spline regression with a knot designated on 9 May, after which linear growth was evident (Fig. 3.3; linear spline regression $R^2 = 0.15$, $F_{2,39} = 4.64$, $P = 0.012$). Mean root:shoot ratios therefore increased from less than 15 to greater than 20 over the time of the experiment.

Total plant N from experimental cores increased over the experiment from a low of 0.6 mg core^{-1} on 29 April to 2.2 mg core^{-1} on 30 May (Fig. 3.4; linear regression $R^2 = 0.55$, $F_{1,40} = 50.89$, $P < 0.001$) revealing that in the order of 1.6 mg N was taken up over a 33-day interval. Microbial biomass levels in these cores decreased from above 300 mg microbial C m^{-3} to below 200 mg m^{-3} during the experiment (Fig. 3.4; linear regression $R^2 = 0.35$, $F_{1,40} = 22.75$, $P < 0.001$). This decline was similar to that documented for the larger site, but the magnitude of the decline was dampened, and the absolute values were higher in experimental cores, relative to other areas of the site, at least on a per-volume basis.

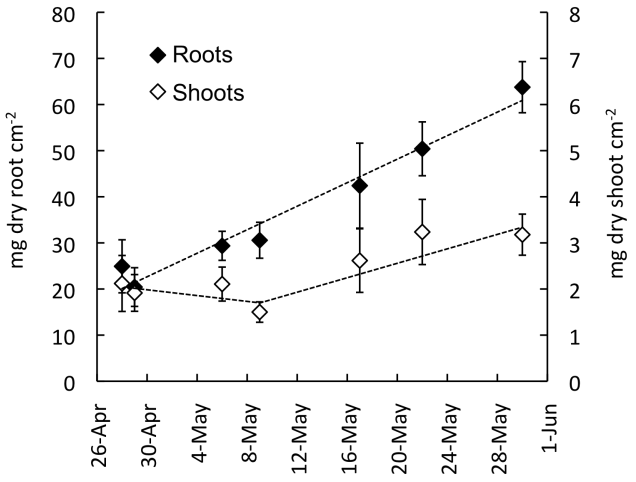

Fig. 3.3. Biomass of shoots and roots from experimental soil cores processed between 28 April and 30 May 2007 that were sampled on three occasions in April and May 2007 from a wet sedge meadow near Churchill, Manitoba, Canada, and then incubated for 0, 1 or 8 days before plant tissue harvesting. Root biomass increases linearly over the period ($R^2 = 0.53$, $P < 0.0001$) while shoot biomass is best fitted to a spline regression model containing a knot at 9 May ($R^2 = 0.15$, $P = 0.016$). Untransformed means and standard errors are plotted (n = 6) with linear regression lines.

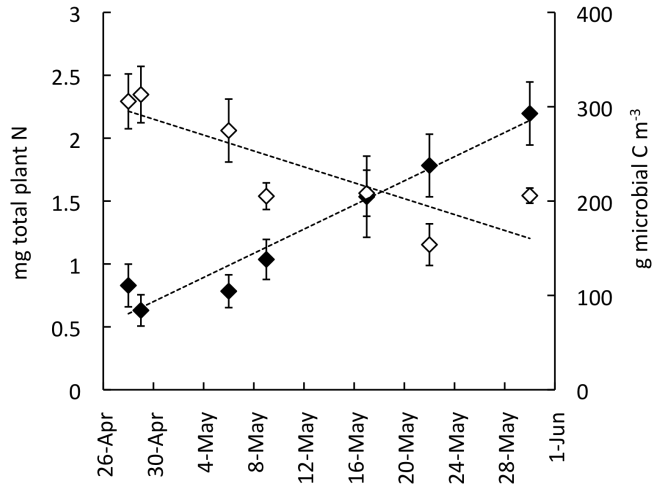

Fig. 3.4. Microbial carbon and total plant nitrogen from experimental soil cores processed between 28 April and 30 May 2007 that were sampled on three occasions in April and May 2007 from a wet sedge meadow near Churchill, Manitoba, Canada, and then incubated for 0, 1 or 8 days before plant tissue harvesting. Microbial C (open diamonds) declined over the period ($R^2 = 0.35$, $P < 0.001$) while plant N (black diamonds) increased ($R^2 = 0.55$, $P < 0.001$). Untransformed means and standard errors are plotted (n = 6) with linear regression lines.

Control cores (injected with water instead of $^{15}NH_4^+$) were not different than treatment cores in terms of plant biomass, plant N concentration or MB (data not shown), suggesting that the injection of NH_4^+ did not produce a fertilization effect and that our results are not due to the small increase in inorganic N provided by the isotope tracer.

Discussion

Carex aquatilis took up large proportions of added ^{15}N-ammonium over short-term incubations during the seasonal transition between winter and spring, when snowmelt was well underway but the soil was still partially frozen at the site. *Carex aquatilis* is not known to be mycorrhizal (Muthukumar *et al.* 1994), so N-uptake reported here is not likely to be attributable to fungal associations.

The ability of plants to take up nutrients at or below freezing temperatures is well known. Billings *et al.* (1977) demonstrated that *C. aquatilis* and *Eriophorum angustifolium* roots in Barrow, Alaska, USA, could grow at temperatures below 1 °C, and roots of *E. angustifolium* grew along the interface between the active layer and frozen soil. Further, roots from these two species as well as *Dupontia fischeri* that were frozen for several days were able to recover to normal root elongation rates after being thawed for 12 h. More recently it has been observed that *Corydalis conorhiza*, an alpine herb, has snow roots that are specifically adapted to take up N in the snowpack (Onipchenko *et al.* 2009).

We have demonstrated here that *C. aquatilis* roots can acquire significant quantities of inorganic N within a few hours of soil thaw. Since we thawed soils prior to treatment, we cannot be sure that N-uptake by plants occurred earlier than this; however, it is quite likely that plants can take up nutrients during soil thaw, when channels of liquid water form around plant roots, accelerated by early snowmelt around standing dead plant material (caused by lowered albedo). Additional heat produced by increased plant and microbial metabolic activity in the rhizosphere may also contribute to this localized warming, providing suitable microsite conditions for nutrient uptake by plant roots even when much of the bulk soil water is frozen.

Carex aquatilis accumulated more NH_4^+ during the winter–spring transition than did soil microbes, in contrast to the prevailing wisdom that over short-term intervals (days to weeks) soil microbes outcompete plants for N (Jackson *et al.* 1989; Lipson *et al.* 1999; Zak *et al.* 1990;

McFarland *et al.* 2002; Bardgett *et al.* 2003; Nordin *et al.* 2004; Andresen and Michelsen 2005; Grogan and Jonasson 2005; Harrison et al. 2007; Nasholm et al. 2009). However, some studies have found plant uptake of ^{15}N-labelled N to be comparable to net microbial immobilization (Norton and Firestone 1996; Lipson and Monson 1998), and in an Arctic salt marsh just east of our experimental site, 29% of injected NH_4^+ was recovered in the dominant graminoid, *Puccinellia phryganodes*, while 34% was immobilized by soil microbes (using a K_N correction, for incomplete microbial recovery, of 0.35; Henry and Jefferies 2003).

Previous authors have suggested that over the long term, plants may compensate for their short-term competitive disadvantage by maintaining the ability to acquire nutrients throughout the year (Andresen and Michelsen 2005). This view is supported by studies that show decreasing microbial sink strength during experiments lasting several months to one year (Hart et al. 1993), and by experiments demonstrating the importance of pulsed nutrient supplies as compared with steady supplies of nutrients for spring plant growth (Bilbrough and Caldwell 1997). Our study lends further support to the view that ephemeral nutrient supplies are an important component of the annual N budget of at least some plants.

Several features of the late winter and early spring period in wet sedge meadows make this a favourable time for plant nutrient uptake, namely, large pools of nutrients, moderately flowing water, and weakened microbial populations. Nutrient pools are at or near annual peaks at this time of year (Chapter 2, Lipson et al. 2002; Nemergut et al. 2005; Buckeridge and Grogan 2008). The presence of moderately flowing water during spring snow melt facilitates nutrient mobility, increasing nutrient flux to root surfaces (Chapin *et al.* 1988). Moving water may also temporarily increase levels of dissolved oxygen in sites that are ice-bound in winter and water-logged throughout much of the summer. However, in some cases spring runoff may result in significant N losses, particularly when large volumes of water are flowing rapidly, where nitrate-N is abundant, and where N is deposited on soil surfaces, i.e. by atmospheric deposition (Joseph and Henry 2008,2009). With soil microbes in decline during the transition from winter to spring, the competitive ability of soil microbes for nutrient acquisition may be lower than it would be during the rest of the growing season, when turnover of N by microbes is relatively high despite low MB (Buckeridge and Jefferies 2007). However, data from a *Carex bigelowii* – dominated heath site in Scotland suggested that microbes in spring could be strongly N-limited, and therefore be intense competitors for N, despite low MB at this time of year (Bardgett et al. 2002).

Our results reveal that about 1.6 mg of N was taken up in 33 days by the two or three small *C. aquatilis* ramets contained in each experimental core. These ramets would each accumulate about 15 mg of N by the end of the growing season (Edwards, unpublished), so this early season uptake represents on the order of 5% of the total N for the season. This initial gain early in the season would be important given the rapid growth that follows once temperatures warm. Daily minimum soil temperatures at the end of this experiment were still hovering just above zero (Fig. 3.1), so the time of greatest nutrient uptake likely followed soon after, with maximal growth and tissue N accumulation expected to occur during the first half of the growing season (Jaeger et al. 1999). The 5 % estimate is lower than the mean 12% of annual N uptake reported for alpine tundra graminoid species during snowmelt (Bilbrough et al., 2000), but is much higher than estimates reported by the same authors for Alaskan tundra species (< 0.1%). However, Bilbrough et al. (2000) applied ^{15}N to the snowpack, so it is unclear whether differing results between the two studies are due to methodological differences or due to biological differences in ecosystems, sites or plant species.

Although spring thaw is a favourable time for N uptake by *C. aquatilis*, we observed that the inorganic N that was taken up remained in the roots and was not translocated in significant amounts to other plant tissues such as the rhizome, shoot base or shoot. An increasing amount of N was translocated to shoots throughout the experimental period, but this was a small amount relative to what remained in roots, even after shoot development and growth were observed during the final experimental trial. This is in contrast to several studies conducted later in the growing season that found high rates of tracer- ^{15}N (Schimel and Chapin 1996; Grogan and Jonasson 2003; Sorensen *et al.* 2008) and ^{32}P (Jonasson and Chapin 1991) allocation to graminoid shoots, suggesting that N acquired in early spring is used for root growth, and/or stored in roots for weeks or even months, and that initial shoot growth in the spring relies on N taken up the previous year and stored in rhizomes over the winter (Jaeger and Monson 1992).

Recovery of ^{15}N from injected cores was high, often totalling well over 100%. This is not unexpected or uncommon (Finzi and Berthrong 2005; Clemmensen et al. 2008) given the large volume of soil injected and the difficulty of mixing soils thoroughly and quickly. Based on this, we believe that soil and microbial pools are more prone to error than are plant tissue pools, which are more completely homogenized prior to subsample collection for isotopic collection.

The winter–spring transition is recognized as an important time of year in the functioning of northern ecosystems and especially in understanding the consequences of broad-scale environmental disturbances including climate change. This time of year is marked by fluctuations in temperature, snow depth and the physical state of soil water (Chapter 5), and the phenology of events below ground that impact the summer growing season are likely to be altered in the north as winters become warmer and snow fall increases (IPCC, 2007). The effects of these changes on plant productivity and nutrient cycling are unknown. It is possible that as springtime arrives earlier in the year, nutrient flushes that result from late-winter microbial die-off and spring meltwater discharge will occur before some plants are metabolically ready to utilize resources, and a large proportion of the annual N supply to Arctic plants could be lost through microbial immobilization, gaseous efflux and/or leaching. Alternatively, some plants may respond to these warming events by increasing uptake of N (Turner and Henry, 2009), enhancing primary productivity and carbon sequestration potential. However, if plant nutrient uptake and growth is initiated during a winter warming event followed by subsequent freezing, development and reproduction during the growing season that follows can be dramatically compromised (Bokhorst et al. 2008, 2009; Joseph and Henry 2008). Further monitoring and experimental work, including during the fall and spring shoulder seasons, is needed to properly anticipate the effects that shifting climate patterns will have on soil nitrogen cycling and resultant plant productivity.

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Chapter 4
Nutrient amendments do not sustain high winter microbial biomass in low Arctic wet meadows

Preface

In Chapter 2, a seasonal pattern was described in which soil microbial biomass peaked in winter and declined quickly during soil thaw. Several hypotheses to explain this pattern have been discussed in the literature, and most prominent among them is the suggestion that winter microbes exhaust available carbon substrates and other nutrients, leading to population declines. In this chapter, I present a series of experiments designed to test this hypothesis.

Abstract

Studies conducted in several Arctic ecosystems have documented large soil microbial biomass (MB) in late winter, followed by a reduction in biomass during the transition from winter to spring, and relatively small MB persisting throughout the summer. Several authors have suggested that the observed reduction in soil microbes is caused, at least in part, by the exhaustion of resources, including suitable carbon substrates. I conducted three experiments in low-Arctic sedge meadows near Churchill, Manitoba to test this hypothesis. Field additions of carbon (C), nitrogen (N), and phosphorus (P) in dry sedge meadows confirmed co-limitation of microbial biomass growth in summer by all three resources. In wet sedge meadows the addition of these nutrients failed to maintain large winter microbial biomass pools across the period of soil thaw. A laboratory incubation experiment was conducted in which multiple carbon sources were added to frozen wet sedge meadow soils either in the presence or absence of added water. Microbial biomass C (MBC) declined as soils thawed (incubated at ~4 °C) and over the 7 days of incubation, CO_2 efflux increased and net CH_4 efflux became less negative. Soils did not respond to carbon additions except for increased CO_2 production after 1 and 2 days of incubation.

In a third experiment, frozen soil cores were sampled from wet sedge meadows and treated by injecting a solution into pre-drilled holes containing a mixture of simple and complex C compounds, and inorganic N and P. Control (untreated) cores were injected with water. Cores

were replanted into the ground and five replicate pairs were recovered after four, eight, or twelve days of field incubation. This routine was carried out five times over the seasonal transition between winter and spring, at eight-day intervals. The treatment was effective in boosting levels of phosphate and ammonium throughout the experiment, and C was noticeably elevated in the initial period of the experiment. However, MBC did not respond positively to resource additions. I conclude that resource limitation does not contribute significantly to the late-winter microbial biomass decline in these sites. Instead, I suggest that the influx of liquid water during soil melt is the primary driver of the observed MB reduction.

Introduction

The seasonal transition from winter to spring brings about intriguing biological and biogeochemical changes in Arctic tundra soils that have only recently been characterized in some sites, and remain poorly understood. Studies in both alpine and Arctic tundra have revealed that soil microbial biomass (MB) is large at the end of winter, shrinking during the winter-spring seasonal transition to relatively small amounts during the plant growing season (Chapter 2, Lipson et al. 2000, Grogan and Jonasson 2003, Schmidt and Lipson 2004, Larsen et al. 2007, Buckeridge and Grogan 2008). Equally dynamic are the changes in measured amounts of soil nutrients across this seasonal transition, although there is considerable variation in the timing of nutrient peaks. Some studies report the occurrence of inorganic nitrogen (N_i) peaks after the observed MB decline (Schmidt and Lipson 2004) while others have documented peak amounts of nitrogen (N) in winter, either prior to (Brooks et al. 1998) or concurrent with (Chapter 2) the MB peak. In all cases, MB and nutrient dynamics are inextricably linked, and nutrient recycling by microbial communities during the winter contributes significantly to mineral nutrient supplies in spring that support plant growth (Hobbie and Chapin 1996, Lipson et al. 1999, Brooks et al 1998, Grogan and Jonasson 2003). In order to better understand what drives nutrient fluxes in these cold ecosystems, and how nutrient fluxes may be affected by climate-driven seasonal variability and warming events, it is necessary to understand what drives the seasonal reduction of MB.

Several contributing factors have been suggested as explanations for the early-spring MB reduction in frozen soils, including freeze-thaw cycles, temperature, physical-chemical changes, predation, and resource shortages. Freeze-thaw cycles can cause reductions in soil MB (Schimel

and Clein 1996, Larsen et al 2002, Grogan et al. 2004, Henry 2007, Schimel et al. 2007) due to the stress of both the temperature and moisture fluctuations that characterize freeze-thaw events (Schimel et al. 2007). However, experimental demonstrations of this effect have often used unrealistic temperature fluctuation regimes that would not regularly occur under field conditions (Henry 2007). More moderate and realistic freeze-thaw cycles do not appear to provoke significant microbial die-off, particularly with soils accustomed to moderate freeze-thaw cycles such as those of Arctic tundra sites (Grogan et al. 2004, Henry 2007). Also worth noting is that in wet tundra, where large volumes of frozen water buffer the system against air temperature fluctuations, even large daily fluctuations in air temperature do not typically thaw and refreeze the bulk soil, except at the ground surface (Buckeridge and Grogan, 2009).

Rising soil temperature has been discussed as a potentially lethal stress to the psychrophilic microbes that may be abundant in winter (Lipson et al. 2000). These are microbes that grow below 0 °C but optimal and maximum temperatures for growth are much higher (~15 °C and ~20 °C respectively; Gounot 1986, Robinson 2001, Deming 2002, Beales 2004). Therefore the MB decline, often seen at temperatures in the range of -2 °C to +2 °C, is not attributable to the increase in temperature alone, although temperature may cause other physical-chemical changes to occur that create inhospitable conditions for the winter-adapted microbial community.

Physical-chemical changes brought on by warming temperatures in late winter and early spring have been recently suggested as a potential cause of soil MB reductions (Chapter 5, Schimel et al. 2007, Buckeridge and Grogan 2008). In particular, osmotic changes occur when large amounts of fresh water infiltrate the highly saline films of liquid water that provide microbial habitat in frozen soils (Chapter 5, Schimel et al. 2007). Oxygen concentration may also change dramatically between winter and spring, particularly if soils become very anaerobic over the winter months and are rapidly aerated during thaw.

Faunal predation and/or viral attacks on soil microbial communities may also play a role in the MB decline observed during thaw. The hatching of nematodes, springtails (Collembola), mites, and protozoa in spring can cause declines in MB (Ingham 1986, Sjursen et al. 2005) and the timing of hatch may correspond with the timing of microbial biomass decline at least in some sites (K. Buckeridge, *pers. comm.*) Little is known about this possible trophic interaction at thaw,

and even less is known about the role of soil viruses (Srinivasiah et al. 2008), but this too remains a possible contributor to the observed microbial patterns.

Lastly, several authors have suggested that the seasonal MB decline is the result of resource exhaustion, particularly of carbon (C) (Lipson et al. 2002, Schmidt and Lipson 2004). Different substrates for microbial utilization are available in winter than in summer, which can lead to different composition of the microbial community (Schadt et al. 2003, Lipson et al. 2002) as well as to different limitations on microbial growth between seasons. In summer, C substrates from plant root exudates are readily available for microbial consumption along with the initial products of litter decomposition (Bardgett et al. 2005). High C:N content of these substrates is believed to lead to N-limitation and observed net N immobilization by microbes in summer (Schmidt et al. 1999, Schimel et al. 2004). In the winter, dissolved organic carbon as well as microbial products and necromass have been shown to be important microbial substrates (Schimel and Mikan 2005), and utilization of these substrates having lower C:N content may lead to C-limited growth conditions, and cold-season net mineralization of N (Grogan and Jonasson 2003, Schimel et al. 2004). This may however be more characteristic of early than of late winter (Schimel and Mikan 2005).

Experimental evidence in support of wintertime microbial C-limitation has largely come from studies showing that respiration by these microbial communities responds positively to C amendments (Lipson et al. 2000, Brooks et al. 2004, Grogan and Jonasson 2005). Other studies have shown that C substrate availability also limits methane production in peatlands (Valentine et al. 1994, Bergman et al. 1998). Resource limitations on microbial growth (biomass) have less often been examined for Arctic sites, and Schimel and Weintraub (2003) contend that while respiration may be increased in the presence of added C, growth may still be N-limited due to the demands of exoenzyme production. Respiration can increase in response to added C, without concomitant biomass increase, through “overflow metabolism” (Tempest and Neijssel 1992). However, in an experimental test of these ideas, Buckeridge and Grogan (2008) showed a positive response of both microbial activity and MB to C additions in Arctic heath tundra soils at the end of winter, an effect that was exacerbated under conditions of deepened snow.

In the sedge meadows studied here, MB is reduced over the period of warming between winter and spring, with the completion of this decline occurring once soil water is liquid (Chapter 2,

Chapter 6). In three experiments I have tested the hypothesis that resource shortages cause the observed MB decline. These experiments are all based on the principle that if resource shortages limit MB and if these same resources are added to soil during the decline phase, large soil MB would be maintained through the thaw period. In all cases I hypothesized that adding resources would prevent or reduce the MB reduction when compared with untreated soils, over the period of soil thaw.

Materials and Methods

Field sites

Experiment 1 was conducted in both wet and relatively dry (mesic) sedge meadows, located near the Churchill Northern Studies Centre (CNSC), Churchill, Manitoba (see Chapter 1, Fig. 1.1). The wet meadow is about 2 km east of the CNSC and is characterized by tall dense growth of *Carex aquatilis*, saturated soils, and a deep, peaty organic layer underlain by calcareous gravel. Soils are pH-neutral and moderately anaerobic in summer. The dry sedge meadow is situated approximately 5 km west of the CNSC and is characterized by lower plant productivity, higher plant diversity including several ericaceous species, and a shallow organic layer (10-15 cm) underlain by sand. Soils are pH-neutral and aerobic. More detailed descriptions of these sites are provided in Chapter 6. Experiments 2 and 3 used soils collected only from the wet sedge meadow site.

Experiment 1: Field nutrient additions

Experiment 1 was a field experiment in which nutrients were added to the ground surface at both wet and dry tundra sites. Four replicate plots were set up in each of the two sites. Each plot consisted of four treatment subplots, receiving C and NP additions in a randomized factorial design. Subplots were each 50 cm x 100 cm in size, and nutrients were added in crystalline form. Carbon was added as glucose ($C_6H_{12}O_6$) and 100g / m^2 was applied. Nitrogen was added as ammonium sulphate $(NH_4)_2SO_4$ and 40 g / m^2 was applied. Phosphorus was added as a combination of sodium phosphate dibasic dihydrate ($Na_2HPO_4.2H_20$) and sodium phosphate monobasic ($NaH_2PO_4.H_20$) using 17 g / m^2 and 13 g / m^2 respectively. Salts were weighed and mixed in the lab and were applied in the field on 1 June, 2005 and 15 July, 2005 and again

between 21-29 April, 2006. The 2006 addition was done at the end of snowmelt, when the ground was still frozen and ice-covered in many areas.

Soil was sampled from dry plots on 12 May, 21 May, 29 May, and 18 July 2006. Wet plots were sampled on 15 May, 23 May, 31 May, and 22 July 2006. Soil cores (diameter = 7.4 cm) from dry sites included the entire organic soil horizon, whereas wet site samples utilized soil no deeper than 20 cm below the ground surface. Soils were partially frozen in mid-May at the time of the first sampling, and were completely thawed by the end of May. Soil samples were processed within 2 days of sampling.

Microbial biomass extractions were done using a modified chloroform incubation and extraction technique (Witt et al. 2000) using 25 g fresh soil and 50 mL of 0.5 M K_2SO_4 extracted for 1 h, mixed frequently, and filtered using pre-leached Whatman 1 filter paper. Fumigated portions received 2 mL of ethanol-free $CHCl_3$ and were sealed and incubated at 4 °C for 24 hrs before extraction. K_2SO_4 extracts were analyzed for C using the volumetric dichromate method (Nelson and Sommers 1996), and for phosphate using the ammonium molybdate method (Ames 1966). Organic N was measured by chemoluminesence using a TOC-TN autoanalyzer (Shimadzu, Kyoto, Japan), except for the 15 May sampling (wet site), which was analyzed using alkaline persulphate oxidation followed by nitrate analysis (Cabrera and Beare 1993). Measures from unfumigated samples were subtracted from fumigated samples to estimate microbial C, N, and P pools. Unfumigated samples were used to estimate soil extractable C, N, and P pools. Inorganic N was measured by extracting 10 g fresh soil with 50 mL of 1 M KCl for 2 hrs with frequent mixing, filtered using pre-leached Whatman 1 filter papers. KCl extracts were then analyzed for ammonium and nitrate using an autoanalyzer (Technicon AAII, Tarrytown, NY), in which NO_3^- is reduced with cadmium and the resulting NO_2^- is measured calorimetrically. A sample of fresh soil (8-15 g) was weighed, dried at 50 °C for at least 48 h, and re-weighed to estimate the ratio of fresh weight: dry weight (FW:DW) in order to express quantities per unit dry mass of soil.

Statistics were performed in JMP 7.0 and all significance tests used alpha = 0.05. Variables having non-normal distributions were square-root or log transformed as necessary to reduce or eliminate negative skew in the data. Mixed model ANOVAs were performed for each variable of interest, with plot specified as a random effect, and sampling date and treatment as fixed effects. No “treatment x date” interaction effects were detected so this interaction term was excluded

from the models. When significant effects were detected, differences between effect levels were investigated using Tukey HSD.

Experiment 2: Laboratory incubations

Experiment 2 was a laboratory experiment involving soil incubations in jars, with and without added C and water. Soil was collected from the wet sedge meadow site between 18 April and 1 May, 2007. Soil samples selected for the experiment were taken from a larger pool of samples from this two-week period, and were estimated to contain relatively large MBC pools, based on microbial biomass phosphorus (MBP) measurements that were done in the field between the time of sampling and the initiation of the experiment. Soil was stored frozen (-20°C) until the experiment was conducted between 24 May and 4 June, 2007. Seven replicate soil samples were used, divided into 100 g portions. Soils were incubated in small Mason jars with or without a C treatment for 12, 24, 48, 72, 120, or 168 h, totaling 84 incubations (7 samples x 2 treatments x 6 incubation times). Portions weighing 100 g were cut into 3 or 4 small pieces (frozen) and placed in a Mason Jar with 110 mL of C solution or distilled water. Additionally, 3 of the 7 soil samples were also incubated with or without C, but without the water addition ("ambient water"), for 24, 72, or 168 h, contributing 18 more jars (3 samples x 2 treatments x 3 incubation times). Lids were placed on jars but were removed for a short time each day, so that aerobic conditions were maintained in jar headspace throughout the incubation.

The C treatment was applied as a combination of calcium lactate hydrate, sodium propionate, sodium butyrate, and sodium acetate trihydrate, and the treatment solution was verified to be pH-neutral, as is the *in situ* soil solution. These substrates are somewhat more complex than simple sugars and therefore are more representative of *in situ* sources and are less likely to be quickly exhausted. Ten mL of solution containing 2 mM concentrations of each C source was added to the treatment jars, along with 100 mL of water for treatments receiving water. The amount of added-C totaled 3.2 mg per jar. The addition of water nearly or entirely submerged the soils so that they remained saturated, analogous to *in situ* conditions during thaw. Water and treatment solutions were approximately 10 °C when added to soils. Jars were swirled gently to encourage microbial access to added resources without causing undue disturbance, and were incubated in the fridge (2-5 °C) until processing. Prior to processing the soil, jars were fitted with lids that were modified with rubber septa to enable gas sampling. Jars were sealed and gas samples were

taken after 20, 40 and 60 minutes. After gas sampling, headspace volume was measured, and soils were retrieved and were drained of excess water. Soils were then processed for microbial biomass C, extractable C, and FW:DW using the methods described above for Experiment 1. For some samples, the mass of soil used for extractions was reduced, but ratios of soil:extractant and soil:chloroform:extractant was kept the same for all samples.

Gas samples were analyzed for CO_2 and CH_4 using a gas chromatograph (Shimadzu GC-8A, Kyoto, Japan). Net gas fluxes were calculated by averaging values from the three 20-minute runs, after verifying that flux rates were linear over the 60-minute sampling period. Values from blank jars (containing treated or control water but no soil) were subtracted from experimental jars, and fluxes were calculated in relation to the measured volume of headspace for each jar. Two samples were lost during analysis, with the result that only one replicate is reported for the ambient water + C treatment on incubation day 7 (Fig. 4.6c).

Statistics were performed in JMP 7.0 and all significance tests used alpha = 0.05. Variables having non-normal distributions were square-root or log transformed as necessary to eliminate or reduce the negative skew in the data. For the water-added jars, mixed model ANOVAs were performed, with sample specified as a random effect, and incubation time and treatment specified as fixed effects. Significant "treatment x incubation time" interactions were also included. When significant effects were detected for variables with multiple effect levels, differences between levels were determined using Tukey HSD.

In order to analyze the effect of added water, paired samples of ambient-water and water-added treatments, with and without C, were included in the model (n = 3). Each of the three incubation times was analyzed separately. For each time, sample was specified as a random effect and water and C additions were fixed effects, with "water x C addition" interaction terms included when significant.

Experiment 3: Field-incubated cores

Experiment 3 combined the control of laboratory soil manipulations with the realistic temperature regime of the field, by treating soils in the laboratory and incubating them in the field. Cores were sampled from 5 locations (no closer than 20 m) throughout the wet sedge meadow site using a custom designed CRREL (U.S. Army Cold Regions Research and

Engineering Laboratory) permafrost drill fitted with carbide cutters. On each sampling occasion, 30 cores (vegetation intact; 7.4 cm diameter) were sampled and were put into the freezer (-20 °C) for 8 days. They were then sorted into matching pairs (based on characteristics of soil depth profile, vegetation cover, and ice cover) and cut to 6 cm depth, from the ground surface. Ten holes were drilled into the bottom of each core in a regular pattern, through 4 cm of the soil depth, so as not to breach the surface. Fifteen cores were used as controls, and 5 mL of water was injected into the holes of these samples, while the other 15 cores were treated by injecting 5 mL of a treatment solution.

Injecting was done over a period of approximately 30 minutes, with cores warming slightly in order for the injected volume to be distributed throughout the core. The treatment solution contained carbon (0.4 mM trehalose, 20 mM glucose, and 20 mM ethanol), nitrogen (10mM ammonium sulphate) and phosphorus (3 mM $NaH_2PO_4.2H_20$). Trehalose is a naturally occurring disaccharide, and ethanol is an appropriate substrate for methanogenic organisms (Valentine 1994, Yavitt et al. 2006). Amounts corresponded with each core receiving 0.36 mg trehalose-C, 7.2 mg glucose-C, 2.4 mg ethanol-C, 1.4 mg ammonium-N, and 0.45 mg phosphate-P. Cores were put in small Ziploc bags that had small cuts made in the bottom and remained open at the top, and were planted back into the field into new sampling holes at the locations from which they were sampled. Cores were buried such that the core surface was flush with the surrounding ground surface. Any snow that was removed to gain access to the ground was replaced on top of the cores.

Cores were recovered for processing after 4, 8, and 12 days of incubation in the field. This routine was repeated 5 times (5 “runs”) over the transition from winter to spring, at 8-day intervals; this schedule is outlined in Table 4.1. Soils were processed for microbial biomass C, N, and P, K_2SO_4- extractable C, N, and P, N_i, and FW:DW as for Experiment 1 above. Analysis of extracts was also performed as described for Experiment 1, utilizing the TC/TON method for analysis of N from K_2SO_4 extracts.

Statistics were performed in JMP 7.0 and all significance tests used alpha = 0.05. Some outliers (> 3 standard deviations from the mean) were removed from the dataset, and variables having non-normal distributions were square-root or log transformed as necessary to eliminate or reduce the negative skew in the data. Mixed model ANOVAs were performed for each variable of

interest, with plot specified as a random effect, and treatment, run, and incubation[run] specified as fixed effects (i.e. Incubation nested in run). If a "treatment x run" interaction effect was detected the term was used in the model. When significant effects were detected for variables with multiple effect levels, differences between levels were determined using Tukey HSD.

Table 4.1. Schedule of sampling, planting, and processing for a resource-addition field experiment conducted over the thaw period in wet sedge meadows near Churchill, Manitoba (Experiment 3). Five runs were completed during the thaw of 2008.

Sampled	**Planted**	**4-day incubation**	**8-day incubation**	**12-day incubation**
17 Apr	25 Apr	29 Apr	03 May	07 May
25 Apr	03 May	07 May	11 May	15 May
03 May	11 May	15 May	19 May	23 May
11 May	19 May	23 May	27 May	31 May
19 May	27 May	31 May	04 Jun	08 Jun

Results

Experiment 1: Field nutrient additions

In the dry site, MBC values did not change during May, 2006 but increased by 18 July, 2006, while microbial biomass N (MBN) did not change significantly over time (Fig. 4.1a,b, Table 4.2). Microbial biomass P (MBP) was significantly different between dates, but a Tukey HSD test failed to identify pairwise differences. Across dates, microbial C, N, and P pools were larger in CNP–fertilized plots. The treatment effect appeared to increase over time for MBN and MBC, whereas it decreased for MBP, however, no significant "date x interaction" effects were detected. K_2SO_4-extractable C was significantly different between dates, but a Tukey HSD test did not detect pairwise differences. K_2SO_4-N nearly doubled between 12 and 21 May (Fig. 4.2b). Inorganic N and K_2SO_4-P decreased over time, but these appear to be driven by decreases in treated plots (Fig. 4.2c, d). Recovery of added N and P was evident in NP-amended soils, but extractable C was not more abundant in soils that received C additions.

In the wet site, MBC and MBP decreased by about 50% between 15 May and 23 May, but MBN showed no change over time. There were no significant effects of treatment on any of these

microbial pools (Fig. 4.3, Table 4.2). K_2SO_4-extractable C did not change over time, and although a treatment effect was detected, a Tukey HSD test did not identify specific treatment differences (Fig. 4.4, Table 4.2)). In a similar pattern to that seen in the dry site, K_2SO_4-N increased slightly between 15 May and 23 May, while N_i decreased during the same period (Fig. 4). An NP treatment effect is apparent on 15 May for N_i, but this was not significant based on the three-way ANOVA. However, CNP additions did increase K_2SO_4-P above control and C treatments, particularly during the first two sampling dates (Fig. 4.4, Table 4.2).

Table 4.2. Significant results from mixed model three-way ANOVAs evaluating results of a field nutrient addition experiment (Experiment 1) in dry (Figs. 4.1,4.2) and wet (Figs. 4.3,4.4) sedge meadows. Plot was specified as a random effect and date and treatment as fixed effects. No "date x treatment" interactions were detected, so these were not included in the analyses.

Site	**Variable**	**Effect**	**F ratio (d.f. = 3, 54)**	**P-value**
Dry	MBC	date	8.90	< 0.001
		treatment	5.94	0.001
	MBN	treatment	6.78	0.001
	MBP	date	3.22	0.030
		treatment	15.64	< 0.001
	K2SO4 - C	date	2.83	0.047
	K2SO4 - N	date	22.76	< 0.001
		treatment	14.40	< 0.001
	inorganic N	date	3.54	0.021
		treatment	21.00	< 0.001
	K2SO4 - P	date	3.55	0.020
		treatment	51.38	< 0.001
Wet	MBC	date	7.75	< 0.001
	MBP	date	7.46	< 0.001
	K2SO4 - C	treatment	3.44	0.023
	K2SO4 - N	date	5.48	0.002
	inorganic N	date	10.16	< 0.001
	K2SO4 - P	date	6.22	0.001
		treatment	4.93	0.004

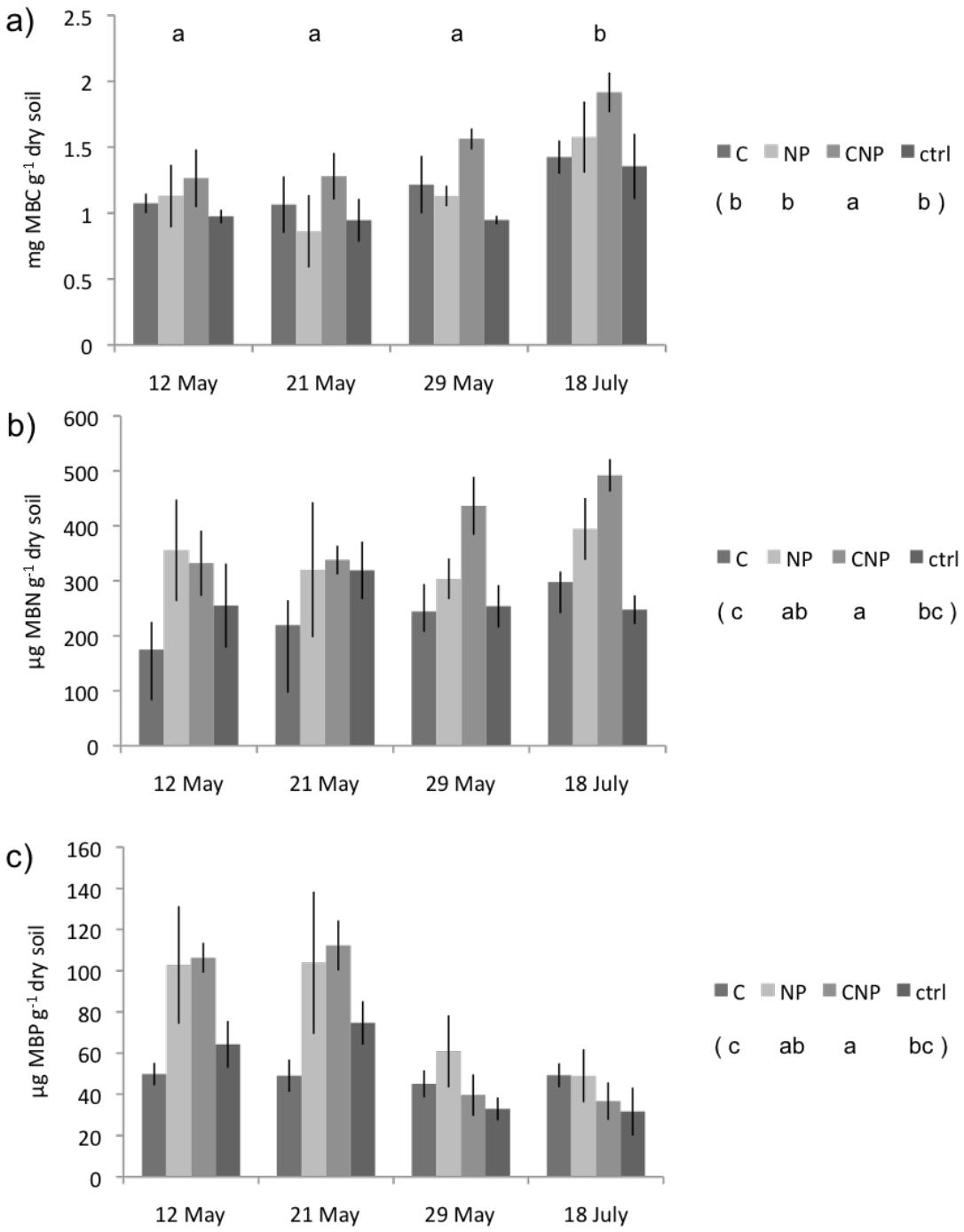

Fig. 4.1. Microbial biomass a) carbon (C), b) nitrogen (N), and c) phosphorus (P) of soils sampled from nutrient addition plots in dry sedge meadows on four dates in 2006 (Experiment 1). Plots received C, NP, CNP or no treatment (ctrl). Means and standard errors are shown (n = 4). Letters above bars and below legend identify statistically different groups (Tukey HSD; $p < 0.05$).

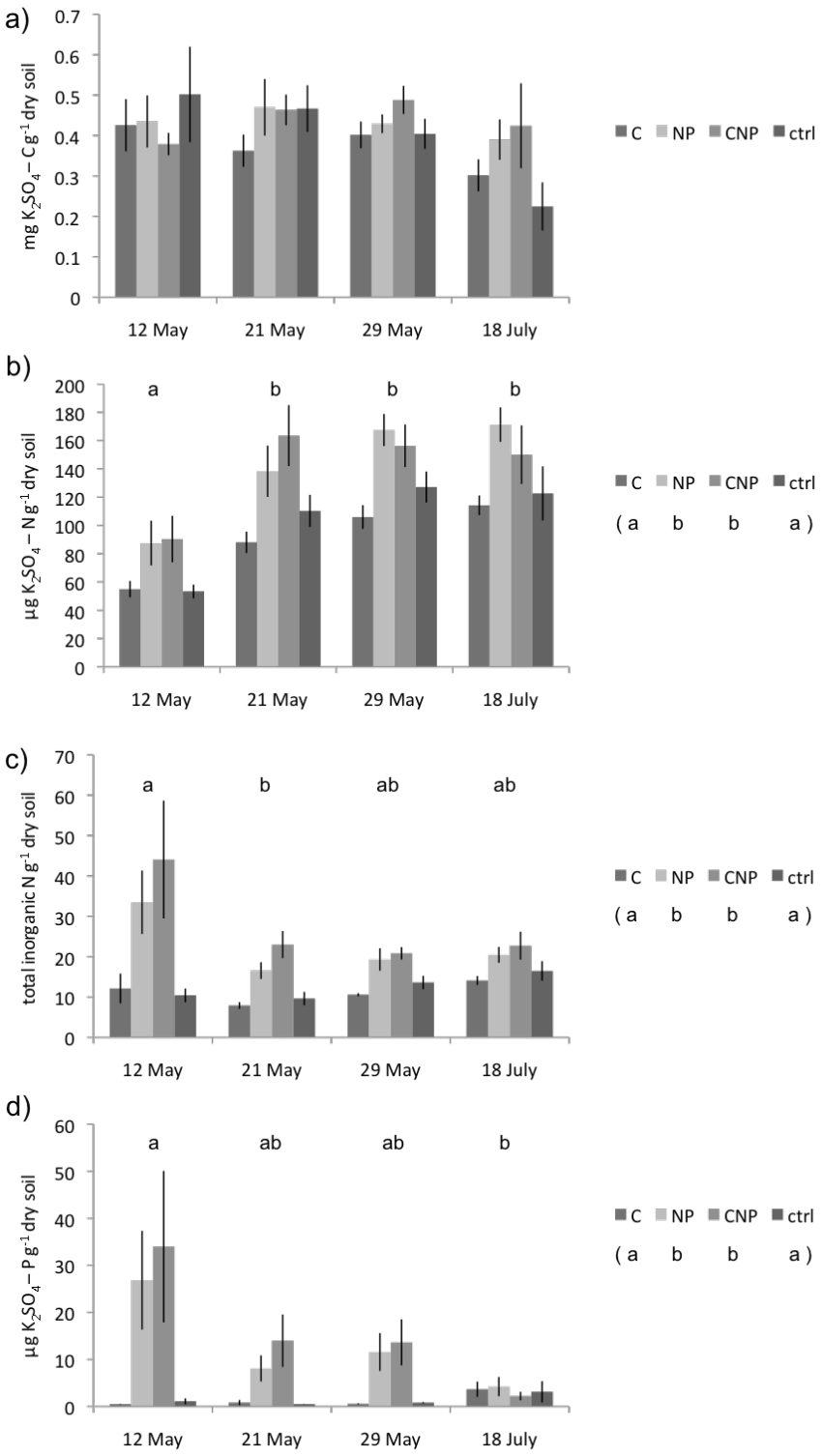

Fig. 4.2. K_2SO_4-extractable a) carbon (C), and b) nitrogen (N), c) inorganic N, and d) K_2SO_4-extractable phosphate (P) of soils sampled from nutrient addition plots in dry sedge meadows on four dates in 2006 (Experiment 1). Plots received C, NP, CNP or no treatment (ctrl). Means and standard errors are shown (n = 4). Letters above bars and below legend identify statistically different groups ($p < 0.05$).

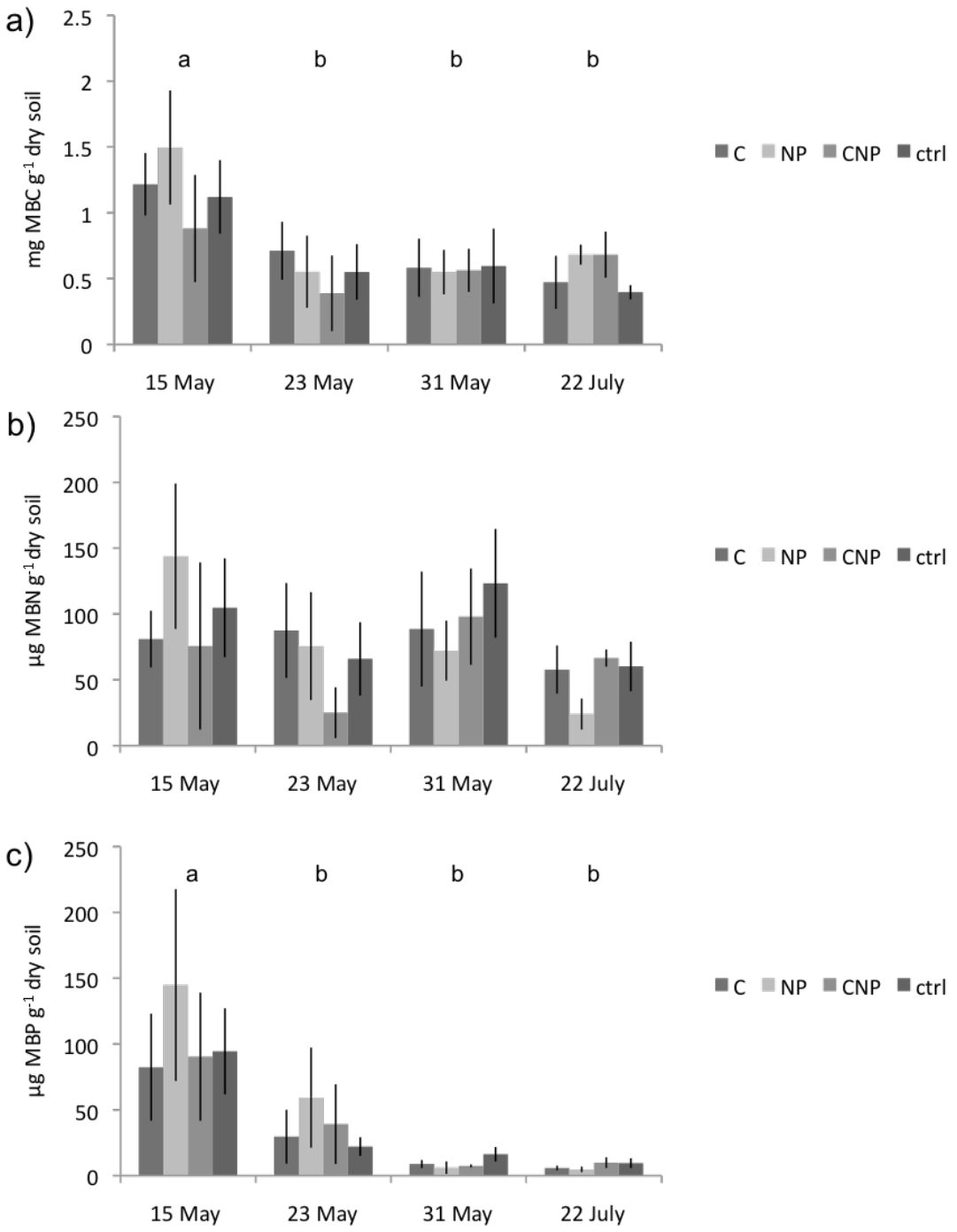

Fig. 4.3. Microbial biomass a) carbon (C), b) nitrogen (N), and c) phosphorus (P) of soils sampled from nutrient addition plots in wet sedge meadows on four dates in 2006 (Experiment 1). Plots received either C, NP, CNP or no treatment (ctrl). Means and standard errors are shown (n = 4). Letters above bars and below legend identify statistically different groups ($p < 0.05$).

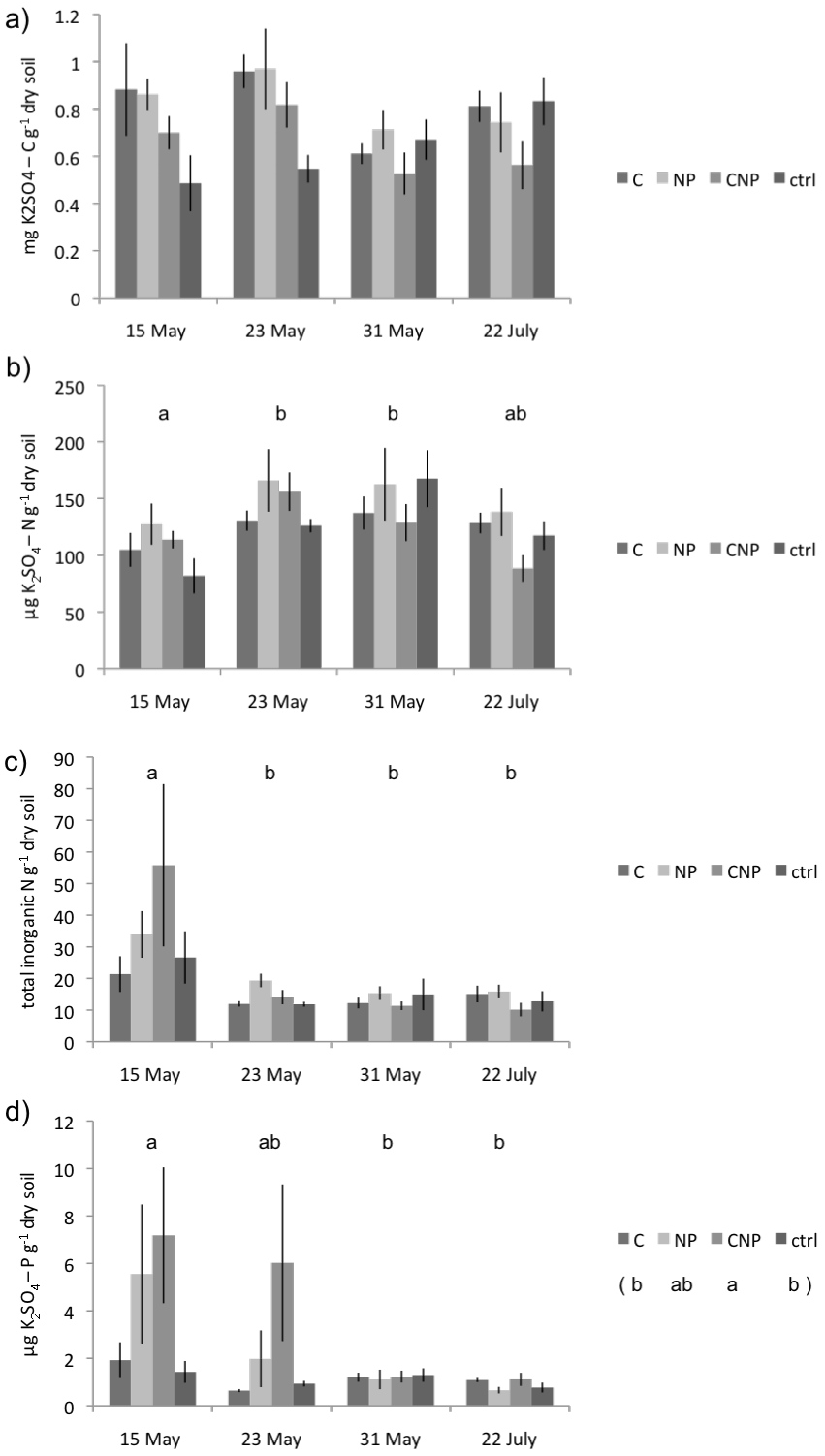

Fig. 4.4. K_2SO_4-extractable a) carbon (C), and b) nitrogen (N), c) inorganic N, and d) K_2SO_4-extractable phosphate (P) of soils sampled from nutrient addition plots in wet sedge meadows on four dates in 2006 (Experiment 1). Plots received C, NP, CNP or no treatment (ctrl). Means and standard errors are shown (n = 4). Letters above bars and below legend identify statistically different groups ($p < 0.05$).

Experiment 2: Laboratory incubations

Carbon added with water

Soils were completely thawed after 2 days of incubation, with many samples thawing after only 1 day. Microbial biomass C decreased by almost 50% between the 12 hr and the 5 d sampling (Fig. 4.5a; 3-way ANOVA, $F_{5,71} = 3.97$, $p = 0.003$), while K_2SO_4-C increased slightly by day 2 of sampling (Fig. 4.5b; 3-way ANOVA, $F_{5,71} = 3.94$, $p = 0.003$). Neither of these variables responded to C treatments across the 7 days of incubation.

Net CO_2 production increased from zero to over 100 ug g^{-1} dry soil hr^{-1} (Fig. 4.5c; 3-way ANOVA, $F_{5,66} = 25.70$, $P < 0.001$) and although there was no treatment effect over this time, there was a "treatment x date" interaction, identifying differences between some treatment-date combinations (Fig. 4.5c; 3-way ANOVA, $F_{5,66} = 2.94$, $p = 0.019$). Net CH_4 flux was approximately -3 ug g^{-1} dry soil hr^{-1} at the beginning of the experiment, rising to near zero by day 7 (Fig. 4.5d; 3-way ANOVA, $F_{5, 71} = 8.89$, $P < 0.001$). Again, no effect of C-addition was detected.

Carbon added with and without water

Microbial biomass C was greater in the ambient water treatment soils than water-added soils on day 3 of the experiment but did not respond to the C treatment (Fig. 4.6a, Table 4.3). K_2SO_4-C was no greater in C-amended soils, but was slightly higher in ambient water conditions than in water-added treatments on days 1 and 3 of the experiment (Fig. 4.6b). CO_2 efflux increased substantially in the absence of added water on days 1 and 3, but water-added samples increased to similar flux levels as their ambient-water counterparts by day 7 (Fig. 4.6c, Table 4.3). Net CH_4 flux was consistently positive in jars without water additions, with replicate means ranging between 13 and 23 ug g^{-1} dry soil hr^{-1}, in contrast to samples with added water, which consumed CH_4, most noticeably at the beginning of the experiment (Fig. 4.6d, Table 4.3).

Table 4.3. Results of mixed-model 3-way ANOVAs evaluating differences between carbon and water additions on soil variables in a laboratory incubation experiment (Experiment 2). Sample was specified as a random effect while carbon and water were designated fixed effects. No carbon or carbon x water interaction effects were significant. Separate analyses were done for each incubation time (1d, 3d, 7d) and only significant results are shown. See Fig. 4.6 for summarized data and post-hoc results.

Variable	**Incubation T**	**Effect**	**d.f.**	**F ratio**	**P-value**
MBC	3 d	water	1, 7	14.40	0.007
K_2SO_4 - C	1 d	water	1, 7	8.57	0.022
	3 d	water	1, 7	25.74	0.001
CO_2	1 d	water	1, 7	332.69	<0.001
	3 d	water	1, 7	14.41	0.006
	7 d	water	1, 5.68	6.29	0.048
CH_4	1 d	water	1, 7	225.32	<0.001
	3 d	water	1, 7	66.97	<0.001
	7 d	water	1, 7	252.34	<0.001

Table 4.4. Significant results of mixed-model ANOVAs evaluating effects of carbon, nitrogen, and phosphorus additions for various soil variables over thaw in a field incubation experiment (Experiment 3). Site was specified as a random effect while treatment, run, and incubation time (nested in run) were fixed effects. Insignificant interaction effects were not included in analyses. See Figs. 4.7, 4.8 for summarized data and results of post-hoc tests.

Variable	**Effect**	**d.f.**	**F ratio**	**P-value**
MBC	incubation (run)	10, 128.1	3.15	0.001
	run	4, 128.1	13.05	< 0.001
MBP	incubation (run)	10, 129	1.92	0.048
K_2SO_4 - C	run	4, 128	9.04	< 0.001
K_2SO_4 - N	treatment	1, 129	12.62	< 0.001
	run	4, 129	6.80	< 0.001
inorganic N	run	4, 125.1	4.22	0.003
	treatment x run	4, 125	3,04	0.020
K_2SO_4 - P	treatment	1, 129	24.75	< 0.001
	incubation (run)	10, 129	4.58	< 0.001
	run	4, 129	9.34	< 0.001

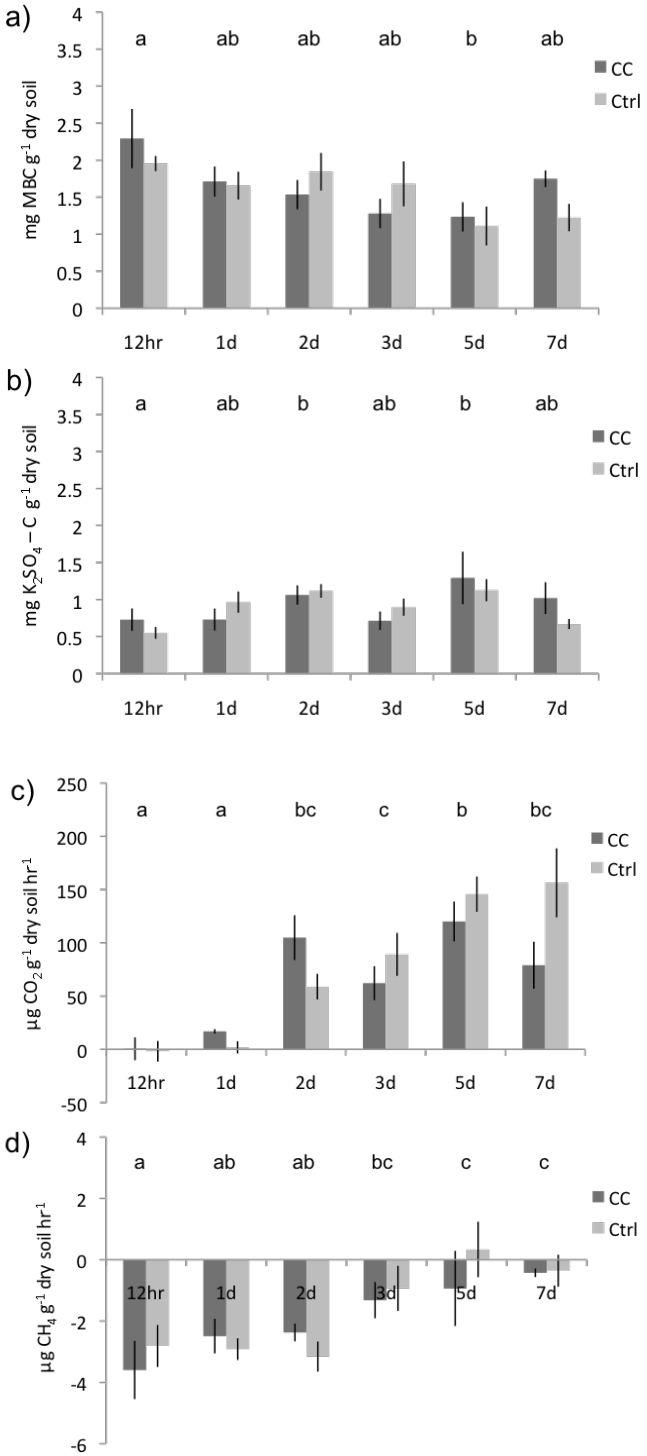

Fig. 4.5. Soil a) microbial biomass carbon, b) K_2SO_4-extractable carbon, c) net CO_2 flux and d) net CH_4 flux from a laboratory incubation experiment (Experiment 2). Soils were collected from a wet sedge meadow, were initially frozen and submerged in water, and were processed after 12 hrs and 1, 2, 3, 5, and 7 days of incubation at ~ 4 °C. Soils received multiple carbon sources (CC) or no carbon (Ctrl). Means and standard errors are shown (n=7). Letters above bars denote significant differences between groups based.

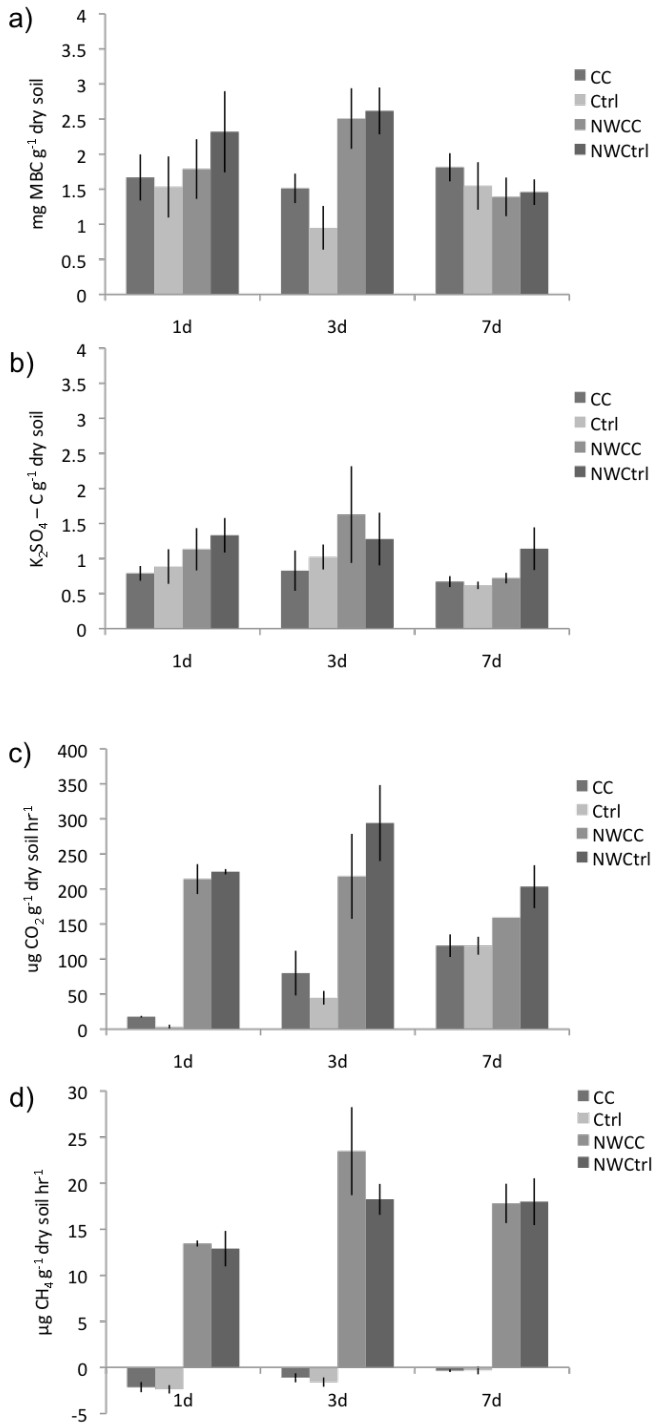

Fig. 4.6. Soil a) microbial biomass carbon, b) K_2SO_4-extractable carbon, c) net CO_2 flux and d) net CH_4 flux from a laboratory incubation experiment (Experiment 2). Soils were sampled from a wet sedge meadow, were initially frozen, and were processed after 12 hrs and 1, 2, 3, 5, and 7 days of incubation at ~ 4 °C. Soils were submerged in water and received multiple carbon sources (CC), in water without carbon addition (Ctrl), not in water with added carbon (NWCC), or not in water without carbon (NWCtrl). Means and standard errors are shown (n=3, see text for one exception).

Experiment 3: Field-incubated cores

Microbial biomass C decreased over time from approximately 4 mg C g^{-1} dry soil to ~ 1 mg C g^{-1} dry soil, and the decrease was similar between treated and untreated cores (Fig. 4.7a, Table 4.4). Differences also occurred within each of the runs, with decreases evident between 4 and 12 days of incubation during the first 3 runs (Fig. 4.7a). MBN and MBP pools did not decrease across the 5 dates sampled, but MBP decreased within runs (Fig. 4.7b, c, Table 4.4). Microbial biomass N was highly variable and although within-run decreases are evident (Fig. 4.7b), these were not statistically verified by the 3-way ANOVA. As with MBC, neither MBN nor MBP pools responded to the CNP addition treatment.

Soil extractable C, N, Ni, and P pools decreased over the course of the experiment (Fig. 4.8, Table 4.4). Some differences were also seen within runs for K_2SO_4–C and K_2SO_4–P (Fig. 4.8a, d) but not for the two measured soil N pools (Fig. 4.8b, c). During the first two sampling dates K_2SO_4–C appeared to be approximately twice as abundant in treated soils, however across the experiment this is not significant (Fig. 4.8; 3-way ANOVA, $F_{1, 128} = 3.37$, $p = 0.069$). Differences between within-run samples (incubation times) were also insignificant across the experiment, but only by a small margin (Fig. 4.8; 3-way ANOVA, $F_{10, 128} = 1.89$, $p = 0.052$). Treated samples contained more K_2SO_4–N and K_2SO_4–P than untreated samples, while the treatment effect as measured by N_i was marginally insignificant (Fig. 4.8; 3-way ANOVA, $F_{1, 125.1} = 3.89$, $p = 0.05$). A "treatment x run" interaction was significant, however, with treated soils being higher in N_i than untreated soils during the second trial (Fig. 4.8c). In all nutrient measures, any detectable treatment effect is clearly weighted to the beginning of the experiment, however this is also when the variation between replicates was typically the highest.

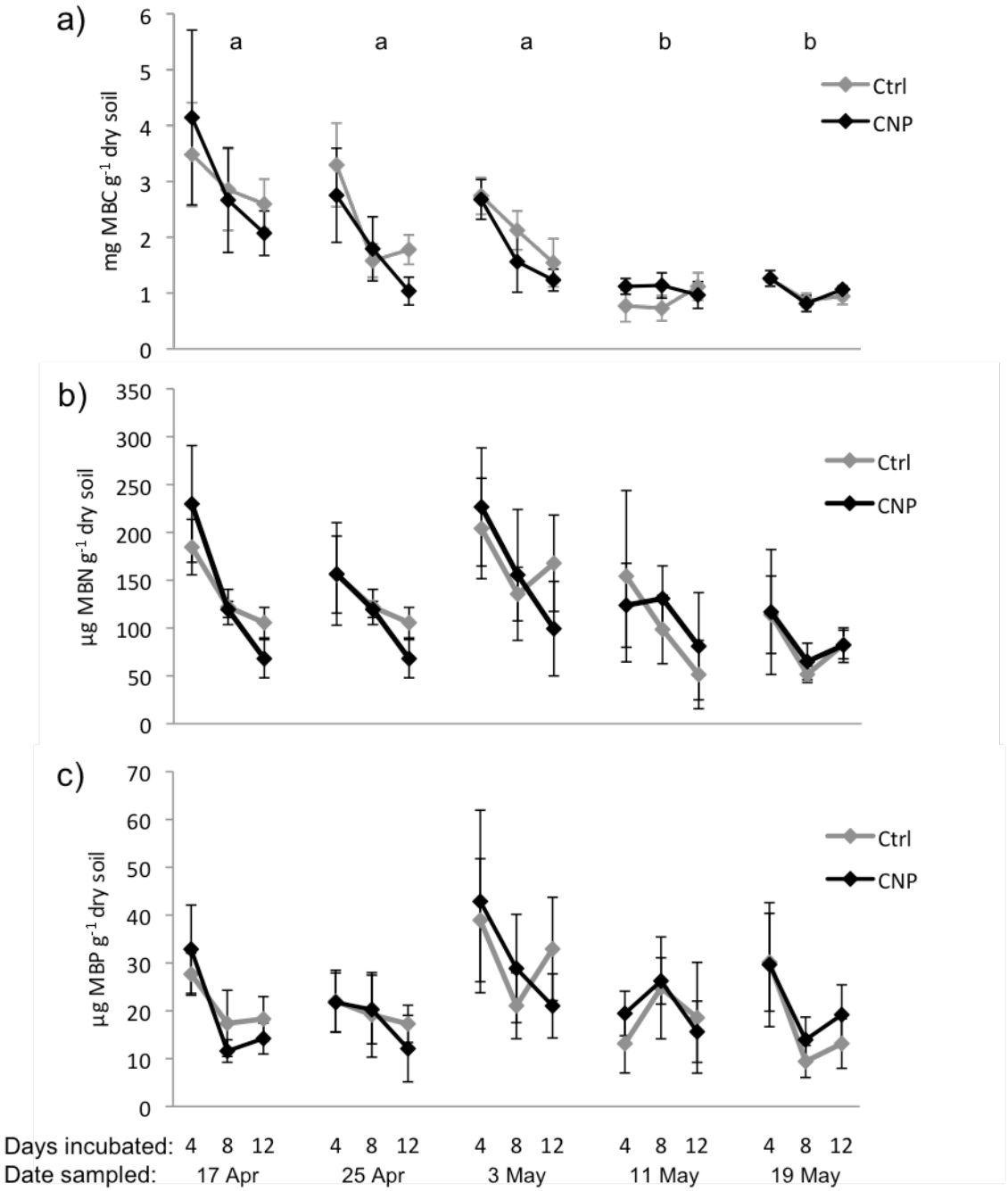

Fig. 4.7. Microbial biomass a) carbon, b) nitrogen and c) phosphorus of soils from an experiment in which soil cores were sampled from a wet sedge meadow, and injected with a solution of carbon, nitrogen, and phosphorus (CNP) or with water (Ctrl) (Experiment 3). Cores were sampled on 5 occasions (runs), replanted after treatment, and recovered for processing after 4, 8, and 12 days (Table 4.1). Means and standard errors are shown (n = 5). Letters indicate significant differences between runs.

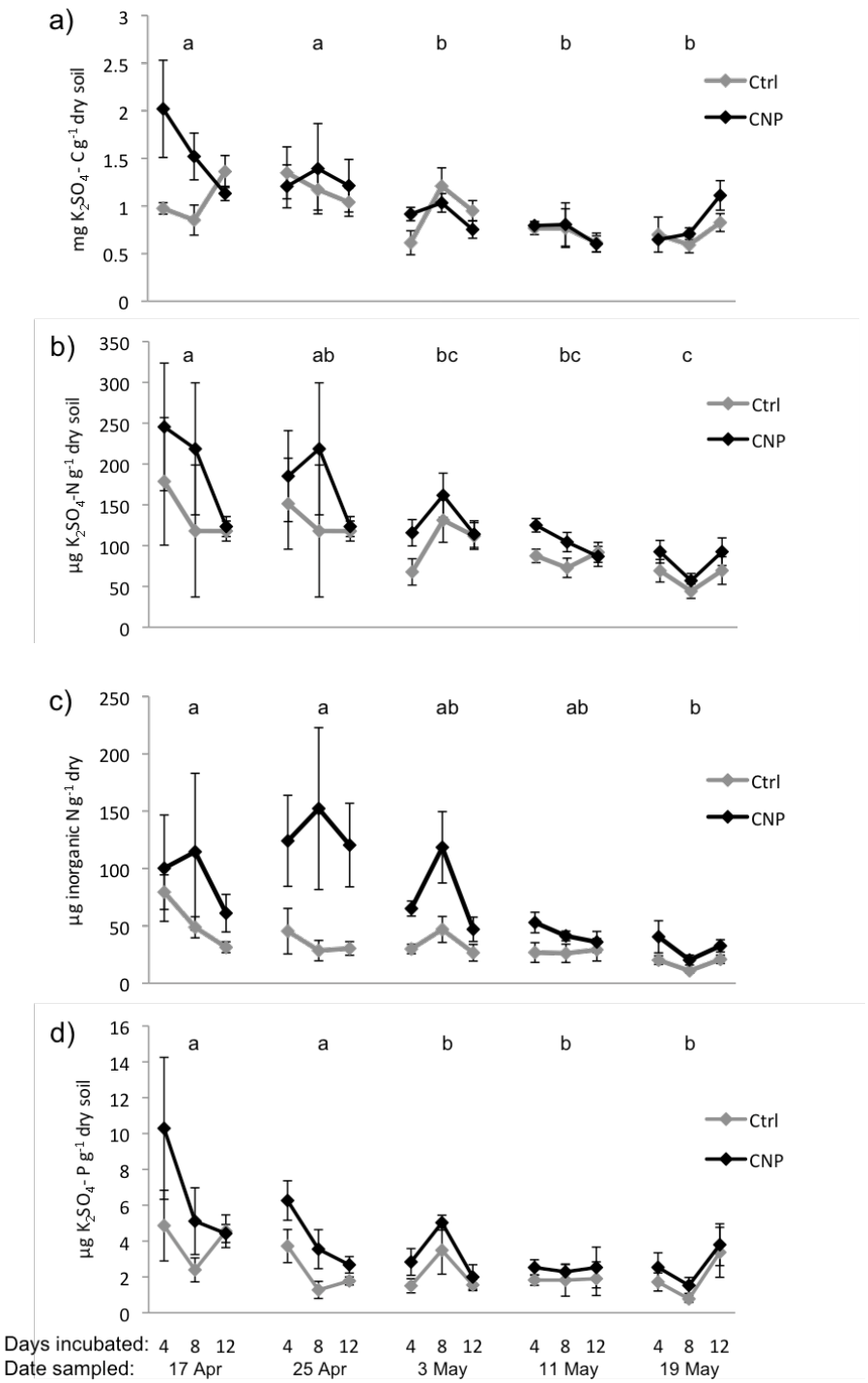

Fig. 4.8. Soil K_2SO_4-extractable a) carbon and b) nitrogen, c) inorganic nitrogen, and d) K_2SO_4-extractable phosphorus of soils from an experiment in which soil cores were collected from a wet sedge meadow and injected with a solution of carbon, nitrogen, and phosphorus (CNP), or with water (Ctrl) (Experiment 3). Cores were sampled on 5 occasions (runs), replanted after treatment, and recovered for processing after 4, 8, and 12 days (Table 4.1). Means and standard errors are shown (n = 5). Letters indicate significant differences between runs.

Discussion

Experiment 1: Field nutrient additions

The field experiment conducted in 2005 and 2006 involved NPC additions over a period of about a year, but soil sampling occurred approximately three weeks after the final fertilization to also capture possible short-term effects. The timing of sampling was intended to include the end of the microbial biomass decline period, and this was achieved in the wet site, with MBC decreasing between the first and second sampling dates. In the dry site, however, a decrease was not observed during the spring sampling period, and long-term records indicate that MBC at the site decreased prior to sampling, and much of this reduction occurred prior to the April 2006 nutrient treatment (see Chapter 6); however, I do not have plot-specific measurements to verify this. The results from the dry site therefore do not address the possibility of late winter resource limitations on microbial growth as intended but are still informative for their examination of spring and summer processes.

Soil extractable N and P levels in the dry site were clearly elevated by the fertilization, but increased extractable C was not recovered in soils that received glucose amendments. Despite this, MB responded positively to the addition of C, N, and P together, revealing co-limitation by both labile C and at least one of N and P. Positive responses of MBN and MBP to CNP additions reveal that all three resources were incorporated into biomass in these treated soils. This result is supported by similar results from nutrient amendments conducted at the same site in summer (E. Horrigan, *pers. comm.*). In this site, rather than being limited either by C (Buckeridge and Grogan 2008) or by N and/or P (Schimel and Weintraub 2003) MB responded only to the combined treatment, revealing multiple resource limitation, at least during the growing season.

In the same site, above-ground biomass of the dominant graminoid in this system, *Carex aquatilis*, is co-limited by both N and P (data not shown). This plant takes up N_i very early in the spring (Chapter 3), so that microbes must compete with plants for N and P immediately after thaw, and possibly earlier. Dissolved nutrients may also be susceptible to leaching when melt-water floods much of the area in spring. Carbon limitation in spring and in summer may be partially explained by the relatively young age of this site, formerly a coastal sand beach. The organic soil is relatively shallow (typically 15 cm deep) and plant roots forage deep into the

mineral layer, presumably limited by water in summer, as well as by mineral nutrients. Carbon input to the soil from excess plant photosynthate and from fine root turnover is probably low compared with more developed and densely vegetated hummock tundra or wet sedge sites.

In the wet site, MBC and MBP decreased between 15 May and 23 May 2006, but MBN did not significantly decrease as expected (Chapter 2, Chapter 6). This may be because of high error associated with MBN measurements (ie. low power of detection) and not due to a different timing of MBN decrease relative to the other variables. In any case, the timing of the experiment was appropriate to addressing the cause of the late-winter microbial biomass decrease, with fertilizer application probably occurring during the early part of the MBC decrease, and the first sampling occurring before the completion of this period. However, nutrient amendments did not contribute to the maintenance of high MBC pools, which decreased similarly in treated and untreated plots.

While CNP fertilization was effective in increasing levels of P in the soil, three weeks after application the amount of N was not clearly greater in treated samples (although a positive trend in N_i is noteworthy), nor was additional C measured in treated soil extracts. In the case of C, the amount added to the ground surface for each of the 3 treatment applications (100 g glucose m^{-2}) amounts to approximately 4 mg C cm^{-2}. Ambient levels of dissolved organic C (DOC) in these soils are on the order of 0.1 mg C cm^{-3} in late winter (see Chapter 6), so the amount that I added should not have been swamped by background levels of soil C. Carbon may have been metabolized quickly by microbes, particularly near the ground surface, and respired as CO_2 or CH_4. Added N was probably taken up, at least in part, by plant roots, which can utilize N early in the spring in this system (Chapter 3). Nitrogen may also have been utilized by mosses, algae, and cyanobacteria, which inhabit the ground surface at this site.

It is likely that some of the added nutrients were leached out of the plots, which were water saturated, and often covered with 10 cm or more of standing water. Given the effectiveness of the phosphate-P additions, at least initially, it would appear that leaching did not remove significant proportions of added nutrients from the system. It is possible, however, that P was quickly incorporated into soil water while N and C remained on the soil surface and were consequently more susceptible to loss by surface runoff at a later date.

Overall, this experiment demonstrated CNP-limited MB in a dry tundra meadow during the growing season, but failed to support the hypothesis that MB decreases during thaw in wet meadows are the result of resource exhaustion. However, because I did not confirm elevated levels of C in the soil after nutrient treatments, our conservative conclusion from this experiment is that N and P amendments do not prevent the MB decline in wet sedge meadow soils during thaw.

Experiment 2: Laboratory incubations

The laboratory experiment conducted in 2007 allowed controlled thawing and measurement of CO_2 and CH_4 fluxes, and utilized complex C sources instead of glucose, which are more realistic substrates than simple sugars at this time of year (Lipson et al. 2002, Schmidt and Lipson 2004, Schimel and Mikan 2005). Approximately 3.2 mg C was added to 100 g of soil having a background extractable C typically between 6 and 12 mg per 100 g FW. This modest addition of C may not have been enough to significantly reduce C-limitation, as the added C was not detected in K_2SO_4-extractable C or in MBC pools, nor was CH_4 production elevated by C-addition. However, CO_2 production in C-amended jars was almost nine times higher after 1 day of incubation and almost doubled after 2 days of incubation as compared with untreated soils, although statistical differences due to treatment were not confirmed. If accurate, however, this difference in CO_2 production early in the experiment easily accounts for the 3.2 mg of added C.

Extractable C was not more abundant in treated soils, even after just 12 hrs of incubation. The amount added may have been too small to detect, however it is also possible that the residence time of soluble-C in soils was very short. Initially much C may have stayed in solution external to the soil, but as soil thawed and C penetrated the soil matrix, it was likely metabolized very quickly, for example through overflow metabolism (Tempest and Neijssel 1992). The results therefore fail to support the theory that microbial biomass is C-limited at the end of winter.

Thawing brought about increases in net CO_2 production and a shift from net CH_4 consumption to near-zero CH_4 fluxes in submerged soils (Fig. 4.5). The modest amount of CH_4 consumption is attributable to methanotrophic microbes and it is inferable from this that methane, and methanogenic microorganisms, are present in the soil. Methanogens are probably active throughout the winter under anaerobic conditions, and once the soil is exposed to oxygen, methanotrophs are able to utilize this C source, particularly near the soil surface (Moore et al.

1994, Le Mer and Roger 2001). Methane fluxes near zero at the end of the experiment suggest either an increase in methanogenesis, a decrease in consumption by methanotrophs, or both. This change could be driven by an increase in anoxic microsites, and/or by the exhaustion of methane supplies by methanotrophs. At the same time, an increase in CO_2 production indicates increasing microbial activity as soils thawed, presumably driven by higher temperatures (Mikan et al. 2002) and establishment on substrate-rich microsites.

Soils that did not receive water at the onset of the experiment had larger MBC by day 3 than submerged soils, appearing not to decline over the period of thaw. As well, K_2SO_4-C was more abundant in ambient-water samples than water-added samples on days 1 and 3, and CH_4 and CO_2 production was relatively high in ambient-water jars when compared with water-added jars (Fig. 4.6). Without added water, CH_4 and CO_2 production were also higher, indicating increased microbial activity relative to water-added samples, including methanogenic activity. Without the addition of water the microbial community as a whole was not disturbed in the same way as the soil that had to contend with a large input of water.

In soils that were submerged in water, MBC decreased over the first 5 days of warming, however similar declines were not observed in K_2SO_4-C, which increased over the same period, probably due to the release of lysed microbial cell contents. This is a different pattern than I have observed from monitoring field conditions, where extractable C tended to decline concurrently with MBC at thaw (Chapter 2, Chapter 6). The difference between these results may be due to experimental conditions, for example soil changes during storage (which occurred for several weeks under sealed conditions and at very cold temperatures), the relatively aerobic conditions maintained throughout the incubation, and/or the prevention of water flow inside jars.

Differences in water and no-water treatments could be exacerbated by different integration of the C treatment. Without water there would be less water present to aid in distribution but also less treatment dilution. Gas diffusion into water should not have had a large impact on our measurements, but could have led to slight underestimates of gas production in jars containing added water. More relevant differences are in the access of oxygen to microbes, and perhaps in the temperature buffering capacity of water, which may have changed the rate of thaw in submerged samples.

Microbial activity in these soils undergoing thaw, including methanogenic activity, appears to be highly susceptible to the addition of liquid water. The presence of water could be stressful for a number of reasons. These include buffering the soil from above 0 °C air temperatures (ie. keeping soils at or near 0 °C for a longer period of time), diluting soluble resources, causing a redistribution of appropriate substrates away from established microbial micro-habitats, and changing osmotic and redox conditions that are presumably quite stable over the winter months. The conditions of water submergence created in this experiment are probably more similar to field conditions at thaw than soil that is free of surrounding water. Indeed, the microbial decline observed in the water-added samples in this experiment was similar to the pattern measured in the field. Thus, while this experiment failed to show maintenance of MB in the presence of added C substrates, it did provide evidence that abiotic changes as a result of the influx of liquid water at thaw can have negative consequences for the microbial community (Chapters 5, 6).

Experiment 3: Field-incubated cores

This experiment combined the realism of *in situ* thawing conditions in the field, with the control of laboratory treatment applications. Additions of C, N, and P appeared to be effective in elevating K_2SO_4-extractable C, N and P, and N_i during the initial part of the experiment, when soils were still cold and soil water was largely frozen. The C treatment was only evident during the first experimental run. Much of the added C that was not recovered from soil cores may have been metabolized during the four days of the first incubation period, especially once soils began to warm and microbial metabolic rates increased. The increase in liquid water over the thaw period may also have contributed to some leaching of added C, as some samples were submerged in liquid water during incubation. The recovery of added nutrients in K_2SO_4-N and N_i, and K_2SO_4-P also diminished over the period of the experiment, and leaching may have played a large role in reducing the retention time of these treatments in the soil. Denitrification by microbes may have also contributed to the loss of N from experimental cores.

Despite elevated levels of extractable C, N, and P during the first experimental run, and elevated N and P for subsequent runs, MBC, MBN and MBP levels were not different between treated and untreated soil cores. Thus the seasonal reduction of MBC at this time of year was not prevented by resource additions, supporting the conclusion that factors other than resource availability ultimately cause the annual microbial decline during thaw.

Microbial biomass C, MBN, and MBP tended to decrease within runs, in some cases to a greater extent than between-run decreases. This can be partially explained by the time-line of the experiment, where samples were frozen for 8 days, then planted and incubated *in situ* for 4, 8, and 12 days (Table 6.1). Therefore 12 - 20 days passed between sampling and soil processing, composed of 8 days of extreme cold (-20°C freezer) and 4 - 12 days of field temperatures, resulting in each run experiencing different overall temperature regimes. Decreases within the 12 days of field incubation are probably the result of increased soil warming experienced on each occasion. Cores are believed to have warmed in the field more quickly than the surrounding intact soil (including in as-yet unsampled locations), due to the disturbance created in the sampling sites. Disturbance of the snow pack in particular could not be avoided and in some cases resulted in pooling of water and a more developed active layer at the previously sampled sites, which were subsequently used for incubation. Within-trial declines may also have been contributed to by the initial stress of storage at a cold temperature followed by warming necessary to apply the treatment, followed again by refreezing before re-planting in the field. The resulting temperature regime experienced by the cores could have been stressful for some portion of the microbial community that failed to be sustained over the 12 days of field incubation. However, this within-run pattern does not diminish the overall lack of response to resource additions. Rather, it supports the alternative hypotheses that abiotic factors brought about by warming, particularly the phase change of ice to liquid water, are the ultimate causes of decreasing microbial biomass.

Comparisons and Conclusions

The three experiments reported here differ in many ways, including the types of resources used for treatment and the thaw regime employed (field or laboratory), but all supported the same conclusion with respect to the wet sedge meadow sites, that C inputs during thaw do not help to maintain high levels of MBC through the seasonal transition from winter to spring. Rather, it appears that when these soils thaw, MB decreases regardless of resource amendments, and from this I conclude that abiotic forces are responsible for this pattern.

I found it difficult in all three experiments to successfully elevate soil resource levels, especially with respect to C. Results from Experiment 2 reveal that added C may be quickly respired by microbes, however the addition of C was modest and the result was not statistically verified.

Increases in respiration as a result of C-amendments have been reported several times for Arctic soils (Grogan and Jonasson 2003, Brooks et al. 2004, Buckeridge and Grogan 2008), so this seems a reasonable explanation for the apparent loss of added-C, if weakly supported by the results presented here. Leaching of nutrients, especially N and P, is also likely to lead to significant dilution of treatment effects, and is an expected difficulty of working in water-logged soils. In the future it would be worthwhile to make a greater effort to capture losses through leaching, and to invest in initial measurements of soil nutrient pools immediately after treatment application, so as to verify the incorporation of added nutrients into soils before substantial leaching or microbial processing occurs.

Other factors that may have affected our measurements of soil resource pools are related to the complex nature of the soil chemistry in these wet, highly organic soils. Several authors have shown that N-fertilization decreases MBC in some soils, and this is attributed to increased C-complexing in the presence of N, reducing the availability of the DOC pool to microbes (Wallenstein et al 2006, Buckeridge and Grogan 2008, Treseder 2008). The soils studied here are rich in Ca, and as a result may be susceptible to DOC complexing (Romkens et al. 1996, Romkens and Dolfing 1998), particularly in the presence of added nutrients or other mineral species liberated during spring thaw. For the most part these should still be extracted by K_2SO_4, but unknown chemical interactions may occur before extraction. Further, flocculation within K_2SO_4 extracts could lead to inaccurate measurement of C, N, and P pools, particularly when automated methods that do not shake samples directly prior to analysis are used.

It is realistic to believe that different sites, with different soil types, nutrient regimes, hydrology, and microbial and vegetation communities, are affected to different degrees by the various processes put forth to explain microbial biomass declines. It is also realistic to assume that inter-annual variation will occur within sites in which different causes of the decline play larger or smaller roles in different years. For example, predation by soil fauna may be the main cause of declines in some years, while specific temperature or precipitation regimes in other years may mean the environmental contributors to the microbial decline are more important. Consistent between years however, is the observation that MB declines between winter and spring, and the timing coincides with warming air and soil temperatures (Chapter 6). Based on the experimental work reported here, I believe that this decline is primarily driven by the increase in liquid water that comes with snowmelt and soil thaw (Chapter 5).

Soil microbes are critical players in the regulation of nutrient cycling, ecosystem productivity, and greenhouse gas fluxes in northern tundra systems. Given that many of the controls on microbial processes are linked with seasonal changes, and that seasonal patterns are being altered by climate change, efforts to document and understand the larger contributions of these cold environments to the earth system require that we uncover the processes by which microbial communities function. Under warming conditions, mid-winter thaws that are more typical of regions to the south are likely to become more frequent in many areas of the Arctic (ACIA 2004). Based on the work presented here, these thaws may have large impacts on winter microbial communities that are in turn likely to alter decomposition rates, C emissions, and nutrient availability to plants.

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Chapter 5
Is the decline of soil microbial biomass in late winter coupled to changes in the physical state of cold soils?

Preface

The previous chapter tested the hypothesis that the observed decrease in soil microbial biomass is caused by the exhaustion of carbon substrates and other nutrients at the end of winter. I did not find support for this hypothesis, but the experiments pointed to the possibility that abiotic drivers and particularly liquid water could be causing the observed microbial decline. This idea is explored in this chapter by providing a theoretical description of how soil microbial cells could be lysed as a result of the influx of liquid water during soil thaw.

Abstract

During winter when the active layer of Arctic and alpine soils is below 0 °C, soil microbes are alive but metabolizing slowly, presumably in contact with unfrozen water. This unfrozen water is at the same negative chemical potential as the ice. While both the hydrostatic and the osmotic components of the chemical potential will contribute to this negative value, we argue that the osmotic component (osmotic potential) is the significant contributor. Hence, the soil microorganisms need to be at least halotolerant and psychrotolerant to survive in seasonally frozen soils. The low osmotic potential of unfrozen soil water will lead to the withdrawal of cell water, unless balanced by accumulation of compatible solutes. Many microbes appear to survive this dehydration, since microbial biomass in some situations is high, and rising, in winter. In late winter however, before the soil temperature rises above zero, there can be a considerable decline in soil microbial biomass due to the loss of compatible solutes from viable cells or to cell rupture. This decline may be caused by changes in the physical state of the system, specifically by sudden fluxes of melt water down channels in frozen soil, rapidly raising the chemical potential. The dehydrated cells may be unable to accommodate a rapid rise in osmotic potential so that cell membranes rupture and cells lyse. The exhaustion of soluble substrates released from

senescing plant and microbial tissues in autumn and winter may also limit microbial growth, while in addition the rising temperatures may terminate a winter bloom of psychrophiles.

Climate change is predicted to cause a decline in plant production in these northern soils, due to summer drought and to an increase in freeze-thaw cycles. Both of these may be expected to reduce soil microbial biomass in late winter. After lysis of microbial cells this biomass provides nutrients for plant growth in early spring. These feedbacks, in turn, could affect herbivory and production at higher trophic levels.

Introduction

Studies during the last decade have established that at least at some alpine and Arctic sites, microbial biomass reaches a peak in late winter and then declines during the period when soil temperatures rise to 0 °C (Chapter 2; Brooks, 1998; Lipson et al., 2000; Larsen et al., 2007). In addition, a number of indirect measures of microbial activity at that season, based on CO_2 fluxes and/or microbial nitrogen pools, are broadly consistent with such changes in microbial biomass (Brooks et al., 1998; Fahnestock et al., 1998). These and other authors have attributed the decline in biomass at the winter-spring transition to a variety of causes, including the low level of soluble organic nutrients and the rupture of cell membranes by freeze-thaw cycles.

In this paper, we examine the decline in relation to the physical state of frozen soils, specifically changes in the hydraulic and osmotic environments, and how these physical changes during thaw may reduce the biomass of microbes. In order to discuss this topic fully, it is necessary to give an account of the physical changes that occur in organic cold soils from freeze-up to melt, as some important aspects of these changes have not been widely discussed. Here we discuss largely the situation in wet Arctic soils, drawing from four years of observations in *Carex aquatilis* – dominated wet meadows near Churchill, Manitoba, Canada (Edwards, unpublished observations). However, bearing in mind that the phenomenon of spring microbial decline is also seen in drier soils and in Alpine sites we feel that this discussion is broadly relevant to cold organic soils.

Characteristics of organic wetland soils in high latitudes

The widespread presence of organic soils in Arctic and sub-Arctic wetlands reflects the low rates of decomposition and often the poor drainage associated with perennially frozen soils (permafrost) characteristic of low-lying gently sloping landscapes (Slaughter and Kane, 1979; Quinton and Marsh, 1999; Carey and Woo, 2001). The porosity of these organic soils in the active layer is often large (up to 80% by volume) and individual pores may be larger than 1 mm in diameter (Luxmore, 1981). The hydraulic mean radius of the pores declines rapidly with depth as a result of compression of organic material (Quinton et al., 2000; Carey and Woo, 2001). Only those pores that are interconnected contribute to the flow of water through the peat, so that not all pores conduct water. As discussed later, rapid movement of snow-and-ice melt-water in surface peats (active layer) at the end of winter may lead to abrupt changes in the physical state of these soils that microorganisms must accommodate if they are to survive (Dunn and Phillips, 1991; Carey et al., 2007). Overall, the hydraulic conductivity in saturated organic soils is low (Quinton et al., 2000; Carey and Woo, 2001; Carey and Quinton, 2005) and of course it will be lower still when they are frozen.

Although we discuss wet organic soils in general here, there is of course great variation between different Arctic wetland types, in important variables such as hydrology and snow depth, pH and redox chemistry, slope, and vegetation (Hinzman et al., 1996; Carey and Pomeroy, 2009). Substantial variation also exists at the microsite scale, which is relevant to understanding the environment experienced by microorganisms. During the thaw period the tundra landscape consists of a patchwork of areas that can vary considerably in terms of progression of thaw. Inter-annual variation also adds to the complexity of these systems, with snow accumulation, spatial patterns of water movement, and active layer development varying year to year.

The freezing process

At the onset of the freezing process in saturated peaty soils in autumn, a thin layer of frozen water overlies unfrozen soil, and a temperature gradient is established between the sub-zero air and the unfrozen soil. Latent heat is conducted away from the soil along this gradient, and the surface layer of soil freezes downwards. The water in the soil freezes progressively as its

temperature falls over a range of values below zero (i.e. the soil water does not have a single definite freezing point).

Three factors depress the freezing point of water in soils below 0 °C:

The Gibbs-Thomson effect: A small radius of curvature on a convex ice surface raises its chemical potential, favouring the liquid state over the solid state in small (nm radius) hydrophilic pores. This depresses the freezing point of water by an amount that depends on the pore radius. This is relevant for water in nanometre pores, so not directly relevant to micrometre-sized organisms.

Pre-melting: the presence of unfrozen films of water on ice surfaces, both facing other solids and facing water vapour: Proposed by Faraday, these films are at a low chemical potential (i.e. in equilibrium with the ice) because they are under tension, a negative hydrostatic pressure generated by the repulsion between their surfaces (Dash 1989, Rempel et al., 2004). This negative pressure is just that required to bring the water film into equilibrium with the ice. Its value can be calculated from equilibrium thermodynamics. The thickness of these films, which falls with temperature, is only of the order of a nanometre. However in finely porous media the negative pressure in the film sets up a hydrostatic pressure gradient causing water to flow to the ice surface. Here it freezes, forming an ice lens of macroscopic size and leading to frost heave (Wettlaufer, 1999; for a contrary view, see Torrance and Schelekens, 2006). However little frost heave is seen in the peaty, coarsely porous soils we are dealing with here. We attribute this absence of frost heave to cavitation in the soil water under tension in these peats, a cavitation facilitated by degassing as the water freezes and by plentiful ice nucleation centres in the soil. For this reason, we suggest that tension in water in this soil is likely confined to the pre-melting layers themselves, rather than being transmitted, as in finely porous media, to surrounding water. It is not thought relevant to the question of survival and growth of micron-scale organisms.

The osmotic depression of the freezing-point: This is the incidental result of solutes in soil water lowering its chemical potential and hence its freezing point. As the soil water freezes, solutes are excluded from the ice, which is a poor solvent, increasing the solute concentration in the remaining liquid water and reducing its freezing point. At a given temperature freezing will continue until the chemical potential of the soil water is just that required to equal that of ice at

the same temperature (in water potential units), so that it falls at about 1.1MPa per °C fall in temperature[1]. The solute concentration in unfrozen water at soil temperatures just below 0 °C is likely to lead to only a small increase in the local solute concentration, as water steadily freezes at the interface of the ice, excluding its solutes (Stähli and Stadler, 1997; Watanabe et al., 2001). In contrast, when the soil temperature falls to around -10 °C, the solute concentration in caches of unfrozen water may approach saturation (Stähli and Stadler, 1997) and lead to very low water potentials of the order of -10 MPa.

This fall in osmotic potential as the soil water freezes is expected to directly affect microorganisms, withdrawing water from the cell contents, first reducing the turgor pressure and then the cell volume, so increasing internal solute concentrations (Csonka, 1989; Wolfe and Bryant, 1992; 1999; 2008). Internal concentrations of compatible solutes will also rise in microorganisms due to synthesis and uptake (Csonka, 1989; Csonka and Hansen, 1991). The effects of these physical changes on the soil microbial flora will be discussed in greater detail below.

Snow and ice melt, and soil thaw

At the winter-spring transition these physical changes do not just happen in reverse order. With increasing soil temperatures, the solute concentration in the caches of unfrozen water falls, brought about by the inflow of snow-melt water along interconnected channels in the surface ice and soil, and the melting of the soil ice. The soil temperature rises very slowly just below the freezing point, resulting in quasi iso-thermal conditions. When all the ice has melted the temperature leaves this iso-thermal state, the so-called “zero-curtain envelope” (Muller, 1947; Washburn, 1973), and rises above 0 °C.

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[1] Matric potential is used by some authors to discuss effects in mixed solid-solution phases. Passioura (1980) points out that this term is often not well defined, and that when it is defined, it includes terms that already appear in the definitions of either hydrostatic potential, osmotic potential or both. Following Passioura, we therefore refer only to hydrostatic potential and osmotic potential in this discussion.

In late February and March, increases in the amount of solar radiation begin to impact on landscape surfaces resulting in considerable sublimation. Snow cover and depth may vary considerably across the landscape, depending on previous amounts of precipitation, wind speeds, topography, and vegetative cover (Hinzman et al., 1996). Both snow and ice allow some transmission of short-wave radiation, although some absorption occurs within the volume of snow and ice (Oke, 1987).

Radiative heat transfer dominates over heat conduction in the upper 50 cm of snow and the upper 5 m of ice because short-wave radiation is transmitted much more readily than long-wave radiation in these media (Oke, 1987). Although fresh snow has a high albedo, the accumulation of material on the surface, such as soot, plant material, and dust, appreciably lowers the albedo. In addition, in late afternoon films of free water are often present on snow or ice surfaces that reduce the albedo close to that of water (c. 0.1) (Oke 1987). Thus, the surface properties of snow and ice determine the transmission or reflexion of incoming short-wave radiation.

Living vegetation and dead shoots buried in ice and snow absorb transmitted radiation, becoming an anomalous thermal feature compared with surrounding snow and ice because of their different albedo values, leading to the melting of snow and the establishment of drain holes surrounding plant tissues (Kane and Stein, 1983). There is increasing build-up of unfrozen water beneath snow as melt proceeds. Snow has a high pore capacity and there are numerous dendritic channels though which unfrozen water drains into frozen ground beneath surface ice (see Kane and Stein, 1983 and Marsh and Woo, 1984 for a detailed description). Once the finger wetting front (Marsh and Woo 1984) reaches the snow-soil interface, water may infiltrate the soil or form a saturated layer at the interface which freezes due to the low soil surface temperature (Woo and Heron, 1981). A significant portion of the latent heat released upon this freezing raises the temperature of the ground (Marsh and Woo 1984). Some of the water that infiltrates into the soil will be refrozen. With increasing soil temperatures in April and May in the range of -10 °C to -3 °C, the water potential of this unfrozen layer is less negative as free water becomes available and the solute concentration is diluted. Under the increased radiation load in the daytime and the presence of free water, soil ice melts and more water penetrates the less frozen ground through channels and cracks that have developed in the surface ice. The anomalous thermal properties of living or dead plant tissues embedded in the ice, which have a different albedo from that of ice, also influence the penetration of water into the frozen ground as described above for snow. At a

microsite scale this means that some areas will be inundated with fresh water while other microsites remain frozen for a longer time, with differing consequences for soil microorganisms inhabiting different microsites. Respiration of living below-ground tissues may contribute locally to heat transfer resulting in the development and a widening of water channels through the ice.

As indicated earlier, the pore space of these peaty soils is high (c. 80% by volume or more) and they are water-saturated for most of the snow-free season (Luxmore, 1981; Carey et al. 2007). During the early stages of the winter-spring transition, the temperature in the upper 15 cm of soil rises rapidly towards 0 °C. This is thought to be a consequence of non-conductive heat transfer within the mat due to solar radiation (Hinkel and Outcalt 1994; Romanovsky and Osterkamp 2000; Kane et al., 2001). The steepness of the rise depends on the thermal properties of the substratum and the ability of solar radiation to penetrate the snow and ice leading to an increase in the amount of unfrozen water in the bulk frozen ground. The temperature rise leads to the zero-curtain envelope and iso-thermal conditions (Muller, 1947; Washburn, 1973; Hinkel et al., 2001). The temperature in the upper layer of the soil is within a few degrees of the freezing point of water and shows a steady slow climb to 0 °C over 10 days to two weeks.

Seasonal patterns of microbial biomass: cold-adapted microbes, and their physiological responses to cold

During the late fall, when plants have senesced and there is a ready supply of substrate from fresh plant litter (including roots), the microbial flora continues to be active, in some cases resulting in higher microbial biomass in winter than in other seasons (Chapter 2; Brooks et al., 1998; Lipson et al., 2000; Nemergut et al., 2005). Snow cover insulates the ground, moderating soil temperatures relative to air temperatures, and constraining minimum soil temperatures (Groffman et al., 2001; Buckeridge and Grogan, 2008). Where snow depth reaches at least 50 cm, air and soil temperatures become decoupled (Larsen et al., 2007) so that soil temperatures can remain at -10 °C or higher despite fluctuating and much colder air temperatures. At these soil temperatures, as discussed above, some unfrozen water occurs in the soil (Romanovsky and Osterkamp, 2000; Price and Sowers, 2004) in which microorganisms are present (Coxson and Parkinson, 1987; Rivkina et al., 2000; Mikan et al., 2002).

Microbial activity declines substantially as soil temperatures in the active layer approach -10 °C (Mikan et al., 2002), as occurs in mid-winter in Arctic sites. Evidence from Arctic soils during the winter (Chapter 2) suggests that, as in alpine sites (Brooks et al., 1998; Lipson et al., 2000; Schmidt and Lipson 2004), peak microbial biomass tends to occur in the latter half of the winter season. In spite of the high biomass, the turnover rate of microbial nitrogen is extremely slow in late winter (Buckeridge and Jefferies, 2007). Where microbial biomass is known to be high in late winter, levels decline markedly during the winter-spring transition (Lipson, 2000; Edwards, 2006; Larsen, 2007). For example, the decline in biomass from peak values in Arctic wet sedge meadows at Churchill, Manitoba appears to start in most years just prior or at the onset of the zero-curtain period when iso-thermal conditions prevail (Fig. 5.1, redrawn from Edwards et al., 2006 [Chapter 2]). By the time soil temperatures rise above 0 °C at the end of the zero-curtain period, microbial biomass values are very low (Fig. 5.1). This decline in biomass may be accompanied by broad changes in the microbial community composition, as has been shown in Alpine sites where fungi are more dominant in winter and bacteria are more active in summer (Lipson et al. 2002, Schadt et al., 2003); however, the same was not observed in Arctic tundra soils where seasonal shifts occurred only at much finer taxonomic scales (Wallenstein et al., 2007).

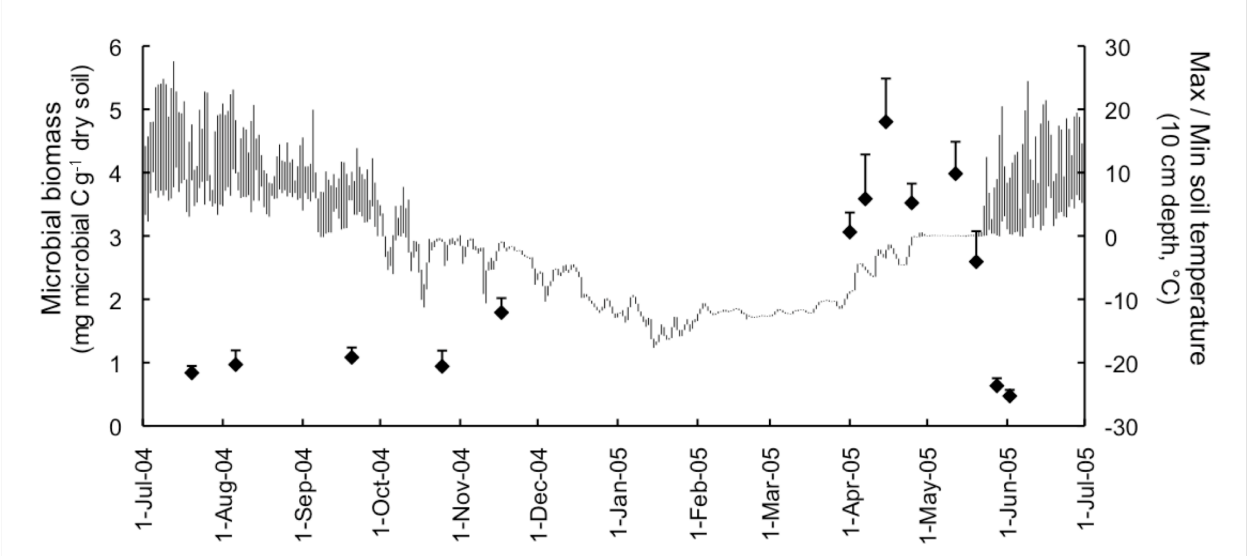

Fig. 5.1. Seasonal patterns of microbial biomass (values shown as solid diamonds ± S.E, n=6) and daily maximum and minimum soil temperatures in the active layer (10 cm depth; solid line) in a wet sedge meadow on the Churchill Peninsula, Manitoba, Canada between July, 2004 and June 2005. The zero-curtain envelope is from 25 April to 20 May during which time iso-thermal conditions prevail and the temperature is just below 0 °C.

Quantifying and characterizing the microbes that exist in frozen soils is difficult using traditional techniques that may require soil to be thawed or incubated above 0 °C. Determining viability of cells (ie. those that are capable of living but may be dormant) and whether cells are actively metabolizing and even growing are significant challenges (Gilichinsky, 1995). In an Arctic tundra birch hummock site, Buckeridge and Grogan (2008) estimated total bacterial cell numbers in the active layer at the end of winter to be approximately 5 x 10^9 cell g^{-1} dry soil, roughly consistent with both alpine sites (Lipson et al. 2002) and a little higher than estimates from permafrost soils (up to 10^8 cells g^{-1}; Rivkina et al., 1998). In both the Arctic and the alpine case, fungal estimates of biomass were much higher than those for bacteria, with wintertime fungal:bacterial ratios of 4.3 and 1.6 respectively (Lipson et al., 2002; Buckeridge and Grogan, 2008). Measuring culturable bacteria from Arctic tundra soils, Vishnivetskaya (2000) estimated roughly 10^5 cells g^{-1} wet sediment, with estimates from some permafrost samples being similar. Viable organisms identified from permafrost have been found to be predominantly aerobic and anaerobic bacteria, but cyanobacteria, algae, yeasts, filamentous fungi, and Archaea have also been isolated from these perennially frozen soils (Gilichinsky et al. 1995; Rivkina et al., 2004). Based on the temperature classification of Morita (1975), most organisms are believed to be psychrotrophic (pyschrotolerant), capable of growing below 0 °C but also above 20 °C (Gounot, 1986; Gilichinsky et al., 1995; 2007; Rivkina et al., 2004; Steven et al., 2006). There is evidence of microbial growth (basidiomycetous yeasts, ascomycetous fungi, and bacteria represented by *Pseudomonas, Arthrobacter* and *Polaromonas*) at temperatures between -18 °C and -35 °C (Panikov and Sizova, 2007), and microbial respiration has been measured down to -40 °C (Panikov et al., 2006). In order to survive conditions of high solute concentration organisms may also be halotolerant, such as *Psychrobacter* and *Exiguobacterium* isolates from Siberian permafrost that displayed metabolic activity at 5 osMolar (2.79 M NaCl i.e. -11 MPa) (Ponder et al., 2008).

At the onset of winter, the microbial flora must contend with both decreasing temperatures, and increasing solute concentration of the soil water as solutes are excluded from developing ice in the soils - hence the organisms must be both psychrotolerant and halotolerant to survive (Franks, 1990; Deming, 2002). Several physiological adjustments take place for cells to survive cold

temperatures, including increasing enzymatic activities, membrane bilayer changes that maintain membrane fluidity and enable solute transport, and changes in gene expression known collectively as the cold shock response (Franks, 1990; Beales, 2004). Physiological responses to desiccation stress under cold conditions mirror those required for survival during other situations of cellular drought in which the extracellular environment is characterized by high osmolarity, or low water potential (Csonka, 1989; Potts et al., 1994; Schimel et al., 2007). In general, microorganisms are able to survive cold-induced dehydration and resist intracellular ice formation by supercooling and/or reduction of freezing point by the production and uptake of compatible organic solutes and changes in the composition of membranes (Franks 1990; Wolfe and Bryant, 1992; 2008; Beales 2004).

As ice forms in the bulk soil and extracellular osmolarity is increased, cellular turgor pressure may be lost temporarily, but can be regained through osmoregulation: an increase in intracellular osmotic concentrations through the withdrawal of water and the build-up of compatible solutes. Compatible solutes allow for the continued functioning of cellular enzymes and can be synthesized within the cell (such as glutamate, proline, glutathione, trehalose, and K^+) or can be taken up from the extracellular environment (glycine betaine and proline) (Csonka 1989; Csonka and Hansen, 1991; Beales 2004; Schimel et al. 2007). In addition, a low external osmotic potential can cause a large reduction in the aqueous volume of the cell and in the area of the plasma membrane. The extent of osmotic contraction of cells depends upon the cooling rate and the prevailing temperatures (Mazur, 1963). The integrity of the plasma membrane and endoplasmic membranes is critical in the adjustment of cells and involves changes in the lipid composition of the plasma membrane, as the cells adjust to these low osmotic potentials (Uemura et al., 1995). Phospholipids are incorporated into the existing membrane when an increase in area is required (Wolfe and Steponkus, 1983); however, cells may still succumb upon osmotic expansion because of the loss of ability of cold-tolerant, dehydrated cells to alter their membrane composition rapidly. In bacterial membranes there is an increase in membrane *cis*-unsaturated lipid fatty acid composition in response to sub-zero temperatures (Russell, 2007). The modification of the lipid composition of the membranes maintains the fluidity of the lipid bilayer by inducing a kink in the acetyl chains that reduce contact between adjacent chains and alter the organization of the membrane bilayer (Feller, 2006; Russell, 2007). Some, but not all, psychrotolerant microorganisms have the ability to convert *cis*- to *trans*-unsaturated fatty acids in

less than 2 hrs when an increase in free water occurs and temperatures rise. However, the response is not reversible during freeze-thaw cycles (Okuyama et al., 1997; Russell, 2007). Desiccation-induced changes in membrane lipids further to those caused by low temperature stress include an increase in anionic phospholipids in the membrane bilayer relative to neutral, zwitterionic lipids (Beales 2004).

Intact semi-permeable membranes are essential for maintaining the different solute composition of intra- compared with the extra-cellular solutions, and helping to prevent intracellular ice nucleation. However, at the end of winter, the input of melt water in ice channels may lead to a rapid increase in the external osmotic potential (see below). This will cause a rupture of cell membranes and rapid cell death if the plasma membrane cannot expand quickly enough to accommodate the osmotic excursion, or if the cell cannot gracefully release its accumulated compatible solutes. The release of intracellular solutes occurs under situations of acute hypoosmotic stress by way of mechanosensitive (MS) channels in the membrane, which are activated when a sudden drop in extracellular osmotic concentration is detected (Morback and Reinhard, 2002). However, cell rupture may occur if the increase in osmotic potential is too great and too rapid for MS channels to compensate for, as can happen when microorganisms in desiccated soil experience rewetting (Morbach and Reinhard, 2002; Schimel et al., 2007). Membranes have a limited ability to re-expand rapidly, because the rate of expansion is determined by the rate at which the cell can add material to the membrane. Membranes cannot stretch appreciably and can support a tension of only several mN m^{-1} without rupturing (Wolfe and Bryant, 1992; 2008). It has long been known that cells of some plant species have a limited ability to re-expand osmotically (Levitt and Scarth, 1936). The use of plant protoplasts has shown that when they are placed in hypertonic solutions, they become spherical with a reduction in surface area. The plasma membrane has a small resting-state surface tension which is less than 1 mN m^{-1}. Upon dilution of the external solution, a large influx of water into the cell occurs, but the membrane is capable of expansion of only a few percent without rupture (Wolfe and Bryant, 2008). When the membrane reaches a tension of 4-6 mN m^{-1} lysis will occur (Dowgert and Steponkus, 1984).

Possible causes of microbial decline during the winter-spring transition

Various hypotheses have been suggested to explain the decline of microbial biomass in many seasonally frozen soils during the winter-spring transition, including starvation, temperature rise, and freeze-thaw cycles. We suggest that cell rupture as a consequence of hypoosmotic shock should also be considered as a potential contributor to the observed microbial decline.

Lipson et al. (2000) argue that the decline in microbial biomass at the winter-spring transition is at least, in part, caused by a depletion of labile C substrates produced as a result of decomposition in fall and during the winter. Indeed, there is experimental evidence that provides some support for this hypothesis (Brooks 2004; Buckeridge and Grogan, 2008) but in some situations the addition of carbon has not prevented the microbial decline from occurring (Chapter 4). The lack of resources may contribute to the microbial decline, but this is likely not the only stressor that results in loss of biomass at the onset of spring.

Increasing temperatures in spring have also been invoked as a potentially lethal stress on any microbes that require cold temperatures (psychrophiles) (Lipson et al., 2000). Currently, the proportion of winter microbes that are psychrophiles and might be vulnerable to spring and summer soil temperatures is unknown. Studies of the microbial communities of permafrost soils indicate that organisms are predominantly psychrotrophic (Rivkina et al., 2004). However, the isolation of microorganisms from permafrost sediments is extremely difficult and true psychrophiles are rarely isolated (Vishnivetskaya, 2000). Because most extractions and isolations take place at temperatures above 0 °C, psychrophiles may not survive and consequently are underrepresented in the isolates. Therefore, the possibility that a rise of biomass in winter followed by a decline at the end of winter could be caused by a bloom of psychrophiles adapted to soil temperatures below 0 °C cannot be ruled out.

Freeze-thaw cycles have also been implicated in the death of soil microbes during the winter-spring transition, but their effects on soil microbes remain unclear, as experimental studies have produced mixed results (Henry, 2007). In some experimental studies a single freeze-thaw event killed up to 50% of the viable soil microbes (Soulides and Allison, 1961; Skogland et al., 1988; DeLuca et al., 1992). However, many experimental freeze-thaw studies fail to properly simulate natural freeze-thaw cycles, exposing microbes to temperature fluctuations that were much more

severe than would typically be encountered in nature (Henry, 2007). Regardless of the potential effects of freeze-thaw cycles on the late-winter microbial population, occurrences of re-freezing events in water-saturated soils below 10 cm depth are believed to be relatively uncommon due to the insulation of wet soils from fluctuating air temperatures. Soil refreezing can occur on occasions but by this stage much of the microbial biomass has already been lost prior to soil temperatures rising above 0°C.

As discussed earlier, there is a dendritic network of channels and cracks in surface ice that continue into the frozen ground beneath the ice. These are likely to be more evident at the end of winter when stresses and strains in old ice promote channel formation and the widening of the conduits that maybe supplemented by additional channels that establish around shoots of wetland plants. The net effect of melt water release from snow and above surface ground ice is that there is a downward movement of water of low solute content into the soil during the zero-curtain envelope period when the unfrozen water content of the soil rises and iso-thermal conditions prevail. At this time the rapid movement of melt water into soil via ice channels may lead to membrane rupture in the manner described above, if dehydrated cells are unable to adjust to the abrupt rise in soil osmotic potential. For cells that do survive, the loss of significant amounts of intracellular solutes may also reduce their biomass. Both cell rupture and loss of internal solutes, driven by hydrologic and osmotic changes in the soil, are likely contributors to the loss of microbial biomass at the onset of spring.

Both alpine and Arctic environments are very heterogeneous with respect to vegetation types and soil conditions and the paucity of studies on seasonal changes, especially in relation to intensive spatial-temporal sampling and realistic experimental conditions suggests caution in attributing the microbial decline to a single cause. In this connection it is notable that on rewetting of desiccated soil, there is a loss ranging up to 50% of the biomass (Schimel et al., 2007; but see Fierer and Schimel, 2003 for a different result). This loss is attributed to dumping of compatible solutes without cell death (Halverson et al., 2000). The close analogy between loss of water due to desiccation and that due to freezing means that we should consider cellular responses to rapid osmotic shifts to be at least partially responsible for the microbial biomass decline during the winter-spring transition.

Conclusion and Implications

In summary, a factor in a decline in microbial biomass at the winter-spring transition may be the deleterious consequences of an abrupt change in osmotic potential before the soil temperature exceeds 0 ˚C. Loss of psychrophilic organisms as the temperature rises may also contribute to the decline in microbial biomass in cold soils, as may a reduction in substrate availability, and the potentially disruptive effects of freeze-thaw cycles. The relative contributions of these different causes can be expected to vary across sites and years, and indeed may also vary at the microsite-scale. If this is correct, a decrease in the activity of microorganisms capable of growing at sub-zero temperatures in northern soils may be anticipated as the number of winter warming events increases. Climate change is expected to cause the loss of an extended period where the soil temperature is sub-zero (IPCC 2007). In addition, any decline in plant primary production associated with climate change, such as increased summer drought, may be expected to limit the availability of substrates in soils in the following autumn and winter for microbial growth. The steep decline in microbial biomass in late winter is frequently followed by a pulse of soluble nutrients, such as nitrogen and phosphorus, resulting from the lysis of cells. This release of nutrients represents the largest single annual input of available nitrogen into these cold soils and provides a significant source of this element to plants for summer growth (Jaeger et al., 1999; Lipson et al., 1999, 2000). It is known that some roots can take up N under the snowpack in Arctic sites (Bilbrough et al., 2000; Edwards, unpublished observations). However, reduced microbial growth in winter associated with soil temperatures reaching or exceeding 0 °C could result in a decline in the nutrient pulse. This will reduce the quantity of nutrients for spring plant growth and adversely affect production at higher trophic levels in these northern environments.

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Chapter 6
Year-round seasonal patterns of soil microbial and nutrient dynamics: Four years of observations from wet and dry low Arctic sedge meadows

Preface

Chapter 2 described a seasonal pattern observed in 2005 in which soil microbial biomass and various nutrient pools were large in late winter and declined during thaw. Chapter 3 described that these microbial and nutrient dynamics are relevant for plant nutrient uptake early in the spring, and Chapters 4 and 5 point to temperature and resulting soil water thaw as the main controls on the timing of microbial and nutrient pool decreases. In this chapter I investigate the variability of the pattern described in Chapter 2 over 4 years, and also the variability across the landscape, examining two ecotypes that differ in terms of hydrology, vegetation, and soil characteristics. This provides a more general perspective from which to examine current and future responses to climate-induced ecosystem change.

Abstract

Biogeochemical activity occurs year-round in Arctic soils, and previous studies from both alpine and Arctic tundra sites have revealed that soil microbial biomass (MBC) in the active layer reaches an annual peak at the end of winter, and decreases during or shortly after soil thaw. This occurs concurrently with, or is followed by, a peak in soil nutrients that provide an important nutritional resource for plant growth. I did an observational study in dry sedge tundra and wet sedge tundra to monitor the annual pattern of soil MBC and nutrient resources over four consecutive years, sampling at all times of year but with sampling effort focused on the dynamic period of the winter-spring transition. Soils were collected on approximately 55 occasions between June 2004 and June 2008 from both wet and dry sites, and 6 replicates were usually sampled from each ecotype. Soil MBC, microbial nitrogen (MBN), and microbial phosphorus (MBP) (chloroform fumigation and extraction), extractable carbon (C), nitrogen (N), and phosphate (P), inorganic N (ammonium and nitrate), fresh weight/dry weight, and bulk density

were quantified. Total soil C and N, redox potential and pH measurements were taken for some but not all time points. A consistent pattern was observed in wet sedge meadows wherein large late winter MBC, MBN, and MBP pools decreased steeply during the period of soil thaw. Soil temperatures from a nearby and comparable site shows a consistent correlation between MBC, MBN and MBP declines, and soil temperature warming, both at thaw and during warming events in April each year, suggesting that soil physical factors drive the observed microbial declines. Nutrient pools showed similar patterns, however a post-thaw nutrient-N increase was observed in 2006, particularly in the dry site, but not in other years. Dry site results were comparable to those from the wet site but changes in pool sizes were generally dampened relative to wet sites. The N increase in 2006 was coupled with exceptionally high MBN values during the summer of that year. Over four years, peak winter values of all variables declined in both ecotypes. Soils appeared to become drier, suggesting that if these ecosystems experience long-term drying trends, winter decomposition could be negatively affected, jeopardizing the nutrient supply to plants in spring.

Introduction

Successful monitoring of the impacts of climate change in northern regions and prediction-based management of these ecosystems requires knowledge of patterns and processes across substantial temporal and spatial variability. This variability exists at several scales, and a comprehensive understanding across these scales is limited in part by a shortage of integrated long-term datasets (Hinzman et al. 2005). Year-round seasonal variation in northern areas is one type of variability that is recognized as being a critical component to describing and quantifying ecosystem functioning, but has not been widely explored by ecologists. The lack of research conducted during the Arctic winter is primarily due to a historical assumption that outside of the plant-growing season, frozen landscapes are largely inactive and biogeochemical cycling is minimal, and also because of logistical constraints including site access and limited infrastructure, and even academic teaching schedules. However, freeze-up, winter, and thaw, are now known to be times of significant below-ground biogeochemical activity (Hobbie and Chapin 1996, Fahnestock et al. 1999, Larsen et al. 2007, Schmidt et al. 2007) and understanding the responses and contributions of these systems to climate requires closer attention to cold-season soil processes.

Microbial activity occurs in frozen soils as cold as -40 °C (Price and Sowers 2004, Panikov et al. 2006) and at ecologically significant rates at least as cold as -8 °C (Mikan et al. 2002, Elberling et al. 2003). Soil microorganisms in cold ecosystems therefore carry out biogeochemical processes during most or all months of the year. Following plant senescence, microbes decompose readily available plant litter, while competition for mineral nutrients from plants is waning (Grogan et al. 2001, Nemergut et al. 2005, Schmidt et al. 2007). This period can last several months as the active layer cools and freezes. Microbial processes may be significantly slowed for a period of deep cold in the winter in areas where the active layer freezes below -10 °C, but activity increases again when soils warm, often well before snowmelt (Clein and Schimel 1995, Brooks et al. 1997, Larsen et al. 2007). Winter microbial activity results in significant carbon (C) losses from Arctic sites during the long, cold season, which may result in some sites being a net C source to the atmosphere (Fahnestock et al. 1999, Oechel et al. 2000, Welker 2000, but see Aurela et al. 2004). The highest rates of CO_2 production occur during the shoulder seasons rather than during the period of deep cold (Aurela et al. 2004, Brooks 2004, Sullivan et al. 2008).

When soils are frozen, microbes survive in films of unfrozen water, and the activity of these 'eutechtophiles' (Deming 2002) is limited directly by temperature effects on biochemical processes, but also indirectly by limited transport of substrates and waste products through the network of narrow liquid water films in which they reside (Deming 2002, Agren and Wetterstedt 2007, Oquist et al. 2009). When soils thaw, a shift in the microbial community is likely to occur, as has been described in alpine tundra sites in Colorado (Lipson et al. 2002, Schadt et al. 2003, Lipson and Schmidt 2004). The transition from winter to summer is also accompanied by increasing rates of microbial biomass (MB) turnover (Schmidt et al. 2007, Buckeridge and Jefferies 2007) and changes in substrate use by the microbial community (Lipson et al. 2002, Schimel and Mikan 2005, Schmidt et al. 2007). Plant root exudates supply labile C in summer, while winter microbes tend to rely on dead plant material (Grogan et al. 2001, Lipson et al. 2002, Schmidt et al. 2007) and microbial cellular products (Schimel and Mikan, 2005).

Despite ample evidence of microbial activity in winter, few studies have measured the changes in microbial and nutrient pool sizes outside of the summer growing season in cold regions. It is often assumed that soil respiration is a direct measure of microbial activity, and that activity rates are directly correlated with MB (Anderson and Domsch 1978, Panikov and Sizova 1996).

However Santruckova and Straskraba (1991) reviewed findings worldwide and concluded that MB and soil respiration are not necessarily related, but that as MB increases, the respiration activity per microbial cell tends to decrease, probably resulting from growth inhibition at high CO_2 levels. Similarly, Sparling et al. (1981) found no relationship between MB and microbial activity in glucose-amended soils. Furthermore, under frozen conditions, given temperature, osmotic, and transport constraints, usual assumptions about microbial metabolism may not be appropriate (Mikan et al. 2002).

Studies conducted in sub-Arctic birch forest and heath (Larsen et al. 2007), low-Arctic birch-hummock (K. Buckeridge, *pers. comm.*) and low-Arctic wet sedge (Chapter 2) have all observed MB and nutrient peaks at the end of winter, followed by declines as soils thaw. Similarly, Lipson et al. (1999) found that in an alpine tundra site, MB peaked in late winter and declined after soil thaw, but inorganic N (N_i) did not peak until after the MB decrease. Although such phenological variations are not unexpected between different cold systems and between sites, winter peaks of MB are apparently common across many seasonally frozen systems, and an annual pattern for northern ecosystems is emerging that may be widespread across the low- and sub-Arctic, if not many areas of the boreal, and high-Arctic regions. The true spatial extent of this pattern requires further study, and it is also unclear whether this pattern is consistent between years, and to what extent different climatic and weather conditions can alter the intra-annual dynamics of soil microbial and nutrient pools.

For a given site, the suite of conditions faced by soil microbes during the fall freeze and spring thaw shoulder seasons can vary widely between years, as different temperature and precipitation regimes play out. Snowpack dynamics over winter (Schimel et al. 2004, Groffman et al. 2006, Buckeridge and Grogan 2008), winter temperatures and warming events (Joseph and Henry 2008, Turner and Henry 2010), and the temperature and precipitation regimes during freeze-up and thaw (Aurela et al. 2004, Hinzman et al. 2005) can all vary inter-annually and have significant effects on soil biogeochemistry and ecosystem functioning. Understanding both intra- and inter-annual variation is therefore essential to understanding the effects of the changing climate on the biogeochemistry of these systems.

I conducted a four-year study to monitor the dynamics of various biogeochemical pools, addressing the need for both intra- and inter-annual data. Measurements were taken from two

low-Arctic ecosystems near Churchill, Manitoba. Data from wet sedge meadow and dry sedge meadow sites are reported, both of which have vegetation communities dominated by *Carex aquatilis Wahlenb.*, a common rhizomatous sedge species that is not known to be mycorrhizal (Muthukumar 2004). The sites differ by plant community composition, hydrology, and soil characteristics (see Methods for site details). The wet sedge meadow sites are representative in many ways of the vast system of boreal and Arctic peatlands that cover an area of approximately 2.1×10^6 km^2 across the circumpolar north (Oechel 1989). I took measurements year-round, but focused sampling effort on the transition between winter and spring. This time of year is dynamic and is also sensitive to environmental changes relative to other seasonal periods (Olsson et al. 2003). Because it precedes the summer growing season, the changes in biogeochemical processes at this time of year are also directly relevant to plant productivity.

As much of this paper discusses seasonal patterns, a nomenclature of seasonal descriptors is valuable. Olsson et al. (2003) describe five stages of the Arctic cold season based on data collected at Imnavait Creek, Alaska, and I will generally follow their nomenclature here, with one modification to further delineate the final stage. The five stages are "early snow", "early cold", "deep cold", "late cold", and "thaw". These stages correspond respectively with soil temperatures near 0°C, freezing of soils, declining soil temperatures, modest increases in soil temperatures, and rapid increases in soil temperatures. Most of the discussion in this chapter pertains to the late cold and thaw stages. Air temperatures are rising throughout these periods and as snow melts, soil temperatures become responsive to these increases. Thaw is defined by Olsson et al. (2003) as the period of snowmelt (until 95% completion). As I am concerned with soil processes, and the relationship between soil thaw and snow thaw is not equivalent between years and sites, I define the beginning of "early thaw" to occur when soil temperatures step up from the coldest winter values (Buckeridge and Grogan, 2009), usually occurring at the beginning of April and resulting in a rise approximately from -10 °C to -5°C. This period ends when soil temperatures reach near-zero temperatures, entering the zero-curtain envelope, which I call "late thaw". During late thaw, the soil water becomes liquid, which happens over time throughout the soil profile, and often occurs after most of the snowpack has melted. This is followed by spring, when temperatures in the plant rooting zone are consistently above zero, and above-ground plant green-up and growth is evident.

Materials and Methods

Site descriptions

Location

Research was carried out near Churchill, Manitoba. The region covers a transitional area from sub-Arctic boreal forest (inland) to low-Arctic tundra (coastal), with the sites used here most closely representing the latter. Between the years 1971 and 2000, average daily temperatures on a monthly basis ranged between -27 °C in January to 12 °C in July, annual rainfall and snowfall averaged 264 mm and 191 cm respectively, and mean snow depth was 15 cm. These and other detailed climate records are accessible from the Environment Canada's National Climate Data and Information Archive (Environment Canada website).

Wet sedge meadows

Three sites were sampled that are collectively referred to as wet sedge meadow, but could also be called fen or calcareous mire sites (Oechel et al. 1989). Two are adjacent to each other, located approximately 200 m apart, and are 2 km east of the Churchill Northern Studies centre. The third site is a small low-lying area northeast of the large dry meadow described below (Chapter 1). All sites are characterized by the dominance and high productivity of *Carex aquatilis*, which is nitrogen (N) and phosphorus (P) co-limited, and reaches an above-ground biomass of 140 g dry tissue m^{-2} (K. Edwards, unpublished data, collected 2004, 2005). The fibrous *C. aquatilis* root system inhabits an organic soil layer typically 30 cm deep, which is underlain with a mix of sand, silt, and limestone fragments.

Nomenclature of vascular plants listed below follows Porsild and Cody (1980). Vascular species present include *Carex glareosa* Wahlenb., *C. gynocrates* Wormsk. ex Drejer*, Eriophorum angustifolium* Honck*., and Triglochin palustris* L.. Bryophyte cover is high in some areas, and includes primarily *Tomentypnum nitens* (Hedw.) Loeske, *Drepanocladus revolvens* (Sw.) Warnst. and *Blepharostoma trichophyllum* (L.) Dum. Some areas contain tussocks and other raised features that contain several non-graminoid vascular plant species, but these features were avoided when sampling.

Dry sedge meadows

Three adjacent sites were used within a large meadow located approximately 5 km west of the Churchill Northern Studies Centre, Churchill, Canada. The three sites are approximately 500 m apart and each covers an area of about 100 m^2. The meadow is less than 1 km from the Hudson Bay coastline and the 5-15 cm of organic soil is underlain with sand. Although referred to as "dry" for comparative purposes, these sites are generally mesic tundra, although some areas can dry significantly in late summer. Some but not all areas have moderate development of raised tussocks. *Carex aquatilis* is the most abundant vascular plant species, but the flora is relatively diverse and common plants include *Scirpus caespitosus* L. ssp. austriacus (Pallas) Asch. and Graeb., *Kobresia myosuroides* (Vill.) Fiori and Paol., *Andromeda polifolia* L., *Dryas integrifolia* M. Vahl, *Rhododendron lapponicum* (L.) Wahlenb., *Salix reticulata* L., *Carex capillaris* L. ssp. capillaris, *Carex norvegica* Retz., *Carex rariflora* (Wahlenb.) Sm., and *Tofieldia pusilla* (Michx.) Pers. Above-ground *C. aquatilis* growth is limited by the availability of both N and P and presumably by water, given that wetter areas of the site support visibly higher plant biomass, and micronutrient additions did not increase above-ground production (K. Edwards, unpublished data). Above-ground biomass of *C. aquatilis* in these sites reaches approximately 35 g dry tissue m^{-2} at the end of the growing season, about one quarter that of the wet sites (K. Edwards, unpublished data, collected 2004, 2005).

Local soil temperature and moisture data

Daily soil temperatures

Soil temperatures were measured as described in Chapter 2. Briefly, soil temperatures at 10 cm depth were recorded by a Campbell Scientific International CR10X using type T thermocouples. Readings were taken every 5 minutes to determine the minimum and maximum daily temperatures. Temperatures were taken from a wet sedge area within 500 m of the larger wet sedge area used for soil sampling (see Chapter 1, Fig. 1.1). The temperature probe is in the immediate vicinity of a shrub (G. P. Kershaw, *pers. comm.*)

Temperature and moisture relationship

Simultaneous soil temperature and moisture (volumetric water content) data were collected from one of the two wet sedge sites that are adjacent to each other ("R" site; Chapter 1). Five ECH20 TM probes connected to an EM50 datalogger (Decagon Devices Inc., Pullman, WA) were inserted vertically in the ground within a 2 m^2 area. Probes were planted such that measurements were integrated over 5-10 cm soil depth, and readings were recorded hourly from which daily means were calculated. The probes remained in the ground between October 2007 and June 2008, but the datalogger was removed for the coldest months of the year. Absolute moisture measurements were not calibrated for these organic soils but the timing of changes in moisture content are believed to be accurate without this calibration.

Soil sampling and processing

Soils were sampled between 11 June 2004 and 3 June 2008 with a total of 53 sampling times in the wet meadows and 55 collections from the dry meadows. All times of year were captured for microbial and nutrient variables, but sampling effort was concentrated between April and June when samples were often taken weekly or more frequently. Sampling was usually done at all three wet and all three dry sites with 2 samples collected from each site, resulting in n = 6 per ecotype. Replication was as low as n = 2 on 1 March 2004 (no standard error bars graphed) and was n = 3 on 20 Sep 2004, 25 Oct 2004, 1 Apr 2005, and 7 Apr 2005. July 2005 means are represented by 4 replicates, as are 28 April 2006, 1 May 2006, and 15 May 2008. All other means are represented by 6 or more replicates. In winter, samples were collected using an axe or custom-built CRREL (U.S. Army Cold Regions Research and Engineering Laboratory) permafrost drill fitted with carbide cutters, while in summer a knife was used. When transport to Toronto, Canada was required (generally September to April), samples were air-freighted in a cooler. Samples collected before freeze-up were kept refrigerated, while frozen samples were kept frozen, made possible because of refrigeration and freezer storage at all airports en route. Processing was then done within 5 days of sampling for transported soils, and within 2 days of sampling for soils processed at the Churchill Northern Studies Centre on-site laboratory (usually April to August).

After removing the upper 1-2 cm of soil surface (living plant material and litter) the entire organic layer of soil from the dry sites was utilized for soil analyses, whereas in the wet site the

upper 15 cm of soil was used. Soils were processed at field moisture levels and those that were sampled frozen were not thawed before processing, but were cut into pieces no larger than 1 cm^3 and extracted from the frozen condition. In the case of fumigated samples, chloroform was added directly to frozen soil pieces. Large roots, sticks, etc. were removed from soil prior to weighing for extractions and although soils were not thoroughly mixed, an attempt was made to portion the soil for all extractions in a representative way. Soil samples were analyzed for MB, organic C, N, and phosphate-P, N_i, bulk density, and fresh weight, dry weight ratio (FW/DW). Some but not all samples were used to estimate soil pH and redox levels, and these efforts were concentrated during the winter-spring transitions of 2007 and 2008.

For all extractions using frozen soils, an additional 15 minutes of extraction time was allowed, which is approximately how long it took for frozen soil pieces to thaw in solution. Microbial biomass C, N, and P pools were estimated using a chloroform-fumigation and extraction method modified for wet soils (Witt et al. 2000). Briefly, two 25 g portions of fresh soil were weighed, and one was extracted immediately with 50 mL 0.5 M K_2SO_4 for 1 hr, mixing frequently, followed by filtration through pre-leached Whatman No. 1 filter papers. The other 25 g portion was put in a 250 mL bottle and 2 mL of ethanol-free chloroform was added to the soil surface. The bottle was quickly sealed and refrigerated (4 °C) for 24 hrs, followed by extraction with 0.5 M K_2SO_4 in the same manner as the initial portion. The difference in C, N, and P pools from the two extracts gave an estimate of microbial contents. No correction factor was used for fumigation efficiency, so these estimates likely under-represent true microbial pool sizes. Extractable organic C, N, and P were estimated from initial K_2SO_4 extractions. Inorganic N was estimated from KCl extracts, in which 10 g of soil was added to 50 mL 0.5 M KCl and mixed frequently for 2 hrs, followed by filtration through pre-leached Whatman No. 1 filter papers. All extracts were kept frozen until further analysis.

Bulk density and FW/DW estimates were made by cutting, measuring, and weighing a cuboid of soil approximately 30 cm^3 and drying it at approximately 50 °C for at least 48 hrs, then re-weighing the sample. Readings of pH and redox were taken using an Accumet portable pH meter (Fisher Scientific, Waltham, Massachusetts, USA). Measurements of pH were done from a 2:1 slurry of water:fresh soil mixed frequently for 1 hr before reading. Redox potential (ROP) was measured using a platinum combination electrode (Fisher Scientific, Waltham, Massachusetts, USA) calibrated with Zobell's solution (Zobell 1946). Readings were taken from a freshly cut

soil surface and at least 6 readings were taken per sample at 10 cm soil depth, from which a mean was calculated. Dried soils were ground and used for total C and total N determination using an Elemental Combustion System 4010 CHN Analyser (COSTECH International, Valencia, CA).

Analyses of soil extracts

KCl extracts were analyzed for ammonium (NH_4^+) and nitrate (NO_3^-) using an autoanalyzer (Technicon AAII, Tarrytown, NY), in which NO_3^- is reduced with cadmium and the resulting NO_2^- is measured calorimetrically. Organic C was measured from K_2SO_4 samples using the volumetric dichromate method (Nelson and Sommers 1996) and P was measured as phosphate from the same extracts using the ammonium molybdate method (Ames 1966). Organic N was measured in two different but comparable ways. Most samples collected in 2004 and 2005 were analyzed through alkaline persulphate oxidation (Cabrera and Beare 1993) followed by nitrate analysis as described above, while the majority of the samples from 2006 onward were quantified using a TOC-TN autoanalyzer (Shimadzu, Kyoto, Japan) by chemiluminesence. This switching of techniques occurred because low-N potassium persulphate could not be obtained to complete all analyses as initiated. The relationship between these two methods is linear, at least for soils from the dry site, but TN measurements can underestimate persulphate-N by nearly 30% (E. Horrigan, *pers. comm.*).

Statistics

All statistics were done using JMP 7.0.1 (SAS Institute, Inc.). For both dry sites and wet sites, pH and ROP estimates during the late thaw period were compared between 2007 and 2008 using mixed-model ANOVAs with ‘year’ specified as a fixed effect and within-ecotype ‘site’ specified as a random effect. Variables were checked for normality using the Shapiro-Wilks goodness of fit test. Similarly, ROP measurements were compared between June and July in each of 2007 and 2008 to identify changes over the growing season.

Pairwise correlations (Pearson’s r) between pH, ROP, and MBC were examined during the transition period from early thaw to spring in 2007 and 2008. Changes over time (2004-2008) in FW/DW and BD estimates were evaluated using regression analyses, with separate analyses done for the seasons of summer, cold (late cold and deep cold), early thaw, and late thaw. Early

snow and early cold seasons (ie. fall) were not evaluated due to relatively few sampling dates during this time of year. Adjusted R^2 values along with t-test results are reported (Table 6.1).

Results

Soil temperatures

Wet sedge sites

The soil temperatures used to indicate conditions in the wet sedge sites are shown in Fig. 6.1. The period of thawed soils, defined as the end of the spring zero-curtain envelope until the end of the fall zero-curtain envelope, lasted between 10 June to 13 October 2004 (126 d), 23 May to 11 November 2005 (173 d), 12 May to 31 October 2006 (173 d), and 3 May to 26 October 2007 (177 d), with soils in spring 2008 rising above 0 °C on 23 May. Most years, cold soil temperatures were reached during the early cold season, but in the 2006-2007 winter soils did not drop below -5 °C until January 2007. Minimum soil temperatures were as low as -18 °C in January 2005 whereas the lowest temperature reached in the winter of 2005 was -12 °C. The duration of the zero-curtain envelope during late thaw ranged from 10 days (2004) to 27 days (2005). The time of soil freeze was more variable, with the zero-curtain envelope lasting only one day in 2004 and 42 days in 2005.

Dry sedge sites

Soil temperature data are not available for the dry sedge sites or any comparable sites in the area. However, data from a nearby beach ridge site reveals that under very dry conditions, soil temperatures are higher in summer (up to 50 °C) and colder in winter (-30 °C and colder) when compared with the wet sedge temperature record (data not shown). Diurnal fluctuations are also larger, and although freeze-up occurs at the same time in the dry beach ridge site, melt occurs up to three weeks earlier than in the wet sedge site. This beach ridge represents extremely dry conditions, but similar differences, albeit of lower magnitude, would be expected at the dry sedge site as compared with the wet sedge site. I usually observed soils from dry sites to thaw approximately one week earlier than soils from wet sites.

Soil temperature and moisture

The relationship between soil temperature and soil liquid water was examined in a wet site during freeze-up 2007 and thaw 2008 (Fig. 6.2). The proportion of water in the liquid state changes dramatically over approximately 1 week at both freeze-up and thaw, with the majority of the transition occurring over 3 days. Soil water froze when temperatures dropped below 0 °C at the onset of the early cold season, while soil water melted when temperatures rose above 0 °C, marking the onset of spring.

Onsite soil temperature measurements (wet site) were generally similar to those used for longer-term records, which were taken nearby (see Fig. 1.1, "R" site and "Long term soil T" site respectively). Temperatures during freeze-up in 2007 were different, being colder at the long-term record site by up to 7 °C. During thaw however, onsite soil measurements were always within 2 °C and usually within 1 °C of the long-term temperature measurements.

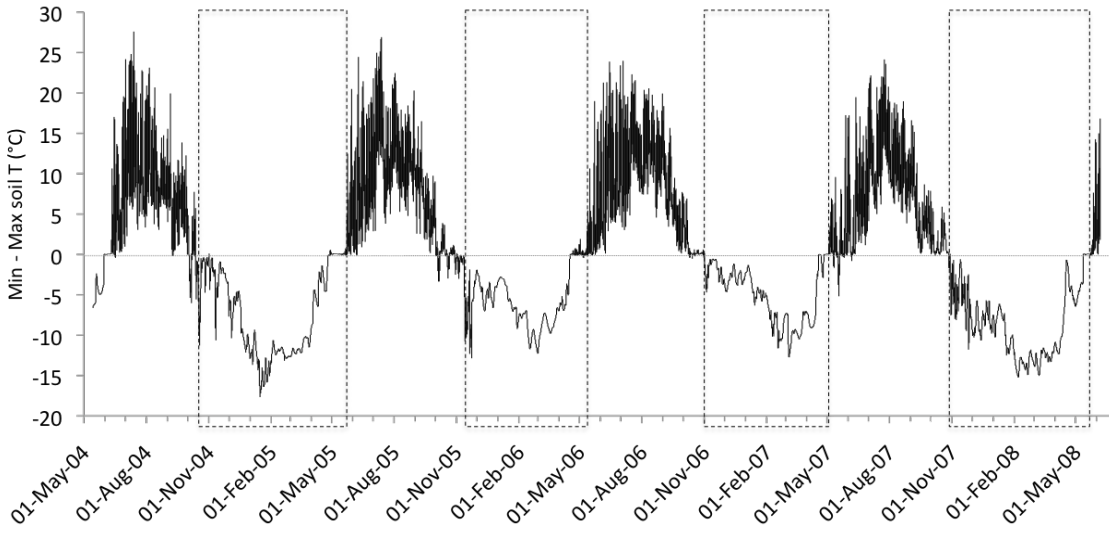

Fig. 6.1. Soil temperatures from a wet sedge site near to sites used for soil sampling, east of Churchill, Manitoba. Bars represent daily Min. - Max. ranges at 10 cm depth. Dashed boxes indicate the periods during which soil was frozen, beginning when soil temperatures drop below 0 °C and ending when temperatures rise above 0 °C.

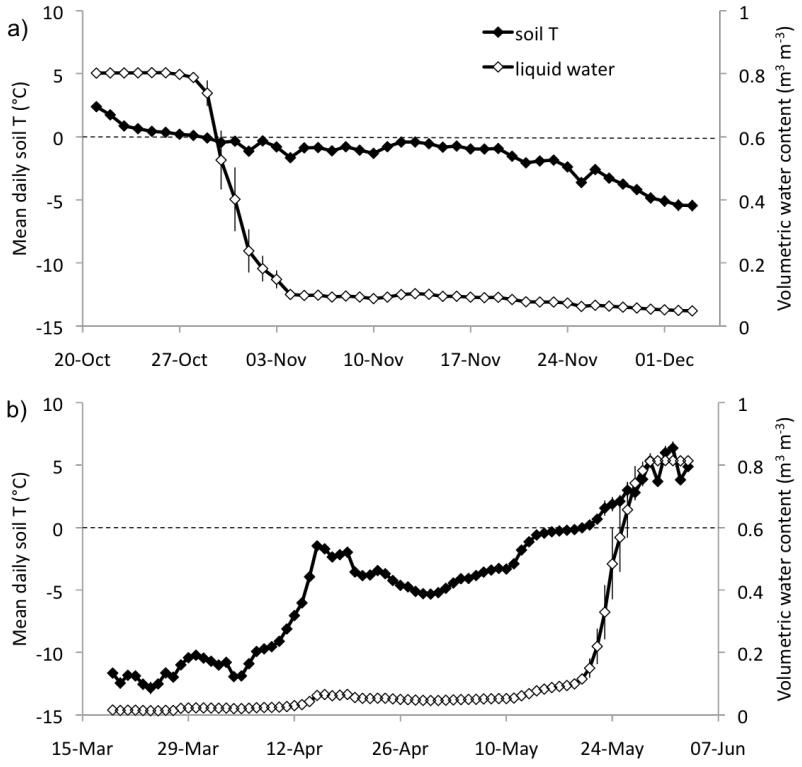

Fig. 6.2. Soil temperature and moisture records from a wet sedge site near Churchill, Manitoba during a) soil freeze-up, 2007 and b) soil thaw, 2008. Closed diamonds represent mean daily soil temperature (°C) and open diamonds represent mean daily volumetric water content (m^3 m^{-3}) measured from 5 probes inserted in the soil at 5 to 10 cm depth. Means and standard errors (n=5) are plotted.

Soil pH

Soil pH was measured primarily between April and June in 2007 and 2008 (Fig. 6.3). Mean values were 7.2 in dry sites and 6.8 in wet sites, revealing near-neutral conditions but slightly more acidic soils in the wet sites. Minimum values also differed, reaching 5.7 in the wet sites whereas the dry site soils were not observed to drop below 6.7. In 2007 pH was measured during and after the zero-curtain envelope (late thaw), while in 2008 the period prior to thaw was also captured (Fig. 6.3). In the wet sites, soils in 2007 were more acidic during late thaw than those collected in 2008 during the same period ($n = 41$, $F_{1,29.95} = 12.98$, $p = 0.0011$). A brief increase in pH from approximately 6.6 during late thaw to ~ 7.0 after thaw was observed in 2007, but the opposite trend was observed in 2008, with a decline from 7.2 to approximately 6.6 during the late thaw of 2008.

In the dry sites, pH values were similar between years during late thaw (n = 18, $F_{1,15.11} = 0.31$, p = 0.58). Measurements varied between dates in both years, but whereas pH in 2007 was higher after thaw than during, in 2008 pH dropped from ~ 7.3 before the zero curtain to ~ 6.8 after the completion of thaw. Therefore in both wet and dry ecotypes, the completion of thaw and onset of spring coincided with an increase in pH in 2007, but a decrease in pH in 2008.

During the 2007 and 2008 measurement periods, pH values showed no relationship with MBC in either wet sites (2007: n =43, r = -0.04, p = 0.80; 2008: n = 55, r = 0.04, p = 0.80) or dry sites (2007: n = 33, r = 0.274, p = 0.12; 2008: n = 36, r = 0.24, p = 0.16).

Soil redox potential

Redox measurements, like pH, were taken during the winter-spring transition periods of 2007 and 2008, but were also collected during the summers of 2004 and 2005 (Fig. 6.3). Redox levels were generally higher in the dry site than the wet site, where standing water during the snow-free season is usual. Summer measurements in 2004 and 2005 were done in June, shortly after the completion of thaw, and approximately one month later in July, during the height of the plant growing season. In the wet sites, no change was observed over this time in 2004 (ANOVA: $F_{1,16} = 0.26$, p = 0.62) while in 2005 mean June values were higher than those in July, but this difference was insignificant (ANOVA: $F_{1,16} = 3.88$, p = 0.067). In the dry sites, redox levels increased in 2004 between June and July (ANOVA; $F_{1,26} = 19.0$, $p < 0.001$), but the reverse trend was observed in 2005 ($F_{1,16} = 7.89$, p = 0.013).

Redox values in wet sites were higher during the late thaw in 2008 than in 2007 (ANOVA: $F_{1,32.16} = 6.12$, p = 0.019), but there was no difference between years in the dry sites (ANOVA: $F_{1,14} = 0.038$, p = 0.85). In the wet site, ROP declined between late thaw (levels above 0 mV) and spring (levels below -100 mV). The same decline did not occur in 2008, when ROP levels remained above 0 mV before, during, and after the late thaw. Similarly in dry sites, late thaw was followed by a drop in redox in 2007, but not in 2008 when ROP levels remained near 150 mV throughout the thaw-spring transitional period. Thus, as with the summer measurements, different patterns were also observed in different years during the winter-spring transition.

ROP did not correlate with MBC in the dry sites during the winter-spring transition of either 2007 (n = 33, r = 0.11, p = 0.55) or 2008 (n = 36, r = -0.11, p = 0.53). However, ROP was

negatively correlated with pH in these dry sites in both 2007 (n = 33, r = -0.39, p = 0.027) and 2008 (n = 36, r = -0.43, p = 0.0082). By contrast, in the wet sites, ROP and MBC were positively correlated in 2007, with both declining during the measurement period (n = 35, r = 0.65, p < 0.0001), although no relationship between ROP and MBC was detected in 2008 (n = 47, r = 0.10, p = 0.51). No relationship was observed between ROP and pH in the wet sites during the winter-spring transition in either 2007 (n = 35, r = -0.19, p = 0.27) or 2008 (n = 47, r = 0.24, p = 0.10).

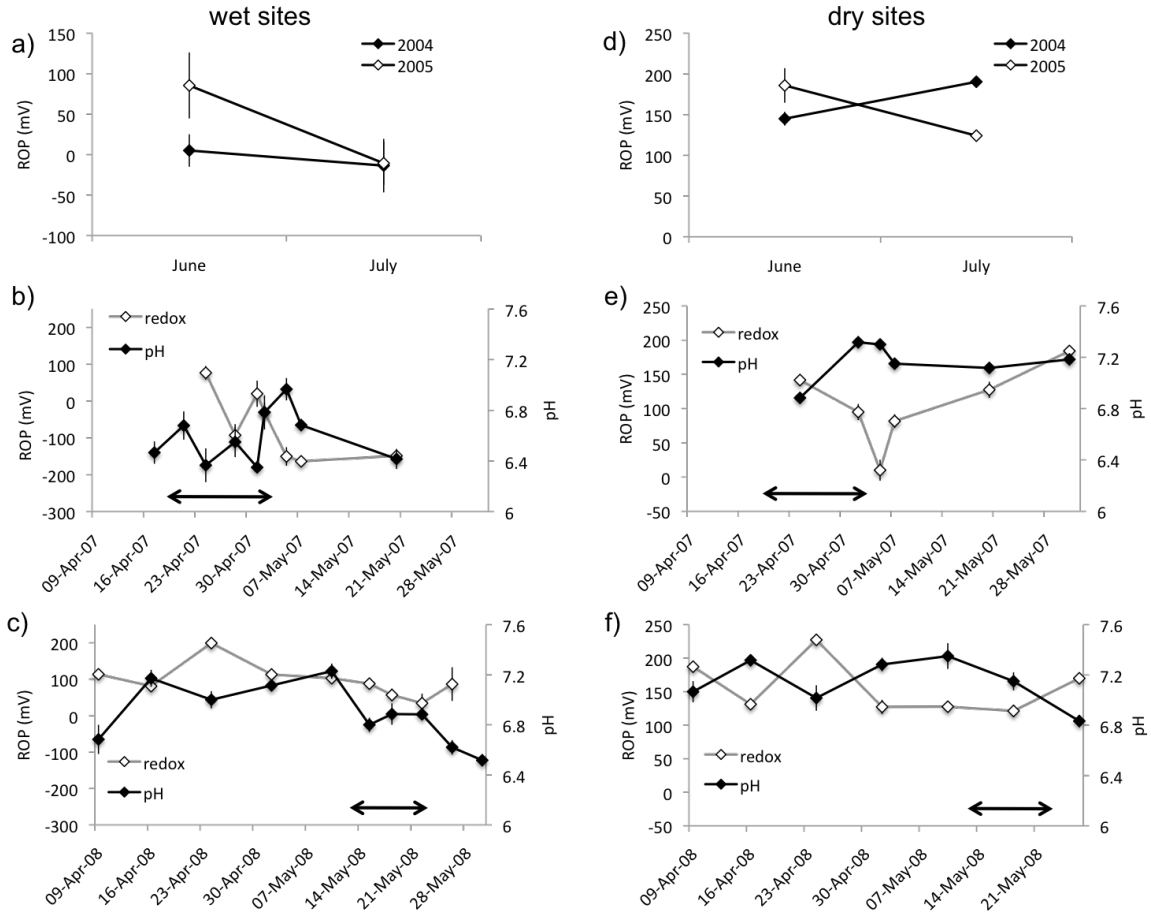

Fig. 6.3. Redox potential (ROP) and pH of soils sampled from wet [a), b), c)] and dry [d), e), f)] sedge meadows near Churchill, Manitoba. Summer ROP values from 2004 and 2005, and thaw and spring ROP and pH from 2007 and 2008 are shown. Means and standard errors are plotted (n ≥ 6, except 5 May 2007 when n = 3). Horizontal arrows identify the period of soil temperatures at 0 °C (ie. late thaw).

Soil water content and Bulk Density

Soil FW/DW and bulk density (BD) estimates were collected at all times of year between June 2004 and June 2008 (Figs. 6.4, 6.5). Wet site FW/DW values ranged between approximately 5 and 10, while dry site values were between 2 and 7 (Figs. 6.4a, 6.5a). FW/DW generally increased between summer and mid-winter and decreased during thaw. Bulk density in the peaty soils of the wet sites was typically less than half that of dry site soils (Figs. 6.4b, 6.5b). Both sites were extremely variable with respect to BD, with maximum and minumum values differing by a factor greater than 10 for the dry sites, and by almost 30 times in the wet sites. Bulk density was notably large in both sites during the summer of 2005, compared with other summer values. Soil BD tended to decrease during winter, particularly in 2008 when BD was large during early cold, decreased during early thaw, and then increased again at the end of soil thaw. Some of these seasonal changes are likely the consequence of comparing soils sampled in the frozen state with those sampled once the ground is thawed, the latter being susceptible to water loss during sampling and handling (see Chapter 7 for a discussion of this).

Regression analyses were done to examine trends over time during different seasons (Table 6.1). In wet sites, a slight downward trend was observed in peak levels between 2005 and 2008 but the only significant change over time occurred in summer, when a slight increase in FW/DW was detected, accompanied by a more pronounced decrease in BD (Table 6.1). A similar trend was observed in dry sites, where there were also modest decreases in FW/DW (and increases in BD) observed during both the cold season and the early thaw. Thus there is evidence that soils became drier in winter between 2004 and 2008 in the dry ecotype. This was not detected in the wet sites, probably because soils were saturated throughout these times. However I did observe a lowering of the water table during spring and summer over the course of monitoring these sites, indicating at least qualitatively that the wet sedge meadows also experienced a gradual drying.

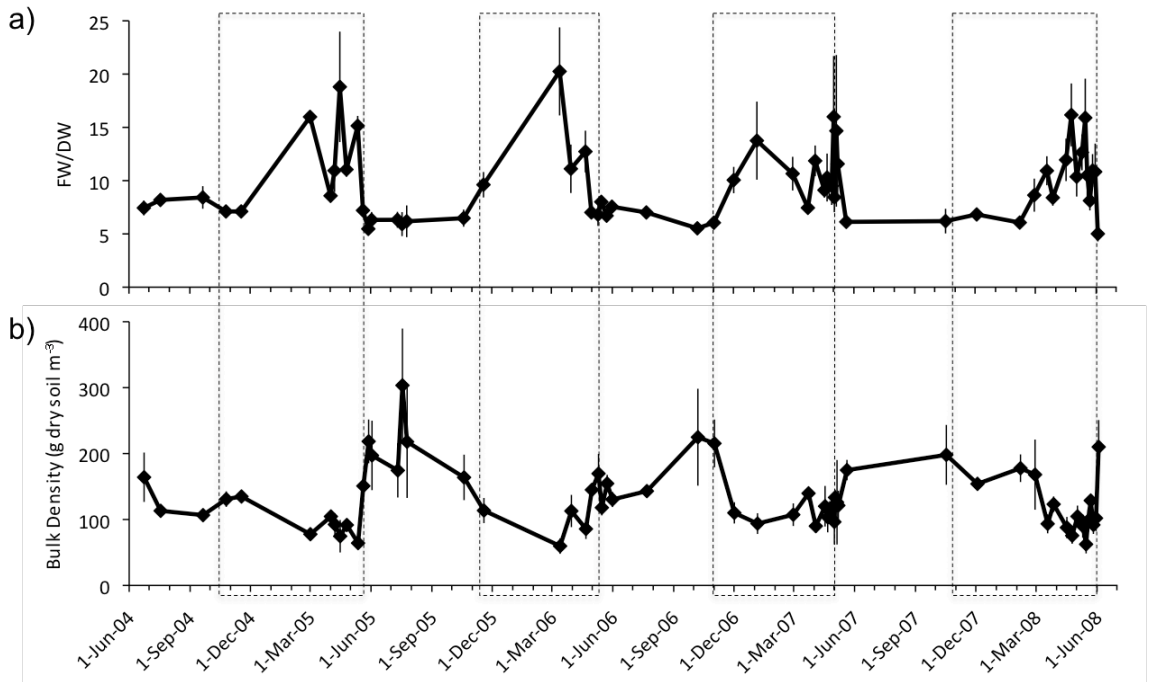

Fig. 6.4. Soil a) fresh weight/ dry weight (FW/DW) and b) bulk density of soil sampled from wet sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often n ≥ 6, but see text for deviations from this.

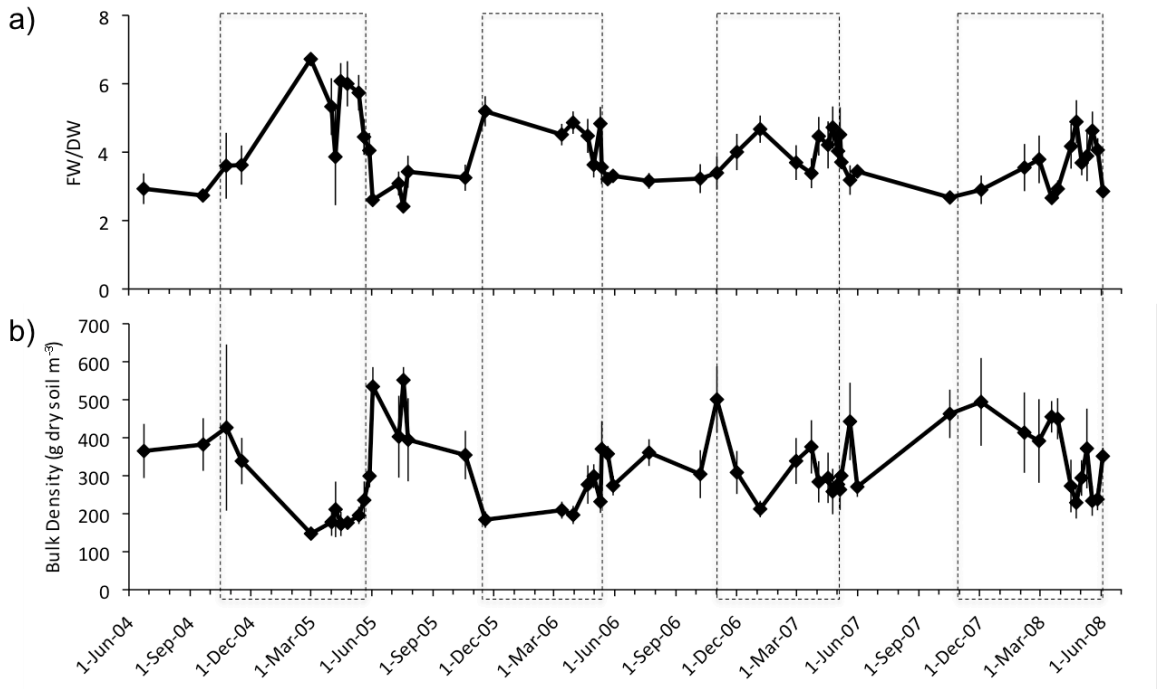

Fig. 6.5. Soil a) fresh weight/ dry weight (FW/DW) and b) bulk density of samples from dry sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often n ≥ 6, but see text for deviations from this.

Table 6.1. Results from regression analyses examining trends in fresh weight/ dry weight (FW/DW) and bulk density (BD) from soils collected between 2004 and 2008 in sedge meadows near Churchill, Manitoba. Four seasons were analyzed separately for the wet sedge and dry sedge ecotypes. Positive t-statistic results indicate a positive trend over time while negative values indicate a decline over the four years of study.

Ecotype	Season	variable	n	R^2_{adj}	t	df	P-value
Wet	Summer	FW/DW	99	0.08	2.40	1, 94.83	0.018 *
		BD	99	0.35	-2.40	1, 94.98	0.018 *
	Cold	FW/DW	98	0.04	-0.23	1, 94.1	0.82
		BD	98	0.06	0.76	1, 94.08	0.45
	Early thaw	FW/DW	46	0.12	-0.69	1, 42.27	0.49
		BD	46	0.10	0.37	1, 42.31	0.71
	Late thaw	FW/DW	80	-0.12	0.41	1, 70.51	0.68
		BD	80	-0.02	-0.78	1, 76.9	0.44
Dry	Summer	FW/DW	89	0.13	2.20	1, 85.17	0.030 *
		BD	89	0.15	-2.90	1, 85.07	0.0047 *
	Cold	FW/DW	98	0.14	-3.07	1, 94.03	0.0028 *
		BD	98	0.19	2.69	1, 94.01	0.0084 *
	Early thaw	FW/DW	51	0.25	-3.42	1, 47.39	0.0013 *
		BD	51	0.19	3.01	1, 47.51	0.0042 *
	Late thaw	FW/DW	48	-0.05	-0.9	1, 44	0.38
		BD	48	-0.08	0.98	1, 44	0.33

Total soil C, N

Soil C and N was estimated from samples between June 2004 and July 2005 (Fig. 6.6a,b). Total soil C was higher in the wet sites, averaging about 250 g kg^{-1} dry soil whereas in the dry sites soil C was generally near 150 g kg^{-1} dry soil. Soil N was also higher in wet sites, averaging about 43 g kg^{-1} dry soil, whereas measurements from dry sites neared 30 g kg^{-1} dry soil.

Soil C and N concentrations showed small changes with season. The pattern for soil C in both wet and dry ecotypes was characterized by a loss in soil C at the beginning of spring (Fig. 6.6a,b). In dry sites, but not in wet sites, this decline was preceded by an increase in soil C during

the cold season. Total soil N declined during early thaw in wet sites and then increased during late thaw. Soil N also fluctuated during this time of year in dry sites, and then declined with total C at the beginning of spring.

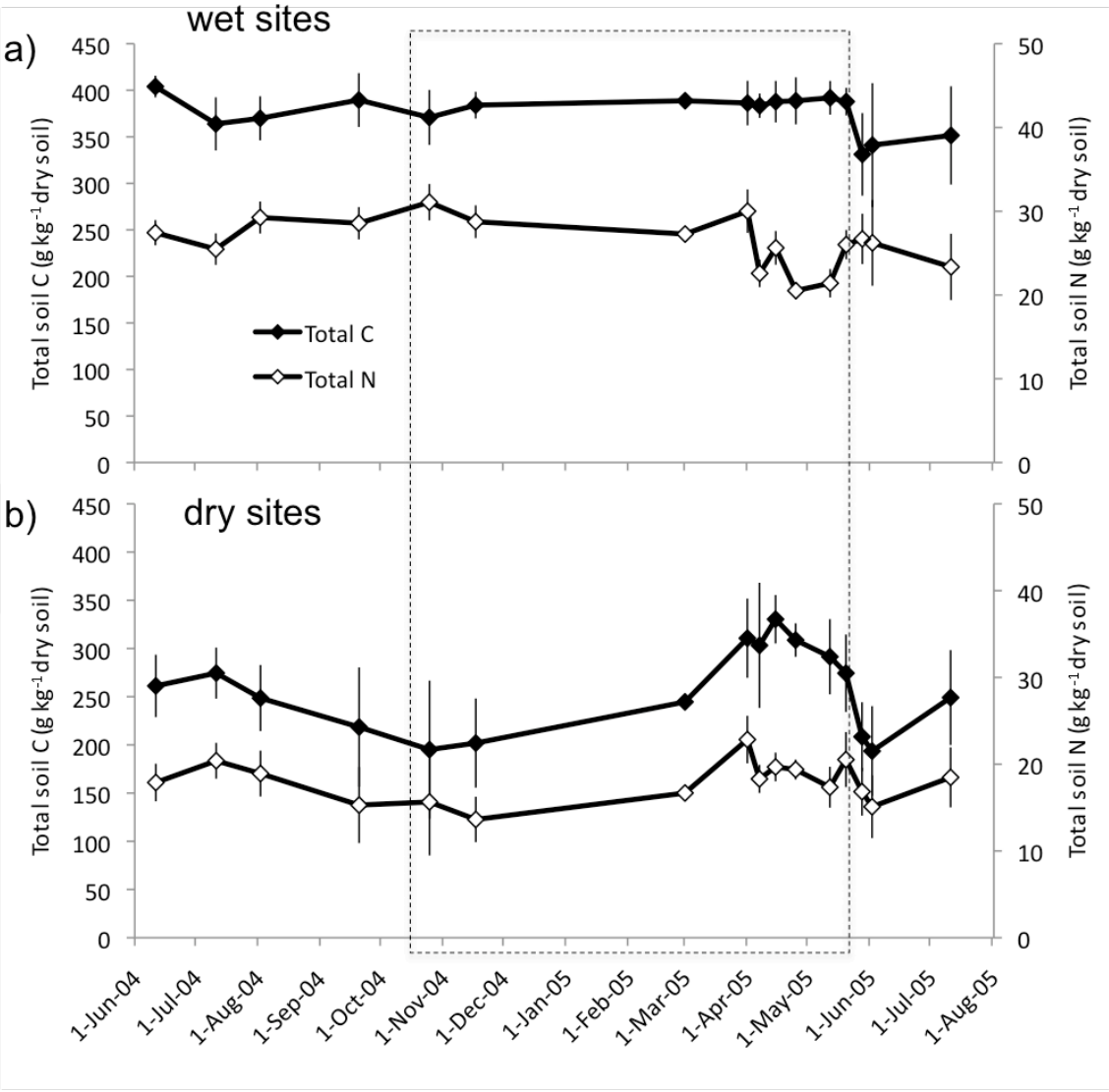

Fig. 6.6. Soil C (closed diamonds) and N (open diamonds) from a) wet sedge and b) dry sedge soils sampled between June 2004 and July 2005. The dashed box indicates the period during which soils were frozen in wet sedge sites (see Fig. 6.1). Means and standard errors are shown. Replication is most often n ≥ 6, but see text for deviations from this.

Microbial Biomass

Microbial biomass carbon (MBC) generally followed a consistent annual pattern, with peaks occurring in late winter and declines observed during thaw (Fig. 6.7a, Fig. 6.8a). This pattern is particularly evident in wet meadows where MBC was up to 5 times higher in winter than in summer (Fig. 6.7a) while winter levels were up to 3 times higher than summer levels in dry sites (Fig. 6.8a). MBC is highly variable in winter, with low values still measured on all sampling dates, but mean values elevated by replicates that are high in MBC (individual data points not shown). Outside of the cold season, large MBC values that were common during winter were never measured, excepting one sample in October 2004 in the dry sedge meadow.

At the onset of early thaw, when soil temperatures rose to -5 °C and above (typically at the beginning of April), a preliminary drop in MBC was observed in both wet and dry sites each year, excepting 2006 in the wet site (but the decline was detected at this time in MBN and MBP pools; Fig. 6.7b,c). MBC typically recovered after this event, and then declined shortly thereafter, during late thaw. In 2006 in the dry site, however, no such recovery was observed. MBC values usually reached annual lows in both ecotypes at the end of late thaw, when the soil warmed above 0 °C.

Microbial biomass nitrogen (MBN) generally followed a pattern very similar to MBC in wet sites (Fig. 6.7b). Winter values were higher than those measured at other times of year, and the highest peak MBN was observed in 2004. Winter variation was significant, with annual peaks occurring during early thaw in 2005 and 2008, and during the deep or late cold in 2006 and 2007. In dry sites, the pattern was somewhat different, and highly variable between years (Fig. 6.8b). MBN generally declined with MBC at the onset of late cold and during the completion of thaw, but in 2006, and to a lesser extent in the two years that followed, MBN subsequently increased at the beginning of spring. MBN was high throughout the growing season of 2006, and an overall decline was observed thereafter, between spring 2006 and fall 2007.

A general decrease in peak MBN levels was observed between 2004 and 2008, particularly in dry sites where, like MBC, seasonal changes are nearly undetectable during the winter-spring transition of 2008. Fall and winter MBN values were also high during the first two years of sampling in dry sites, and reduced during the latter two.

Microbial biomass phosphorus (MBP) closely resembled MBC and MBN patterns in the wet sites (Fig. 6.7c). The temporary decline at the onset of early thaw was particularly steep in 2004, and the decline in peak MBP over the four years was pronounced, with peak pool sizes reduced by about two-thirds in 2008 relative to 2005, and notably low values during the deep cold of 2008. In the dry sites, MBP was similar to MBN, with spring increases observed in 2006-2008, but not in 2005 (Fig. 6.8c). A general decline was observed over the four years of observations, with the highest overall values observed in 2005, not in 2006 as was the case for MBN.

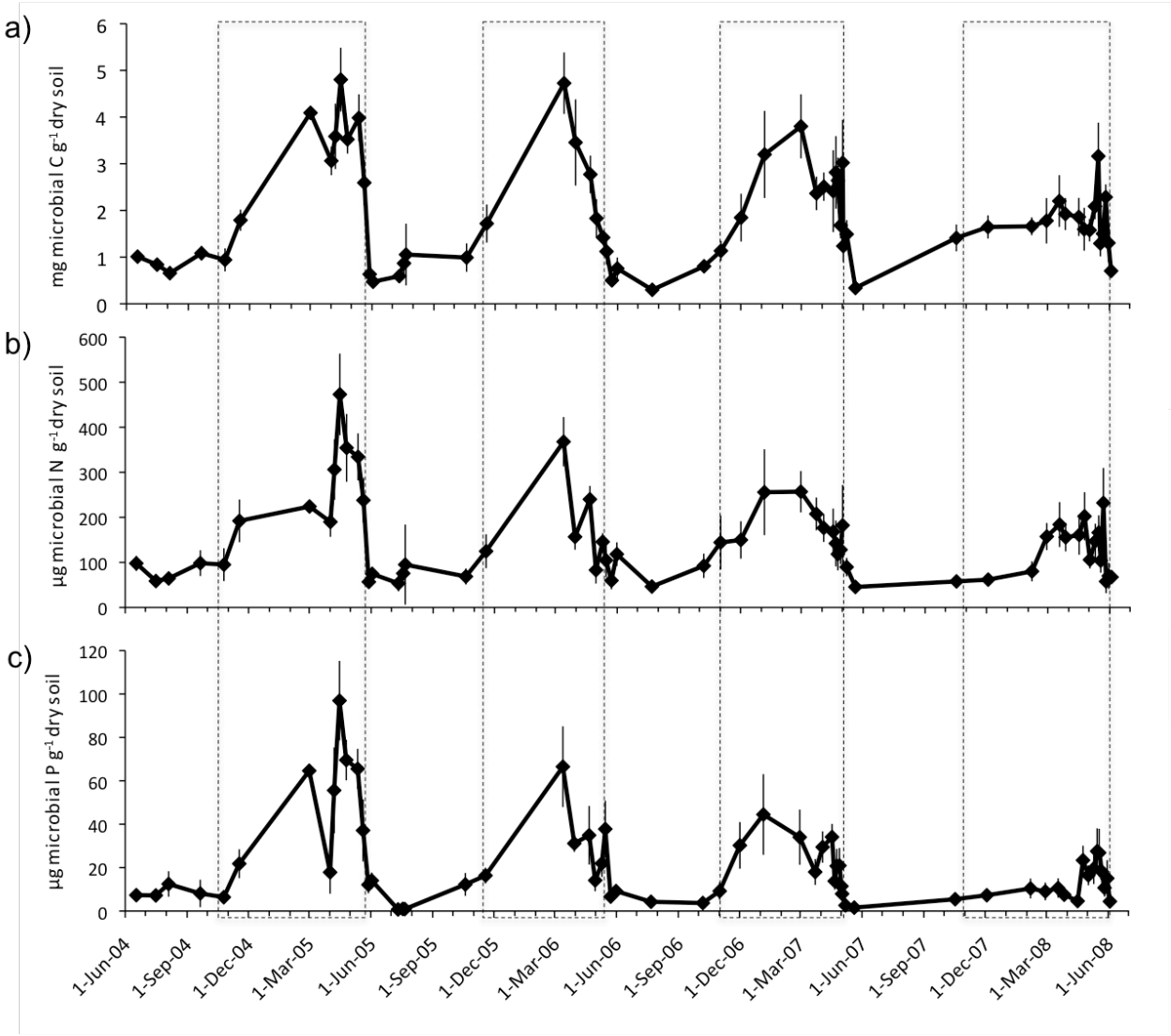

Fig. 6.7. Soil a) microbial biomass carbon, b) microbial biomass nitrogen, and c) microbial biomass phosphorus of samples collected from wet sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often n ≥ 6, but see text for deviations from this.

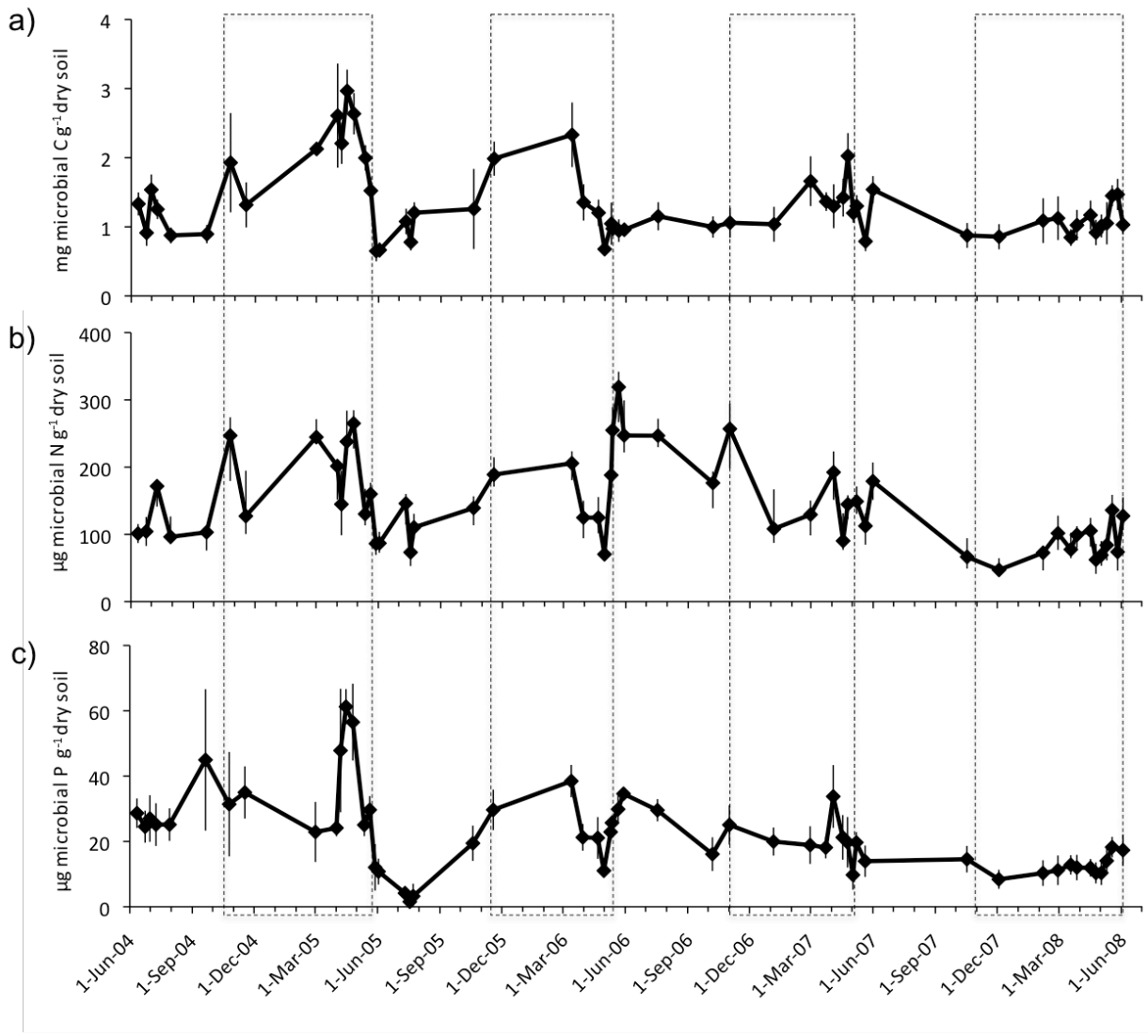

Fig. 6.8. Soil a) microbial biomass carbon, b) microbial biomass nitrogen, and c) microbial biomass phosphorus of samples collected from dry sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often n ≥ 6, but see text for deviations from this.

Nutrients

Soil organic C, N, and inorganic P were estimated from K_2SO_4 extracts, while N_i was estimated from KCl extracts. Patterns of extractable C in the wet site closely resembled MBC, with large pools in winter, brief decreases at the onset of early thaw (including in 2006 when an MBC decrease was not apparent), and further decreases during late thaw (Fig. 6.9a). In dry sites, extractable C patterns were also similar to MBC, with some minor differences, but none consistent between years (Fig. 6.10a).

Extractable N patterns were consistent with MBN patterns in the wet site (Fig. 6.9b). Extractable N increased in spring 2006 and was relatively high during the following summer and fall. Winter 2007 and 2008 peaks were lower than the two years previous. In dry sites, extractable N increased during the winter of 2005 and decreased during the thaw period, similar to MBN and MBC patterns (Fig. 6.10b). However 2006 was different, with large organic N pools measured between June and November 2006, at a time when MBN was also notably high. Extractable N pools decreased thereafter and remained relatively small with a modest increase observed during the late cold of 2008.

Extractable P patterns followed those of MBP in the wet site with a few exceptions (Fig. 6.9c). In winter 2005, extractable P pools were not consistently large, as MBP pools were. In 2006, MBP recovered after the decrease observed at the onset of late cold, however extractable P values did not recover after the same period of decline. As with other variables, peak 2007 and 2008 pools of extractable P were smaller than in previous years. In the dry site, extractable P patterns were similar to MBP and to extractable C patterns (Fig. 6.10c). Exceptionally large pools were measured in November 2004 and 3 April 2007, but otherwise extractable P generally was abundant in winter and decreased during the thaw period.

In wet sites, N_i peaked each year in winter, with some variation in the timing of the peak observed between years (Fig. 6.11a). In 2005 and 2008 the largest N_i pools were observed after the onset of early thaw, while in 2007 peaks occurred both in the early cold season and after the onset of early thaw. In 2006 the largest amounts of N_i were measured during the deep cold, just prior to April. In contrast to other microbial and nutrient pools described so far, N_i did not decline over the 4 years of observation, with 2008 peaks being similar in magnitude to previous years.

In the dry sites, N_i peaked during early thaw in 2005, 2007 and 2008, but in 2006 the largest amounts were observed in November, and relatively small N_i pools were observed during the winter that followed (Fig. 6.12a). The largest summer values were observed during summer 2006, also when large MBN and extractable N pools were measured.

Inorganic N was dominated by NH_4^+, with NO_3^- making up between 3 % and 35 % of the total N_i in wet sites, and between approximately 5 % and 25 % of total N_i in dry sites (Figs. 11b, 12b). In both ecotypes, the proportion of NO_3^- increased during the early thaw period. This is especially

evident in wet sites, where the proportion of NO_3^- decreased from spring to the onset of early thaw, and then increased steeply until the completion of thaw.

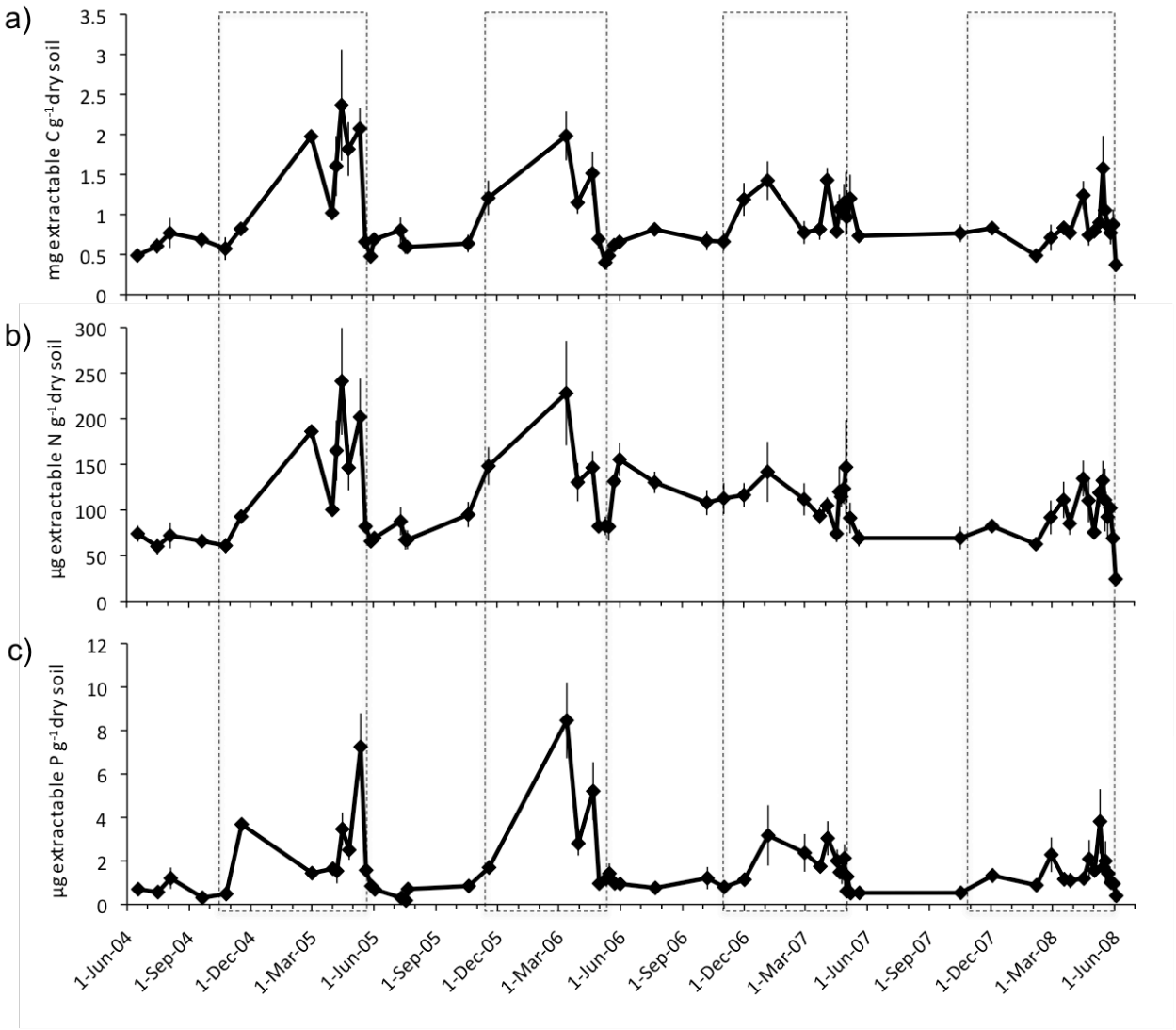

Fig. 6.9. Soil a) K_2SO_4-extractable carbon, b) K_2SO_4-extractable nitrogen, and c) K_2SO_4-extractable phosphate of samples collected from wet sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often $n \geq 6$, but see text for deviations from this.

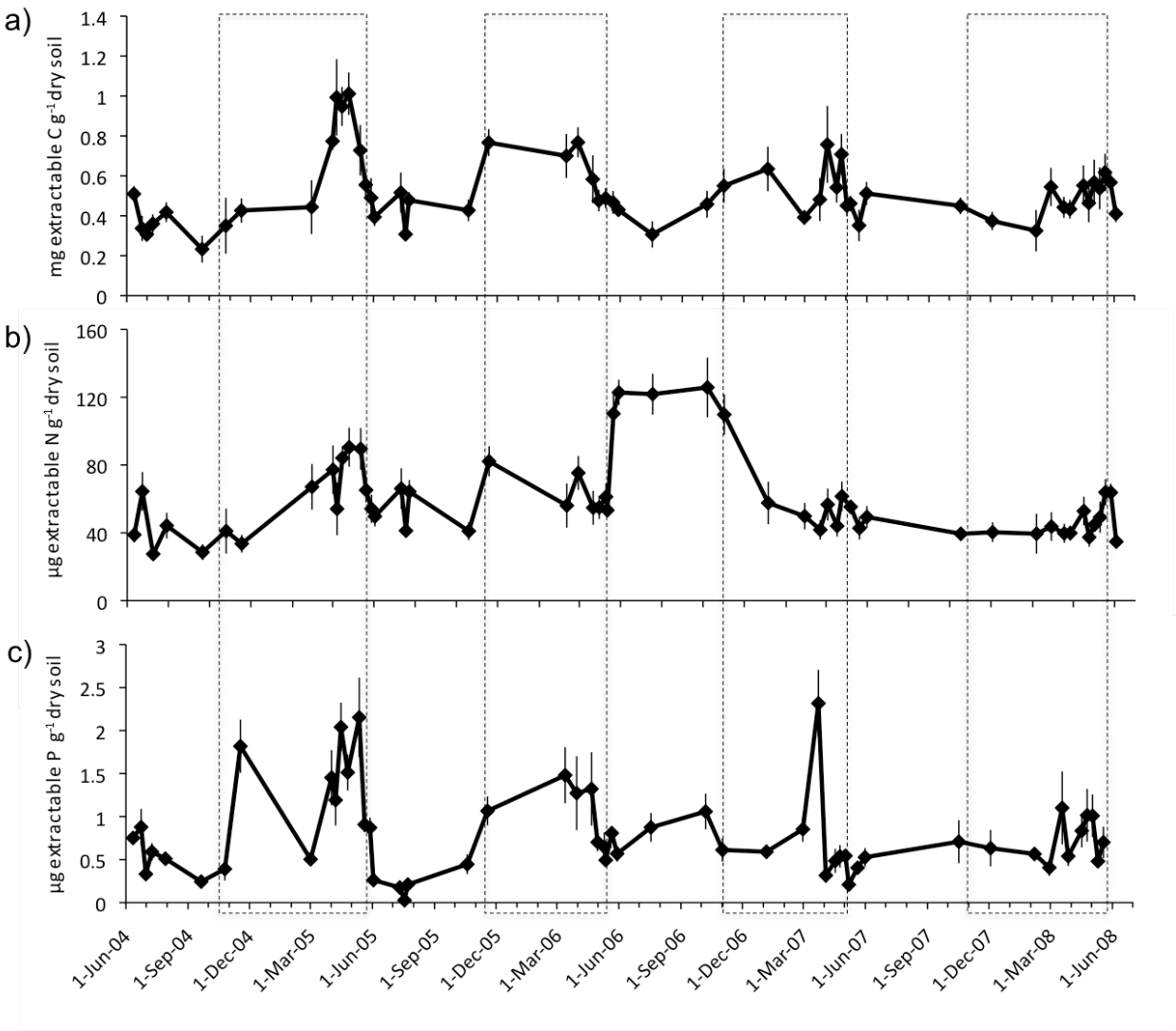

Fig. 6.10. Soil a) K_2SO_4-extractable carbon, b) K_2SO_4-extractable nitrogen, and c) K_2SO_4-extractable phosphate of samples collected from dry sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often $n \geq 6$, but see text for deviations from this.

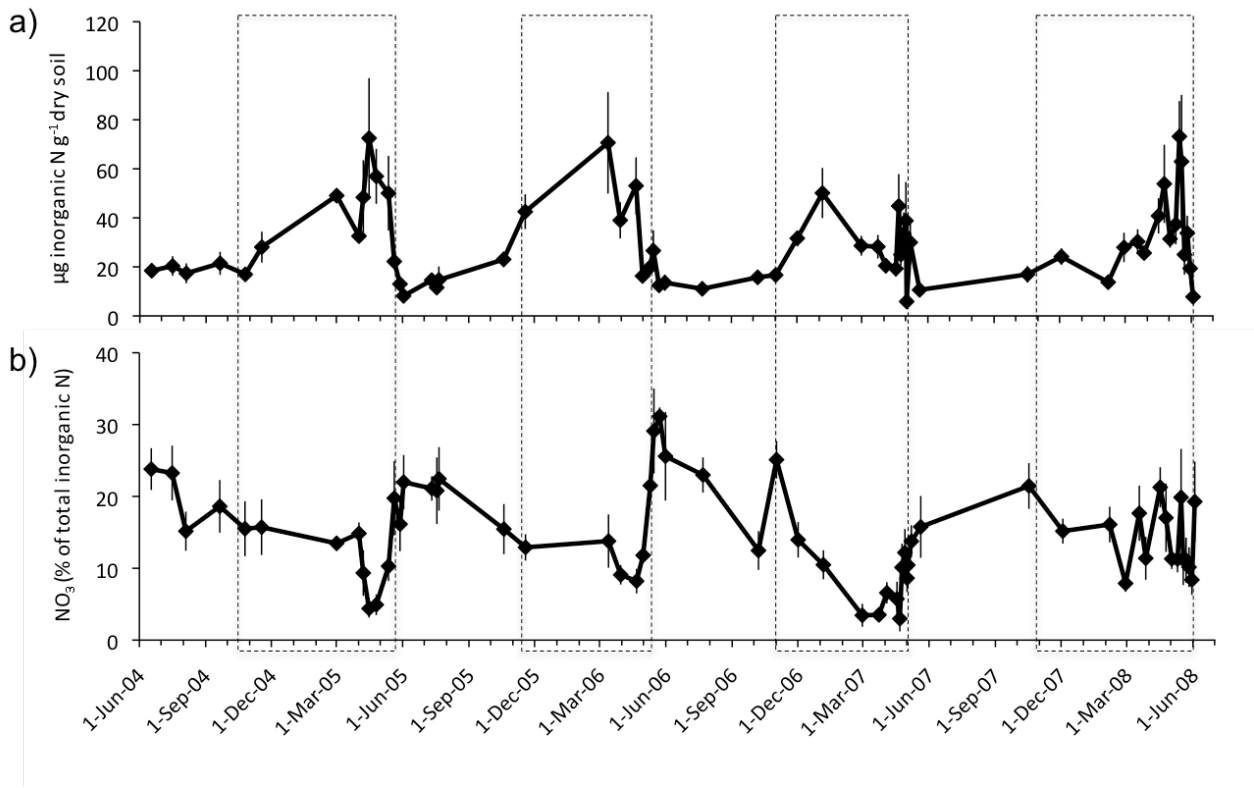

Fig. 6.11. Soil a) total inorganic N and b) percent NO_3 as proportion of total inorganic N of samples collected from wet sedge meadows between June 2004 and June 2008. Dashed boxes indicate the periods during which soils were frozen (see Fig. 6.1). Means and standard errors are shown. Replication is most often n ≥ 6, but see text for deviations from this.

Discussion

Microbial dynamics

The annual pattern presented here from low-Arctic sedge-dominated tundra sites features large pools of MBC, MBN, and MBP and soil nutrients in winter, followed by decreases during thaw and smaller pools maintained throughout summer. The winter-spring decrease of these variables had previously been reported for the wet sites (Chapter 2) but here I establish its repeatability at these sites over 4 years, and place it in the context of annual patterns. I also show that a similar pattern was observed in most years in drier sedge meadow sites, however the magnitude of microbial and nutrient fluctuations is lower and inter-annual variation is higher in the dry sites than in the wet sites.

Microbial biomass generally increased gradually during the early snow and early cold seasons, and these gains are probably supported by an increase in readily available substrates from fresh plant litter (Nemergut et al. 2005, Schmidt et al. 2007). Microbial cell metabolism slows down as temperatures cool to 0 °C and below (Mikan et al. 2002, Panikov and Sizova 2007), and rates of cell turnover decrease (Schmidt et al. 2007). There can also be shifts in the microbial community composition at this time (Nemergut et al. 2005, Schmidt et al. 2007). The observed increase in MB may also be accompanied by a shift in microbial growth rate efficiency, particularly if nutrient supplies are adequate, minimizing wasteful processes such as overflow metabolism and energy spilling (Russell and Cook 1995). Physiological and ecological controls on microbial growth in field situations are not well understood (Koch 1997) and the relationship between growth and activity, in relation to understanding the microbial functioning in seasonally cold and frozen soils, is an important area for further inquiry.

The phenology of early cold (freeze-up) is variable, with soil freezing quickly prior to snowfall in some years, while in other years high rainfall that saturates soils, and/or snow accumulation occurring prior to very cold air temperatures, can insulate soils and slow soil freezing (Fig. 6.1; L. Fishback, *pers. comm.*). Inter-annual differences in ROP estimates that were observed may depend to some extent on conditions at freeze-up and whether soils in winter are covered by a ground surface layer of ice, potentially accelerating the development of anoxic soil conditions. The response of the microbial community to different weather and hydrological regimes during early cold is not revealed by our data and requires further study. During freezing, soluble resources are excluded from the growing ice lenses, and microbes may benefit temporarily from an increased proximity to soluble nutrients within the narrowing liquid water films. Despite temperatures near 0 °C and increasing osmotic pressures as soil water freezes, conditions appear to be favorable for microbial growth, at least until the period of deep cold.

During the deep cold season, the wet site soils reached between -12 °C and -17 °C, depending on the year. At these temperatures, microbes are metabolically active and growth is possible (Panikov and Sizova 2007, Amato 2009) but probably at such a low rate, given both temperature and osmotic constraints, that there is minimal ecological significance (Panikov et al. 2006, Mikan et al. 2002). Still, this season lasts for many microbial generations, and is probably the most stable of all the seasons from the microbial perspective.

The thaw season is both physically and biologically dynamic and MB generally decreased during this period. The rate of thaw across the landscape, and even across sites, is not uniform, and the soil environment becomes a patchwork of microsites that progress through the stages of thaw at slightly different times. Additionally, active layer development throughout the depth of the rooting layer also proceeds variably across the landscape. Early thaw is usually triggered by air temperature maxima above zero for several days in succession, causing snowpack degradation, and an increase in soil temperatures. The development of a thick layer of ice on the ground surface was frequently observed at this time, as liquid water accumulated beneath the snowpack and was re-frozen by relatively cold ground surface temperatures. The onset of early thaw was also observed in the MB signal as a small but predictable decrease in biomass. As thaw progressed each year, the sharpest drop in MB coincided with the period of late thaw, when soil temperatures reached zero, and soil water melted. Microbial biomass pools were generally low by the time soil temperatures rose above zero, at the onset of spring.

Several factors could contribute to the observed MB reduction at thaw, and the apparent susceptibility of the soil microbes to warming temperatures generally. As soils approach 0 °C, significant physical changes occur in the soil. The rapid influx of liquid water during late thaw may cause reductions in the osmotic concentration of the microbial environment that are severe enough to be lethal to microbes (Chapter 5). Increased liquid water also disperses resources in the soil physical environment, which could limit the accessibility of these resources to microbes. In 2005 the nutrient decline appeared to precede the MB decline, suggesting that resource shortages may play a role in the MB crash, but this was not evident in subsequent years. Buckeridge and Grogan (2008) observed experimentally that MB at thaw was limited by C availability in a mesic tussock tundra site. However, resource addition experiments that I conducted during the thaw period in the same wet sites studied here failed to demonstrate that microbial declines were the result of resource shortages at these sites (Chapter 4).

Redox measurements taken while soils are frozen may overestimate *in situ* conditions because soils have to be thawed completely in order to take measurements, and this may allow oxygen to infiltrate even water-saturated samples; however the observed trends are still reflective of *in situ* changes in the soil environment. Observations of pH and redox levels did not reveal clear insights into the role that these variables may play in driving microbial changes, due to inconsistent patterns between years. I observed that redox declined and pH increased just after

the completion of thaw in both ecotypes in 2007 but the same was not observed in 2008. The 2007 result is presumed to be the effect of oxygen being utilized but not replenished in flooded sites, but given the 2008 result this situation is apparently variable depending on annual conditions. In the wet sites in 2007, MBC was positively correlated with ROP, indicating that at least during early thaw, microbial growth may be favored by relatively aerobic conditions. I also observed a proportional increase in NO_3, relative to NH_4, during the early thaw period, which could reflect increasingly aerobic conditions at this time, supporting higher rates of nitrification and NO_3 accumulation. Subsequent ROP declines and increased anoxia during and after late thaw may contribute to the MB decrease, at least in wet sites.

Another potential disturbance with warming temperatures comes with cracking of the ground and soil ice, which not only allows liquid water and oxygen to infiltrate, but also presumably allows the upward movement of gases that may have accumulated beneath the ground ice over winter. These could include potentially toxic metabolic products particularly from deeper anaerobic soils, such as hydrogen sulfide. Sufficient quantities could be released to cause localized cell death to microbes living in the upper soil layers, but this has not been investigated as far as I am aware.

The similarity between MB patterns in wet and dry ecotypes reveals the importance of climate and weather in driving some of these patterns across the landscape. The differences observed between the sites, however, demonstrate the spatial variability of the general pattern, and provides insights about landscape features that may contribute to these site-specific differences. The lower magnitude of MB fluctuations in dry sites relative to wet sites is believed to be the result of lower water content and shallower organic soils. Plant productivity is relatively low and pools of labile resources are smaller, limiting the potential magnitudes of microbial pool fluctuations. Dry conditions also may be particularly stressful to microbial populations during winter, when substantial frozen water could be lost through sublimation or concentrated locally in features such as ice lenses. These conditions lead to freeze-induced dehydration and more severe cold conditions relative to wetter sites, due to reduced insulation by soil water.

Nutrient dynamics

Nutrient dynamics were generally synchronous with microbial pool fluctuations in both wet and dry ecotypes. Large pools that accumulated in winter are presumably the result of microbial

degradation of plant and microbial necromass (Hobbie and Chapin 1996, Schimel and Mikan 2005, Schmidt et al. 2007). Once soils are frozen, leaching of mineral and organic nutrients and organic C ceases, creating conditions for accumulation of decomposition products. Nutrient resources may increase in availability at the onset of plant senescence, and with fall freeze-thaw events (Joseph and Henry, 2008), but once these are exhausted microbes are likely turn to more recalcitrant resources (Loya et al. 2004).

I observed a spring increase in organic N in both wet and dry ecotypes during the spring of 2006, but not in other years. This was pronounced in dry sites, where MBN increased sharply at the end of late thaw, and shortly after it began to decline, an increase in organic N was detected. An increase of organic N was observed at the same time in the wet sites, shortly after a modest increase in MBN. Nutrient pulses during and after thaw have been previously linked to declining MB pools, when the increase in nutrients was presumed to result from the emptying of lysed microbial cells (Schmidt and Lipson 2004). Microbial cells could also be the source of the organic N that we observed at the onset of the 2006 spring. This may have occurred only in 2006, and to greater degree in the dry sites than the wet sites, because of exceptionally dry soil conditions during the thaw of 2006 (K. Edwards, E. Horrigan, *pers. obs.*). The relatively dry conditions at thaw (ie. damp ground but not flooded) would minimize the amount of soluble N lost through leaching, leading to increased nutrient retention in the soil. It is also may have enabled rapid recovery of the microbial populations after declines during thaw, due to warm, moist, and aerated conditions. Alternatively, the elevated levels of organic N in 2006 may have come from a different source altogether, for example the breakdown of cyanobacteria that grew the previous summer. Algal mats and N-fixing *Nostoc sp.* colonies are both observed at the study sites, and inter-annual variability in the productivity of these organisms is likely considerable, but little is known about the frequency of biomass blooms, or the potential for pulsed nutrient input when these organisms decompose.

The period of declining MB and nutrient pools, whether or not this is followed subsequently by a nutrient increase, indicates a large loss from measured C, N, and P pools at thaw. The fate of these nutrients is largely unknown, but plant root uptake, leaching, and gas efflux all probably play a role. Plant roots are able to take up N_i during thaw (Chapter 3) and are also known to utilize organic N in Arctic sites (Henry and Jefferies 2003, Schimel and Bennett 2004). Plant roots are also a likely sink for P during the thaw and early spring, as has been demonstrated in

Arctic soils near 0 °C during fall (Chapin and Bloom 1976). Non-vascular plants including bryophytes and algae may also be nutrient sinks during thaw (Kotanen 2002).

With snowmelt and generally flooded conditions occurring during thaw, there is potential for significant nutrient transport into downstream aquatic systems, either through surface flow or by downward leaching and horizontal flow across the freezing front. Efflux of CO_2, CH_4, and N_2O gases are also likely. I have measured increasing CO_2 production during the thawing of the wet site soils through laboratory incubations (Chapter 4). Several studies have documented net CO_2 efflux during late winter and thaw in Arctic tundra sites (Jones et al. 1999, Nordstroem et al. 2001, Elberling et al. 2003, Lafleur and Humphreys 2007, Elberling et al. 2008, Sullivan et al. 2008). Significant N_2O efflux has also been reported during wintertime (including during snowmelt) from alpine tundra (Brooks et al. 1997) and during soil thaw at an Inner Mongolian steppe site (Holst et al. 2008). I observed that the proportion of total N_i comprised of NO_3 decreased during fall and winter and increased sharply during the thaw. Factors that could contribute to a higher proportion of NO_3 in the total N_i, include increased pools of CO_2 and carbonate substrates and increased activity rates of nitrifying bacteria driven by warming temperatures. The relative abundance of NO_3 suggests that conditions are not highly anaerobic, as indicated by ROP measurements, and this temporary abundance of NO_3 may increase potential N-losses through both denitrification and leaching.

Another pattern common to both ecotypes was a progressive decline in peak annual MBC and especially MBN and MBP pools over the 4 years of study. Causes of this are speculative, but a gradual drying of the landscape that was observed over the 2004-2008 time period may have played a significant role. The spring and summer of 2004 were very wet, relative to the growing seasons that followed, and the winter of 2008 was particularly dry, with soil FW/DW remaining low throughout fall and winter until the early thaw rise in temperature. Dry winter conditions could result from low precipitation volumes in the fall, and also increased fall evaporation and winter sublimation rates. The decline in winter FW/DW is particularly evident in the dry sites, but a lowering of the water table in wet sites was also observed. This lowered water table in the wet sites would likely have impacts on the microbial community composition and functioning, as a result of increased oxidation in surface soils (Blodau et al. 2004). I would expect decomposition rates in summer to increase with drying (Allison 2006, Vitt et al. 2009) but processes in winter are likely to respond differently. Growth-limiting dehydration of microbial

cells in winter may prevent the build-up of large MB pools in wet sites as they become increasingly dry, as was discussed above in relation to the difference between wet and dry ecotypes.

Whatever the cause, smaller MBN and MBP pools at thaw and smaller organic resource pools could eventually reduce the mineral resource supply for spring and summer productivity of microbial and plant communities, leading to reduced net primary productivity. If these trends are due to hydrologic changes, they may be at least partially reversible during exceptionally wet or warm years. How these effects will interact with climate driven changes, such as increased winter precipitation and warming events, is an important area for future study.

Fluctuations in soil water content, as measured by FW/DW estimates, do not only reveal changing conditions, but also have direct effects on estimates that are calculated on a per mass dry soil basis, as are these data. In other words, increases in C, N, and P pool sizes are amplified with increasing FW/DW values. This also means that the higher magnitude of MB changes in the wet site versus the dry site is at least in part due to higher magnitude fluctuations in FW/DW values. If the microbial and nutrient pool data are expressed on a per volume basis, or simply per wet mass, seasonal patterns are somewhat different than those expressed per dry mass. In particular, fall and early cold pool sizes are more comparable with late cold than with summer estimates (data not shown). However, the predominant patterns that I have discussed do not change when the data are expressed differently. I have chosen to display these data on a per dry mass basis in order to maximize precision, which suffers when estimates are expressed by volume, due to the difficulty of getting accurate BD measurements from these wet peaty soils. The error associated with these measurements tends to create substantial noise particularly within N and P patterns, although C pools are large enough to be more robust to the variable BD estimates. Nevertheless, I acknowledge that estimates by volume are essential for incorporating into large-scale ecosystem and global system modeling, and accurate measurements of BD need to be developed and employed for scaling purposes, despite the difficulties presented by working with both frozen and thawed samples in waterlogged organic soils.

I have reported here a seasonal pattern of soil microbial and nutrient dynamics that is generally similar between wet sedge and dry (mesic) sedge tundra sites. Inter-annual patterns that I observed are often common between the two ecotypes, revealing that weather events and patterns

are responsible for much of the year-to-year variation. The consistency of the timing of microbial and nutrient declines in relation to soil temperature patterns suggests that temperature, and its effect on the state changes of water, is the primary driver of the timing of these declines. The amount of water in the system appears to play a critical role, as winter microbial and nutrient pools were much smaller in the dry sites relative to wet sites, and the observed decline in peak winter pools over four years of observations correlates with a landscape-wide drying trend. The integration of inter-annual and intra-annual variation into a general understanding of ecosystem function and response to environmental change is a major challenge for Arctic ecosystem ecology and requires careful monitoring at all times of year to document current patterns and how they are changing. Together with targeted experimental work these studies provide important insights into the functioning of northern systems and their changing role in earth system processes.

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Chapter 7
General Discussion

The generalized annual cycle and suggested future directions

This project has provided the opportunity to study various aspects of the ecosystem ecology of two low-Arctic sedge meadow ecotypes. Work was focused on wet sedge meadows, which are particularly productive areas that share common features with peatlands across the circumpolar north. Monitoring the soil characteristics of a relatively dry ecotype also provided a useful comparison for investigating the generality of patterns across different ecotypes.

Chapters 2 and 6 described an annual pattern of soil microbial biomass and nutrient dynamics and explored the fidelity of this pattern over four years and between wet and mesic tundra sites. Chapter 3 demonstrated that nitrogen that is abundant in the soil in early spring is likely utilized by *Carex aquatilis*, which dominates the plant biomass in the wet sedge sites. In Chapter 4 a series of experiments were discussed that failed to support the hypothesis that resource shortages cause the decline in microbial biomass during soil thaw. Chapter 5 presented the possibility that abrupt osmotic shifts may cause microbial cell lysis during soil thaw, a process that is ultimately driven by increasing soil temperatures and the resultant change of bulk soil water from the solid to the liquid phase. Other contributing factors that could be implicated in the microbial decline are also discussed in Chapters 4 and 5.

This project highlights the importance of the winter-spring transition, as well as the fall and winter, as times of year that are critical, albeit understudied, with respect to biogeochemical cycling and plant-soil interactions. I repeatedly measured large microbial biomass pools in winter, indicating the capacity for soil organic matter (SOM) decomposition to be carried out by microbes during the winter. Further work is needed to characterize the composition and functioning of the microbial community in winter, and how it may change during the spring thaw. In wet tundra sites, the dominance of (non-mycorrhizal) graminoid species coupled with waterlogged conditions would be expected to favor Eubacteria and Archaea over Fungi, while at the dry sites the presence of shrubs and ericoid species (some of which are likely mycorrhizal) and the drier conditions, are likely to be more hospitable to fungi, with lower relative proportions

of Eubacteria and Archaea. Measurements of gas fluxes, changes to the composition of different SOM fractions, and ^{13}C tracing of substrates into microbial groups (eg. via combined ^{13}C-tracing and phospholipid fatty acid [PLFA] techniques) would all be informative as they would help to identify the decompositional processes being carried out at different times of year by particular microbial functional groups. I did not identify substrate or nutrient limitations (Chapter 4) but identifying the preferred substrates and growth limiting factors to the dominant microbial groups would also contribute to a more comprehensive understanding of this system.

Although I have described the annual cycle of microbial biomass and nutrient pools as a general rule, Chapter 6 reveals that this pattern is susceptible to change over a relatively short time. Thus, although I often discuss the data in this thesis as representing a general conceptual model, inter-annual differences were evident. I speculate in Chapter 6 that drying of the landscape may be the cause of the observed decline in winter peak biomass during the latter half of the 4-year project, but this is an untested idea, and the degree to which the pattern is directional, or merely the result of stochastic inter-annual variation, is unclear. If directional environmental change was behind the pattern, then the vegetation in these sites could change relatively quickly as a result. The lack of nutrient peak at the end of winter could lead to decreased growth rates of plants, and would favour species with mycorrhizal associations or other specialized nutrient foraging strategies. On the other hand, if the decrease in peak winter microbial biomass that I observed was due to non-directional environmental variability, we would not expect to see these shifts in plant productivity or species composition. In order to resolve the effects of environmental change on this time scale, seasonal and annual variability must also be integrated with decadal-scale monitoring.

In Chapter 6, I compared two ecotypes that differ with respect to hydrology, vegetation composition and productivity, and features of the soil organic layer. Although pool sizes and some patterns between the sites differed, the timing of microbial and nutrient fluctuations in relation to seasonal changes were similar. Comparable work in other locations is sparse, but research conducted near Daring Lake, NWT, has examined microbial and nutrient patterns primarily in mesic tussock tundra sites. Microbial and nutrient dynamics closely resemble those reported in Chapter 6, including the presence of late-winter microbial and nutrient peaks that decline at the time of soil water melt (K. Buckeridge, *pers. comm.*). Thus, this seasonal pattern may be widespread across the Arctic. Investigating the annual pattern across several Arctic and

northern boreal forest sites would help to further establish its generality, and meta-data provided by studies at multiple sites would provide further insights into the environmental and landscape controls on seasonal biogeochemical processes.

The period of freeze-up

A full understanding of ecosystem functions such as plant productivity or carbon balance depends on integrating the range of biogeochemical processes that occur at all times of the year. I have described and discussed the thaw season extensively throughout this thesis, but have not fully addressed the important elements of freeze-up within the context of the annual cycle.

Generally, when soil bulk water begins to freeze, soluble resources are concentrated in the films of liquid water inhabited by microbes (Chapter 5). This increased proximity to resources, combined with reduced leaching, reduced competition from plants, and inputs of fine root necromass from senescing plants, could lead to microbial growth in autumn. However, the sequence of events in relation to freeze-up can be variable, as shown by the temperature data presented in Chapter 6, in which soil froze over a period of 1 day to several weeks. Precipitation at this time can have dramatic consequences on the timing and rate of ground freezing. Rainfall that saturates the ground can increase the thermal buffering capacity of the soil, slowing the cooling and freezing process relative to air temperature declines and protecting the ground from diurnal freeze-thaw cycles. Snowpack development before the ground is frozen can also insulate the ground, slowing the rate of thaw and dampening diurnal temperature fluctuations in the soil.

The effect of these annual differences on microbial biomass and functioning are likely significant for annual litter and SOM decomposition, and for nutrient turnover and availability in spring. When freezing is delayed, the period between the onset of plant senescence and the time when soils drop below -5 °C can be many weeks longer than in years when soil freezes early in the fall. This would extend the time available for microbial breakdown of plant litter and SOM at relatively warm temperatures. Some plant species can compete for mineralized nutrients at least down to 0 °C (Shaver and Billings 1977) but as soils cool to -5 °C microbes probably dominate any nutrient uptake. Protracted freezing would increase the potential for freeze-thaw cycles, which could increase nitrogen leaching losses (Joseph and Henry 2008, Elliott and Henry 2009),

but would probably not have large impacts on the microbial biomass in wet tundra soils (Grogan et al. 2004, Henry et al. 2007).

It is unknown whether increased microbial activity during the fall, due to higher soil temperatures, could lead to higher microbial biomass and nutrient immobilization at this time, and/or higher rates of CO_2, CH_4, or N_2O efflux. Increased utilization of labile carbon and nitrogen sources in fall could also limit the capacity for microbial growth and nutrient immobilization later in the winter. This in turn would lead to dampened microbial pools and smaller nutrient inputs into soil when microbial cells lyse during thaw. Such a situation would favour plants that could take up nutrients during the fall, and also species that could utilize organic nitrogen sources and store nitrogen for long periods. Further studies are needed to address microbial and plant processes during fall in relation to climate and hydrological variation, and to connect these processes with resource cycling during other times of year.

Effects of increased snow-depth, early onset of spring, and mid-winter warming

Changes to the climate are resulting in Arctic winters that are increasingly shorter, warmer, and snowier than historical norms (IPCC 2007). Increased snowpack insulates the ground from air temperature fluctuations, so that winter soil temperatures are higher, and mid-winter warming events have a dampened warming effect compared with soils that have a shallower snowpack. Although air temperatures are rising earlier in the spring, some of this effect on seasonal soil warming may be attenuated by deepened snow. Snow may also protect plants from re-freezing damage following mid-winter warming events that are increasingly common and severe (Bokhorst et al. 2009). In very wet sites, the presence of ground surface ice, which frequently develops in late winter, can also insulate soils from cold air temperatures, and climatic conditions that mediate the production of this ice cover are also important to consider.

Increased snowpack has been shown to increase the magnitude of nutrient peaks during late thaw, presumeably due to higher soil temperatures and microbial activity rates (Buckeridge et al. 2009). Whether larger pools of available nutrients will have long-term impact on productivity depends on whether these nutrients are retained in the system by plant uptake and microbial re-immobilization, or whether they are lost through leaching and/or gas production (N_2O, NO_x). At least some Arctic plants will probably be able to take up nutrients as they become available

earlier in the year (Chapter 3). However, for some species, photoperiod exerts control over nutrient uptake, at least during fall. Shaver and Billings (1977) found that N uptake in the fall was regulated by daylength for *Eriophorum vaginatum* and *Dupontia fischeri*, but not for *Carex aquatilis*. Different species therefore may respond differently to earlier spring onset, with those that are able to take up nutrients early, and that are able to tolerate subsequent frost events, being favored.

Besides increasing late-winter nutrient pools, warmer winter soil temperatures are also likely to lead to higher rates of SOM decomposition, including degradation of recalcitrant C (Davidson and Janssens, 2006). If winter microbes immobilize this carbon, resulting in larger microbial biomass at the end of winter, turnover of this larger pool of cellular contents at thaw could support higher plant production and growing-season microbial populations. Some of this liberated C could also be transported into downstream aquatic systems through leaching, or released to the atmosphere as CO_2 and CH_4. The degree to which these C losses will be balanced by increased C sequestration by plants due to the longer growing season, is unknown, both in the short and long term (Chapin et al. 2009).

Warming events in mid-winter have already proven damaging to sub-Arctic plant species that suffer frost-damage during subsequent re-freezing events (Bokhorst et al. 2008, 2009). As well as damage to vegetative tissues, frost events can severely impact flower buds, curtailing the berry production and reproductive potential of some species (Bokhorst et al. 2008). In Chapter 6, I showed that warming events in winter are accompanied by moderate reductions in microbial biomass and nutrient pools. However, as these warming events become more severe and soil temperatures approach 0°C, carbon and nutrient fluxes at this time are likely to be significant. Microbial biomass declines are also likely to be more severe, potentially leading to leaching and gaseous carbon losses from the ecosystem. Plant roots may be able to capture and store nutrients that are liberated from microbial cells, but if above-ground plant growth is initiated by warmer conditions, these tissues could be damaged by subsequent frost events.

The interactions of winter warming, increased snow-pack, and potentially increased ice cover in late winter are not well understood, and are likely to vary considerably between years. The long-term impacts of these changing conditions on biogeochemical fluxes and plant production are also unknown; however, projects like this one provide insights into likely scenarios and

directions for further investigations that will reveal a more comprehensive understanding of the ecosystem consequences of these various climate forces.

Methodological challenges of frozen soils

Several challenges arose during this project related to sampling and processing frozen soils. Sampling frozen ground is made difficult by the snow and ice cover that mask important features of local sites. For example, sampling can occur without knowledge of the vegetation community or water saturation level of the ground. This can theoretically result in a wider variety of micro-sites being sampled during the winter than is normally selected during summer. Further, as staff at the Churchill research station often conducted winter sampling, I and the other samplers may have selected precise locations somewhat differently. This issue could have been minimized if suitable sampling areas were marked at the beginning of the project and sampling contained to these areas, and in the future this would be a valuable design element for year-round work. I do not believe that this had a significant impact on the data presented in Chapter 6, as samples that were very different from the rest (for example in terms of ratios of fresh weight: dry weight or vegetation cover) were removed from the dataset.

Sampling of frozen soils was done in two ways, either with an axe or with a permafrost drill. Advantages of the axe are that it is generally safer and cheaper and requires less training, and the number of samples that can be collected is only limited by time and space. The advantages of using the permafrost drill are that the area of disturbance is greatly reduced in comparison to the axe, and consistent soil volumes for experimental purposes can be sampled, including intact plants if desired. However, the drill depends on very sharp blades that dull quickly and are easily damaged, and had to be sharpened professionally, limiting the number of samples that can be collected at any one time. It is also difficult to drill when the ground is only partially frozen.

Experimenting with and processing frozen soil samples present the greatest challenges. Most experimental manipulations and soil analysis methods require that soil be thawed. In Chapter 3 cores were thawed before injection of ^{15}N, and in Chapter 4 cores were warmed before addition of nutrient addition solutions, in order that these solutions be applied and at least partially distributed in the soil. This necessary warming of experimental soils no doubt introduced confounding effects into the experiments, but it is very difficult to estimate the extent of

disturbances that could include increased microbial activity, redistribution of soluble compounds, and oxygen influx. Further methodological studies could be conducted to explore whether a faster or slower rate of thaw would minimize undesirable changes, and what the effects are of thawing different soils under sealed conditions versus being open to the air. It may also be possible to introduce experimental manipulations in other ways, such as using plugs of sponge or thawed soil into frozen cores, or partly submerging cores in solution rather than injecting known quantities of treatment solutions. In any case, employing new methods and testing of current methods should lead to improved ways of experimenting with frozen soils.

Processing frozen soils is made difficult by the inability to mix soils without thawing, and the necessity that soils be thawed in order for liquid extractions to be effective. Again, the consequences of this thawing are unknown, with carbon gas efflux being the only non-destructive measurement that I have measured during thaw (Chapter 4). I chose to limit mixing of soils in favour of minimal thawing before extractions, but other authors thaw soils so that homogenization of samples is possible (Buckeridge et al. 2008). The differences between these techniques has not been quantified, nor is it clear which one is most likely to accurately estimate *in situ* conditions.

A further difficulty when comparing soils that were variously sampled frozen and thawed is that bulk density and related measurements can be affected by the degree of frozenness during processing. When using thawed wetland soils, bulk density estimates suffer because excess water drains from soils as they are sampled and handled during processing. This is particularly relevant for the upper 5 cm of soil that tends to be poorly decomposed moss, and is less an issue for the more compacted soil at 10 cm and greater depths. Frozen soils retain their water, but are difficult to cut into regular shapes with easily measured volumes, and may contain sticks or macropores that are more difficult to work around or remove; thus, bulk density is difficult to measure accurately from both thawed and frozen samples. I would recommend in the future that one method is chosen and used year-round, perhaps depending on the type of soil being processed. For example, for very wet soils, more consistent and accurate estimates would result if bulk density cuboids were cut from all soils when they were frozen. For soils that hold less water, bulk density would be more accurate and consistent if done from thawed samples.

Conclusion

I have described an annual cycle of microbial biomass and nutrient pools in sedge meadows near Churchill, Manitoba, Canada that was reasonably consistent over four years in wet sedge sites, and apparent but less consistent over the same time period in a large mesic sedge site. Peak microbial biomass and nutrient pools occurred in late winter, followed by a sharp decline when soils thawed. The microbial biomass decline appears to be driven ultimately by the thawing of soil water, as subsidizing soils with added carbon, nitrogen, and phosphorus did not alter the timing or rate of microbial biomass decline. The dominant plant in this system, *Carex aquatilis*, is able to take up significant amounts of inorganic nitrogen during the spring thaw, at a time when nitrogen is abundant relative to the growing-season. The repeatability of the late-winter and early-spring microbial and nutrient dynamics suggests that perturbations to the system that interrupt this pattern are likely to have lasting ecosystem effects. Climate change presents the largest threat as it is likely to dramatically change soil temperature and moisture regimes, and will no doubt have consequences for carbon, nitrogen, and phosphorus fluxes, plant productivity, greenhouse gas production, and other ecosystem functions. Further monitoring and experimental work especially outside of the growing season are needed to better understand the intra- and inter- annual biogeochemistry of different northern ecosystems and how they will respond and feed back to the global climate system.

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