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Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra

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ABSTRACT

Microbial activity in the long arctic cold season is low but cumulatively important. In particular, the size of the microbial biomass and soil solution nutrient pool at the end of winter may control the quantity of nutrients available to plants in the following spring. Microbial starvation and lysis as a result of increasingly severe soluble carbon (C) shortages over winter has been hypothesized as a potential mechanism for microbial nutrient release at thaw. These C shortages may be exacerbated by the warmer temperatures and increased winter precipitation that are consistently predicted for a large part of the low Arctic. In particular, warmer soil temperatures due to deeper snow may increase wintertime microbial activity and organic matter decomposition over the winter, potentially resulting in enhanced nutrient availability to plants in the following growing season.

In this study, we investigated nutrient limitations to soil microbial growth and activity in late winter under ambient and experimentally deepened snow (~0.3 and 1 m respectively) in birch hummock tundra within the Canadian low Arctic. We hypothesized that the build-up of moderately deeper snow over winter would exacerbate soluble C-limitation to microbial growth and activity and increase soluble N accumulation, and thus stimulate the growth of bacteria relative to fungi. We measured the *in situ* response of the soil microbial biomass and soil soluble pools in control and snow-fenced plots at the end of winter, and then incubated soils from these plots with added C, nitrogen (N) and phosphorus (P) (at 0–15 °C) to characterize nutrient limitations to microbial growth and activity.

In late winter, deepened snow increased the microbial pool of N, yet decreased soil pools of dissolved organic N and C, and decreased bacterial counts. Fungal mass and hyphal lengths did not change, but remained dominant under both ambient and deepened snow. Deepened snow exacerbated the soluble C-limitation to microbial growth and reduced the P-limitation for microbial respiration. Fungal mass and hyphal length responses to nutrient addition were larger than the bacterial mass or abundance responses and fungi from under deepened snow responded more than those from under ambient snow, indicating a different potential structural and physiological response to substrate availability for these two soil microbial communities. Our results indicate that deeper snow may increase microbial nutrient pools and can alter the physiological functioning of the soil microbial community in late winter, suggesting that microbial N release and its availability to plants during spring thaw may be enhanced.

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1. Introduction

The influence of temporal variation in soil microbial activity and growth on annual N cycling is not well understood in seasonally snow-covered ecosystems (Lipson et al., 2000; Grogan and Jonasson, 2003; Weintraub and Schimel, 2005; Edwards et al., 2006; Buckeridge and Jefferies, 2007). Ongoing low levels of microbial decomposition of organic matter during the long, cold winters that typify tundra ecosystems may be an important contributor to the annual nitrogen (N) supply for plants (Lipson and Monson, 1998; Grogan and Jonasson, 2003). Several studies have documented a decline in the winter-adapted microbial biomass carbon (Brooks et al., 1998; Lipson et al., 2000; Grogan and Jonasson, 2003; Edwards et al., 2006) and microbial biomass nitrogen (Brooks et al., 1998; Edwards et al., 2006) across the spring thaw phase, suggesting that the nutrients released from the microbial cytoplasm *may* be released into the soil solution as a characteristic spring pulse. If release from the microbial pool is a principal mechanism providing the soluble nutrient flush in spring (Herrmann and Witter, 2002), then the composition, size and nutrient content (Schimel et al., 2007) of that winter microbial pool is clearly a critical control on the supply and fate of that flush. This nutrient flush, in turn, may be an important resource for plants and the surviving microbial community at thaw, as well as over the subsequent growing season (Schimel and Clein, 1996).

Several hypothetical mechanisms for microbial lysis and cytoplasmic release at the end of winter have been proposed. Freeze–thaw cycles in laboratory experiments lead to bacterial death and the release of cytoplasmic nutrients (Skogland et al., 1988). However, field and mesocosm studies suggest that soil freeze–thaw cycle regimes that are typical of many tundra environments may adversely affect only a relatively small proportion of the microbial biomass (Lipson and Monson, 1998; Lipson et al., 2000; Herrmann and Witter, 2002; Grogan et al., 2004). Nutrient pulses may also be the result of faunal predation of fungi and bacteria (Ingham et al., 1986), as soil macrofauna often proliferate during thaw (Sjursen et al., 2005). Alternatively, or additionally, the microbial community may be responding osmotically to sudden and enormous increases in soil water at spring thaw (Schimel et al., 2007). Finally, lab incubations with alpine tundra soils (Lipson et al., 2000; Brooks et al., 2004) have suggested that cytoplasmic release at thaw is primarily the result of microbial mortality due to increasingly extreme C-limitation toward the end of winter (Schmidt and Lipson, 2004). Microorganisms may remain active throughout most of the cold season in alpine systems that have deep snow accumulation (~2 m) and/or moderate winter soil temperatures. Under these circumstances, microbial C-limitation increases as inputs from plant litter are depleted, and the winter-adapted microbial community ultimately succumbs to warmer temperatures and C starvation during spring thaw (Lipson et al., 2000). Wintertime conditions in the Arctic may differ significantly from many alpine sites in that soil temperatures are often substantially lower in the Arctic (Brooks et al., 1998; Schimel et al., 2004), and spring thaw drainage is relatively restricted. Here, we focused on investigating the potential for the C-limitation hypothesis to explain microbial turnover and a spring nutrient flush in an arctic ecosystem. In particular, we were looking for evidence that late winter microbial growth in the Arctic is limited by available labile organic substrate.

Strong C-limitation on microbial growth and activity may have conflicting impacts on winter N mineralization and therefore soil N pools. Microbial immobilization of N for growth occurs when microbes are utilizing organic substrates with a relatively high C:N ratio (Weintraub and Schimel, 2003). Tundra plant communities and plant litter typically have high C:N ratios, reflecting the low N availability in these ecosystems (Shaver and Chapin, 1980). A seasonal change between summer net N immobilization and winter net N mineralization is typically recorded in tundra soils (Grogan and Jonasson, 2003; Schimel et al., 2004). Recent evidence suggests winter substrate use may be largely confined to microbial recycling of dead microbial cells and hyphae (Schimel and Mikan, 2005) or endogenous metabolism (the breakdown of living cell constituents/storage compounds for maintenance). If microbial enzymes are primarily decomposing microbial products in the cold season, providing inputs of C and N with a relatively low C:N to the soil solution, then even though the soluble N pool size does not change, the result should be net N mineralization, and a larger inorganic N pool in the soil by late winter (Schimel et al., 2004). Alternatively, intra-seasonal variation in labile substrate availability, as root exudates and plant litter with low C:N inputs decline over the winter months, may lead to initial high rates of net N mineralization in early winter, but net N immobilization by the late winter. Therefore, mobilized winter N may be directly available to plants in spring, or dependent upon controls over microbial survival at spring thaw.

Large increases in snow depth (to 3 m) increased soil temperature minima from −25 to −7 °C, increased respiration (up to 4×) and elevated late winter soil N pools (~3×) (Schimel et al., 2004), indicating that wintertime microbial decomposition of organic matter and mineralization is highly sensitive to soil temperature. However, the potential increases in winter precipitation predicted by climate change scenarios (ACIA, 2004), or snow build-up as a result of shrub-snow feedbacks (Sturm et al., 2005), suggest that relatively moderate increases in snow depth (~1 m) are much more likely. Moderate increases in snow depth may lead to more moderate increases in winter soil temperature. Recent research suggests that there may be a snow depth threshold (~1 m) below which there are strong insulating effects of increasing snow accumulation on soil temperature and above which effects of additional snow accumulation on soil temperature are relatively small (Grogan and Jonasson, 2006). Here, we investigate soil and microbial C and N pools under ambient and moderately deepened snow to determine if more realistic snow depth increases for arctic tundra in future winters will stimulate soil microbial activity and further increase late winter C-limitation.

Soil microbial community change between summer and winter may be a key control on annual patterns of nutrient cycling and plant N uptake in seasonally snow-covered ecosystems (Schmidt et al., 2007). A fungal community phylogenetic study of an alpine tundra soil revealed a diverse and novel soil fungal community in winter as compared to spring and summer, and a larger fungal: bacterial biomass

ratio in winter (Schadt et al., 2003). In contrast, an investigation of the soil microbial community in arctic tundra found that vegetation type was the main control on microbial community structure, with seasonal shifts in species composition only at fine taxonomic scales (Wallenstein et al., 2007). Soil bacterial and fungal groups include species that are psychrophiles (specialists with optima <10 °C, active at subzero temperatures and prevalent in stable, very cold environments) and psychrotolerants (generalists with optima <20 °C, active at subzero temperatures and prevalent in unstable, very cold environments (Morita, 1975)). Little is known about the specific functions of either the specialists or the generalists. In an arctic winter, thin films of liquid water within frozen soils allow for microbial activity at subzero temperatures, at least until −12 °C (Rivkina et al., 2000; Robinson, 2001). Fungi may be favoured over bacteria in the decomposition of organic matter in cold, dry soil, since their mycelial growth habit may allow exploitation beyond individual microsites of liquid water, or across thin films of liquid water in frozen soils. Such differences may have profound effects on soil biochemical cycling during winter and the subsequent growing season, not least because fungi and bacteria often differ strongly in N concentrations, and probably in N storage capabilities (Klionsky et al., 1990; Pokarzhevskii et al., 2003; Schimel et al., 2007). However, to date we are not aware of any substantial studies documenting *relative* fungal and bacterial abundances in winter soils in the Arctic. For instance, if bacterial growth and activity are disproportionately constrained by winter conditions in the Arctic, warmer soils as a result of deeper snow may significantly increase the bacterial component of the active microbial community. Since cold-adapted bacteria may have different nutrient limitations than cold-adapted fungi, they may respond differently to changes in nutrient availability in late winter, and may differ in their susceptibility to the mechanisms that cause microbial turnover and nutrient release at thaw. Thus, changes in wintertime fungal to bacterial ratios could substantially alter annual patterns of tundra ecosystem nitrogen cycling.

In this study, we measured *in situ* late winter soil and microbial nutrient pools, and bacterial and fungal mass and abundances, in soils from ambient and experimentally deepened snow plots. Our objective was to investigate if moderate increases in snow depth, which might realistically be expected as part of interannual variation or climate change, would increase soil biochemical activity over winter, enhancing microbial growth and N accumulation. Secondly, we incubated these soils with factorial combinations of C, N and P additions to determine the effect of deepened winter snow cover on microbial nutrient limitations to activity and growth (net biomass production) during the early spring thaw phase. Finally, we quantified the bacteria and fungi in these soils and investigated the impact of deepened snow on their relative abundances. Specifically, we tested the following hypotheses for a birch hummock tundra system in the Canadian low Arctic:

(1) labile C availability limits the growth and activity of soil microorganisms at the end of winter, and deeper snow exacerbates this limitation;
(2) moderate increases in snow depth enhance soil extractable N pools in late winter;
(3) fungi dominate the soil microbial community mass in late winter; and
(4) bacterial abundances increase under deeper snow.

2. Methods

2.1. Site description

This study was conducted in the late winter (mid-May) of 2005 in a mesic birch hummock ecosystem at the Tundra Ecological Research Station (TERS) at Daring Lake, Northwest Territories, Canada (64°52′N, 111°34′W). Daring Lake is located 300 km northeast of Yellowknife, in the Coppermine River watershed. The area is an important habitat for the Bathurst Caribou herd (*Rangifer tarandus*), which migrates through the local area in large numbers (~1000 s) in the late winter (May–June) and fall (August–September).

The region is underlain by continuous permafrost and is characterized by frequent eskers, boulder fields and Canadian Shield outcrops across the landscape (Rampton, 2000), and a mosaic of vegetation types, whose distribution are determined primarily by topographic effects on hydrology (Walker, 2000). This experiment was located within birch hummock tundra vegetation, common to circumpolar ''Low Arctic'' regions (*sensu*, Bliss and Matveyeva, 1992), where dwarf birch (*Betula glandulosa* (Michx.)) is frequent in both hummocks dominated by *Eriophorum vaginatum* L. and in hollows alongside *Ledum decumbens* (Ait.), *Vaccinium vitis-idaea* L., *Vaccinium uliginosum* L., *Andromeda polifolia* L., and *Carex* spp. A well-developed moss (largely *Sphagnum* spp. and *Aulacomnium turgidum* (Wahlenb.)) and lichen layer occurs in both the hummocks and hollows (Nobrega and Grogan, 2007). The soils in this ecosystem are Histosolic Turbic Cryosols (Soil Classification Working Group, 1998), and roots in this ecosystem are generally confined to the surface Ah-horizon (2–20 cm) but may penetrate the mineral (sandy silt) C-horizon. The total carbon, nitrogen and sulfur contents of the organic horizon are approximately 40, 1.5 and 0.2% of total soil dry weight, respectively (Nobrega and Grogan, 2007).

Climate records from the Daring Lake weather station (1996–2006; Bob Reid, Indian and Northern Affairs Canada, unpublished data) indicate mean daily air temperatures as low as −40 °C in winter and up to 22 °C in summer, with a mean period without snow on the ground of 147 days (June to mid-October). The timing of snow accumulation, soil freeze, and thaw varies interannually. Snow often does not accumulate above 10 cm until the beginning of November, by which time soil temperatures have dropped below 0 °C. The mean monthly snow depth increases from November to April/May, reaching a mean peak of 37 cm, with large intra- and interannual variability in snow depth (10 year mean: maximum peak = 59 cm; minimum peak = 20 cm). Furthermore, there is large interannual variability in the date of complete snow thaw, ranging from early May to early June. Soils undergo freeze–thaw cycles in response to air temperature fluctuations during and after the final stages of snow thaw. Soil thaw to the C-horizon typically follows 1–2 weeks after snow thaw.

2.2. Experimental treatment

A snow experimental treatment was established in the previous summer to increase the depth and duration of snow cover within birch hummock tundra vegetation. The snow fence (1.2 m tall and 15 m long) was constructed on a gentle slope perpendicular to the prevailing winter wind direction (from the north east) using a double layer of diamond mesh construction barrier fence (Quest Plastics DM 1004 X, Mississauga, Ontario) secured by steel T-posts spaced every 3 m. Fence porosity was approximately 50%. A control site (unfenced, 15 m long) was established parallel to the fence in similar vegetation and offset by more than 30 m to ensure clear separation from the snow fence drift area.

Soil temperature over the winter of 2004–2005 was measured every 6 h in hummocks at ~2 cm depth ($n = 2$ each) at an ambient snow depth site using copper-constantan construction thermocouples (T type, OMEGA, Stamford, CT) and CR10 data loggers (Campbell Scientific, Logan, UT). Unfortunately, the data logger failed at the snow fence site over the winter of 2004–2005, but we collected soil temperature data in late winter and early spring 2005 at ~2 cm depth ($n = 1$) using a CR10X logger (Campbell Scientific). In 2005–2006 and 2006–2007 we increased the number of thermocouples and data loggers to better record the effects of deepened snow on soil temperature across the tundra (Fig. 1).

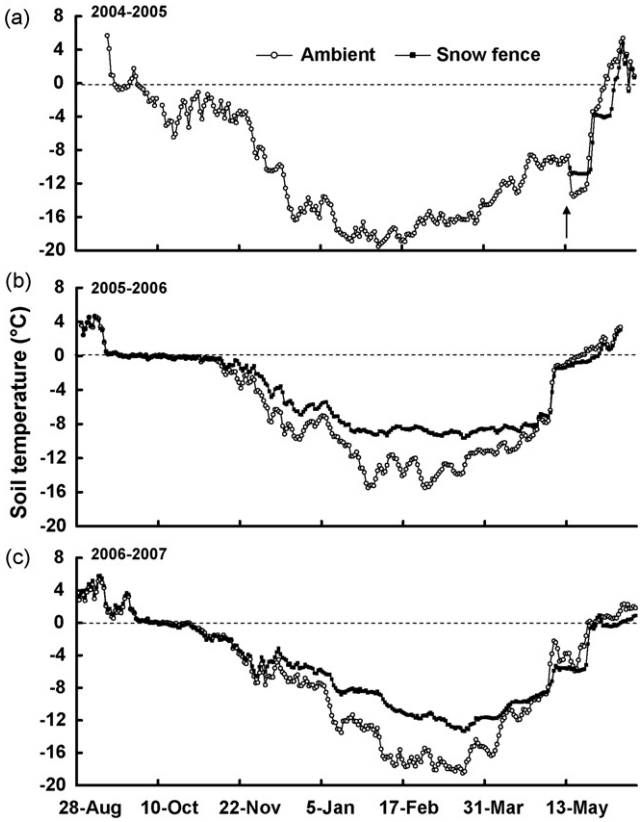

Fig. 1 – Daily mean soil temperature under ambient and deepened snow in: (a) 2004–2005 at 2 cm depth ($n = 2$ and 1 thermocouples, respectively); (b) 2005–2006 at 5 cm depth ($n = 12$ and 8, respectively); (c) 2006–2007 at 5 cm depth ($n = 4$ and 7, respectively). The arrow in 2004–2005 indicates the sample date (May 15) for this experiment.

2.3. Sampling protocol and sample processing

Samples were collected in late winter, before snow thaw on the birch hummock tundra, during the last week of the "late cold" stage of the cold season (*sensu*, Olsson et al., 2003) (Fig. 1). On May 15, 2005, frozen, intact blocks (~450 cm^3, approximately 8 cm × 8 cm) of organic soil to the mineral horizon (5–8.5 cm in depth) were randomly sampled with an axe at locations at least 3 m apart from within snow fence plots ($n = 6$) and control plots ($n = 6$). Two separate experiments were carried out with these soils. In the first experiment, we investigated immediate differences in soil and microbial biochemistry in late winter as a result of the snow fence treatment. For this experiment, subsamples of the frozen soil blocks were processed within 48 h of sampling. In the second experiment, we investigated the effects of factorial nutrient additions (C, N and P) and snow depth on the physiological responses of the soil microbial community and the implications of these microbial responses for soil biochemistry. For this experiment, the remainder of the frozen blocks of soil were stored outside (−10 to −15 °C), sheltered from wind and sun, and processed over a period of 7 days after initial sampling.

2.3.1. Experiment 1: effects of deepened snow on soil biochemistry in late winter

Subsamples were removed from the frozen blocks of soil, approximately 4 cm × 4 cm × the depth of the sample (5–8.5 cm; Table 1), and these 6 control and 6 snow fence subsamples were thawed overnight in the field lab at ~10 °C (the field lab temperature was difficult to control and varied between 5 and 15 °C). Aboveground plant material was removed, and live roots were carefully separated from the organic soils into coarse (≥2 mm) and fine (<2 mm) fractions, in order to reduce root contribution to microbial biomass estimates, and to quantify root mass and volume. Soil microbial biomass C and N contents were determined by the chloroform-fumigation direct-extraction (CFE) technique (Brookes et al., 1985) using 10 g fresh mass of soil and 50 ml 0.5 M K_2SO_4 for each extraction ($n = 12$ plus blanks). Fumigation lasted 24 h in a darkened vacuum desiccator jar at ~10 °C. Non-fumigated samples ($n = 12$ plus blanks) were extracted immediately after sorting. All extract samples ($n = 24$ plus blanks) were shaken manually several times for a minimum of 1 h in extractant, left to settle for 30 min, then filtered through a 1.2 μm pore size glass fiber filter and frozen at −20 °C until analysis. Samples for bacterial and fungal total counts were prepared according to Bloem et al. (1995). Briefly, soil subsamples ($n = 12$, 10 g fresh mass, with roots) and blanks were blended with filter-sterile (0.2 μm pore size) water (90 ml), and the soil solution was then fixed with filter-sterile formalin (3.7%). Fixed samples were stored at ~4 °C until slide preparation (within 2 weeks, described below) and microscopy.

2.3.2. *Experiment 2: physiological responses of soil microorganisms from control and deep snow plots to C × N × P additions*

For logistical reasons we processed the samples in four blocks of three replicates from either the ambient or snow fence plots (on May 16th, 17th, 21st and 22nd, respectively). Because there may be a confounding effect of storage time on the blocks, we maintained non-incubated controls for these soils, to check that response variables did not differ significantly from those in Experiment 1 soils (the *in situ* study). For each block, soils were thawed overnight at ~10 °C and live roots were removed as above. Each replicate was separated into 2 subgroups (destined for fumigation or not), containing 8 subsamples (each 10 g fresh mass). We added 2 ml of solution to each subsample, containing either water or factorial combinations of C (0.25 mM C as glucose), N (0.07 mM N as NH_4NO_3) or P (0.03 mM P as P_2O_5) (as C, N, P, CN, CP, NP, or CNP). These additions were approximately equal to 100 g C, 10 g N and 5 g P m^{-2}, or 7.1 mg C, 0.71 mg N and 0.36 mg P g^{-1} dry mass of soil (mean soil dry mass: fresh mass ratio = 0.23); they were intended to remove short-term microbial limitations to growth or activity, and were in proportion to the typical microbial C:N:P ratios. All subsamples (12 soil samples × 8 factorial combinations × 2 subgroups = 192) were then incubated in sample cups covered with polyethylene film for a total of 45 h: 9 h at ~1 °C (−1 to +2 °C) (initial overnight storage temperature) then 36 h at ~10 °C (5–15 °C). We intended to keep incubation temperatures low enough that psychrophilic microbes would be active, but not so low that temperature would constrain microbial activity in this short incubation period. Although incubation temperature was not easy to control in the field lab cooler and tent, it followed the same temperature pattern for each incubation block (warmer in the day and cooler at night) ranging from 5 to 15 °C. Soil CO_2 production was measured at the end of the incubation by attaching each sample cup to an infrared gas analyzer system (Licor 6400, Lincoln, NE, USA) within a modified chamber in a closed circulation loop. After respiration measurements, half of the incubated soils were extracted immediately, and half were fumigated, as with the samples from Experiment 1, to measure microbial accumulation of nutrients. In addition, an equal mass (fresh weight) from each of the 6 soil replicates was pooled to create a composite snow fence soil sample and similarly to create a composite control plot soil sample. Subsamples from these two composited samples were fertilized (C × N × P) and incubated as above, then fixed and stored for microscopy analysis as with the samples from Experiment 1. By removing soil heterogeneity between plots, these composites allowed us to focus more directly on the different responses of fungi and bacteria within the snow depth treatment to factorial C, N and P additions.

2.4. *Biological and chemical analyses*

NH_4^+–N and NO_3^-–N in the non-fumigated samples and PO_4^{3-}–P in both fumigated and non-fumigated samples, were determined colourimetrically, using automated flow analysis (Bran-Leubbe Autoanalyzer III, Norderstadt, Germany). NH_4^+–N, NO_3^-–N and PO_4^{3-}–P were analyzed with the indophenol (Mulvaney, 1996), sulphanilamide (Mulvaney, 1996) and molybdate—ascorbic acid (Kuo, 1996) methods, respectively. C and N contents in the fumigated and non-fumigated samples were determined by oxidative combustion and infrared (TOC) (Nelson and Sommers, 1996) or chemiluminesence (TN) analysis (TOC-TN autoanalyzer, Shimadzu, Kyoto, Japan). Microbial biomass C and N contents (MBC and MBN) were calculated as the difference between fumigated and non-fumigated extractable C and N samples. Differences were divided by a correction factor to account for microbial C or N that is not susceptible to chloroform fumigation (k_C = 0.35; k_N = 0.4) (Jonasson et al., 1996). All C, N and P concentrations in the extracts were corrected for the dilution associated with the moisture content of each soil sample.

2.5. *Total bacterial and fungal counts*

Slide preparation was according to Bloem et al. (1995) with some modifications. Fixed soil solutions were diluted 10x with filter-sterile water (final concentration approximately 0.2 g soil l^{-1}), then 20 μl was pipetted into duplicate 6 mm wells cut into double-sided tape on a microscope slide, with a slide each for fungi and bacteria, and dried overnight in the dark. The polysaccharide stain fluorescent brightener 28 (FB 28) ($C_{40}H_{44}N_{12}O_{10}S_2$; 2.18 mM with 2 drops 1 M NaOH in water), was used to stain total fungi (i.e. live plus dead fungal mass), and the nucleic acid stain DTAF (5-([4,6-dichlorotriazin-2-yl]amino) fluorescein hydro-chloride, 0.038 M in a phosphate buffer solution (PBS; 0.05 M Na_2HPO_4 and 0.15 M NaCl, pH 9)), was used to stain total bacteria (i.e. live plus dead bacterial mass). The dried soil in the wells was flooded with 20 μl of stain for 30 min (DTAF) or 2 h (FB 28), rinsed in PBS then water (for DTAF) or water alone (for FB 28), then dried overnight in the dark. The double-sided tape was then removed and the dried soil covered with a drop of immersion oil (Type B, Cargill) and a cover slip. All samples were viewed with an epifluorescent microscope (Nikon E600W), and photographed with a cooled 16-bit digital colour camera (QICAM 1394, QImaging, Burnaby, B.C.). Fungal slides were viewed at 400× magnification with a UV filter set (360 nm peak/40 nm wide excitation filter, 400 nm dichroic filter, 460 nm peak/50 nm wide emission filter) and bacterial slides were viewed at 1000× magnification with a blue-light filter set (480 nm peak/40 nm wide excitation filter, 505 nm dichroic filter, 535 nm peak/50 nm wide emission filter). Bacterial cell counts and volume were based upon the average of 10 fields-of-view per slide and fungal hyphal analyses were the average of 30 fields-of-view per slide. Fungal spores or fungi in yeast form were not counted. Enumeration of bacteria and fungi was done semi-automatically using the software program SimplePCI (version 5.3.1, Compix, Cranberry Township, PA), which quantifies the length (L) and breadth (B) of each user-selected organism in a field-of-view, calculated from the object perimeter and pixel area. Length collected in this manner was used to calculate fungal hyphal length. Bacterial and fungal volume per cell (V) were calculated as $V = \pi/4 \times B^2 \times (L - B/3)$ (Bloem et al., 1995). Volumes were converted to mass of C assuming a mean fungal C content of 3.1×10^{-13} g C μm^{-3} and a mean bacterial C content of 1.3×10^{-13} g C μm^{-3} (Bloem et al., 1995).

2.6. Statistical analyses

We tested for significant effects of the snow fence treatment on explanatory variables (Experiment 1—the *in situ* study) using one-way ANOVAs. We used split-plot factorial ANOVAs to investigate the effect of snow fence treatment on incubated responses to C, N and P additions (Experiment 2—the incubation study with C × N × P addition). Snow depth was the fixed between-treatment factor, with two levels, sample number (6) was the random effect nested within this, and C, N and P additions were the three fixed within-treatment factors, with two levels each (Winer et al., 1991). Samples were collected in such a way that within-treatment replicates were closer (~3 m) than between-treatment replicates (~30 m), which increases the chances of spatial autocorrelation. Thus, our approach to the statistical analyses of this experiment (Experiment 2) assumes that the responses of each soil sample within either the snow fence or the control plot was independent of adjacent samples (each of which was ~3 m away). Spatial heterogeneity in major soil characteristics (bulk density, water content and root volume) was just as large within the ambient and snow fence plot as between those plots (Table 1), suggesting that spatial autocorrelation is unlikely to have substantially influenced our results. Data were tested for normality (Shapiro-Wilks), and log transformed when necessary before running the ANOVAs. Traditional (EMS) mixed model ANOVAs were used (Experiment 2) whereby the main effects of between-treatment random factors are tested with mean squares (MS) of the model error but the main effects of between-treatment fixed factors are tested with a MS that involves interaction with a random variable (JMP 7.0, 2007, SAS). In Experiment 2, paired *t*-tests between the deepened snow soils and ambient snow soils were performed on bacterial and fungal mass, since these treatment data were based upon composited samples (no replication). This allowed us to examine if the microbial communities at the snow fence and control plots responded differently to nutrient additions but did not allow us to investigate the factorial response to C, N and P. To provide an indicator of the inherent variability, standard errors expected in these data were derived for the control samples of the second experiment, based upon the variability per unit C mass in the Experiment 1 fungal:bacterial mass. In all data analyses, all significant interactions ($\alpha = 0.05$) are reported.

3. Results

3.1. Environmental conditions

The snow fence successfully deepened snow, promoting snowdrifts of 8 and 15 m on either side (south- and north-facing, respectively), with a consistent maximum snow depth of 1 m along the peak of the drift by late winter (Nobrega and Grogan, 2007). Ambient snow depth in the control site was 30 cm on May 15, 2005. Although our soil temperature data for the winter of 2004–2005 is for an ambient snow site only, complete snow fence and control site temperature data for the following two winters (i.e. 2005–2006 and 2006–2007; Fig. 1) indicated consistent strong ameliorating effects of the deepened snow at several phases of winter (Fig. 1). In particular, there was a slower rate of cooling in the snow fence soil after mid-November, a mean temperature up to 7 °C warmer in the snow fence soil during the ''deep cold'' part of winter (*sensu*, Olsson et al., 2003), more stable soil temperatures throughout this ''deep cold'' period, and cooler soils in the spring (Fig. 1). Other studies have consistently shown that deepened snow increases soil temperature (Brooks and Williams, 1999; Walker et al., 1999; Schimel et al., 2004). Furthermore, soil temperature dynamics in the ambient and snow fence sites during the late winter period of this study were similar to other years. Therefore, we assume that the deepened snow resulted in warmer and more stable soil temperatures over the winter of 2004–2005 and thus that any

Table 1 – Summary of soil characteristics by individual replicate to illustrate plot heterogeneity in birch hummock tundra soils from under ambient (~30 cm snow depth) and deepened snow plots (~1 m snow depth) in late winter at Daring Lake, NWT, May 15, 2005

Treatment	Replicate	Bulk density (g dw soil cm^{-3})	Volumetric soil water (cm^3 water cm^{-3} soil)	Rooting volume (mg dw fine and coarse roots cm^{-3})	Sample depth within organic layer (cm)
Ambient snow	1	0.15	0.62	5.40	8.0
	2	0.14	0.68	14.26	6.0
	3	0.24	0.76	5.55	6.5
	4	0.20	0.80	10.89	6.5
	5	0.11	0.32	4.42	8.4
	6	0.14	0.69	11.50	5.5
	Mean (S.E.)	0.16 (0.02)	0.65 (0.07)	8.67 (1.66)	6.8 (0.5)
Deepened snow	1	0.19	0.67	27.11	7.4
	2	0.13	0.46	5.27	6.7
	3	0.24	0.81	2.63	6.5
	4	0.17	0.61	13.40	5.0
	5	0.15	0.61	8.28	8.5
	6	0.14	0.69	22.10	5.7
	Mean (S.E.)	0.17 (0.02)	0.64 (0.05)	13.13 (3.97)	6.6 (0.5)

There were no significant differences between treatments for any of these variables ($P > 0.3$).

Table 2 – Summary of effects of deepened snow on *in situ* soil biochemical properties in late winter (Experiment 1)

Category	Variable	Ambient mean		Snow fence mean		d.f.	*F* ratio	*P* value	Response to deepened snow
Soil solution	NH_4^+–N	3.2	(1.6)	1.3	(0.2)	1,10	2.6	0.14	ns
	PO_4^{3-}–P	2.1	(0.5)	1.7	(0.4)	1,10	0.5	0.48	ns
	DON	55.8	(8.2)	36.4	(2.7)	1,10	5.0	0.05	Decreased by 35%
	DOC	682	(60)	520	(59)	1,10	3.8	0.08	Decreased by 25%
Microbial pool	MBC	13.4	(0.7)	15.3	(1.3)	1,10	1.6	0.24	ns
	MBN	0.74	(0.05)	1.13	(0.17)	1,10	7.9	0.02	Increased by 53%
	MBC:MBN	18.3	(1.1)	14.0	(0.9)	1,10	9.4	0.01	Decreased by 24%

Soil solution mean values (and standard error) are in $\mu g\ g^{-1}$ dw soil and MBC and MBN are in $mg\ g^{-1}$ dw soil. DON: dissolved organic nitrogen (total dissolved organic N–NH_4^+–N; NO_3–N was below our detection limit); DOC: dissolved organic carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; d.f.: degrees of freedom; ns: not significant.

biochemical or microbial differences between the snow depth treatments reflect winter temperature effects on these soils.

The high spatial heterogeneity of these tundra soils is reflected in Table 1. There was no significant difference ($P > 0.3$) between deepened and ambient snow plots for bulk density, volumetric soil water, rooting volume, or sample depth to the mineral layer.

3.2. *Experiment 1: effects of deepened snow on soil biochemistry in late winter*

Contrary to our expectations of increases in nutrient concentrations in the soil solution under deepened snow, extractable inorganic nutrients were not significantly different and extractable organic nutrients were significantly reduced in the snow fence soils (Table 2). In contrast, deeper snow enhanced microbial nitrogen accumulation, without affecting MBC, leading to a significant decrease in MBC:MBN (Table 2).

Deepened snow did not significantly alter fungal mass, fungal hyphal lengths, or fungal:bacterial mass ratios (Table 3—Experiment 1). Deepened snow significantly lowered bacterial counts, but did not alter bacterial mass (Table 3—Experiment 1). Bacterial mass was calculated as cell counts (abundances) multiplied by mean cell volume per field-of-view. Although bacterial cell counts were significantly smaller under deepened snow (Table 3—Experiment 1), bacterial cell volume was larger under deepened snow ($t_{1,6} = 2.8$, $P = 0.03$) resulting in no significant change in bacterial mass. Despite the large proportion of the microbial mass represented by fungi, fungal mass was not correlated with chloroform-fumigated MBC or MBN, and fungal hyphal length was negatively correlated with chloroform-fumigated MBC ($r^2 = -0.64$, $P = 0.03$). Similarly, bacterial mass was not correlated with MBC or MBN, and bacterial counts were negatively correlated with MBN ($r^2 = -0.80$, $P = 0.002$). MBC:MBN is often used as an indicator for the soil fungal to bacterial ratio. In these tundra soils however, the decrease in MBC:MBN under deeper snow was not reflected by changes in fungal and bacterial mass as measured by epifluorescent microscopy.

3.3. *Experiment 2: physiological responses of soil microorganisms from control and deep snow plots to C × N × P additions*

Our large additions of C, N and P raised the soluble pools of all of these nutrients above ambient conditions by a factor of 3 (dissolved organic carbon, DOC), 170 (NH_4–N + NO_3–N), and 9 (PO_4^{3-}–P) (Table 4) indicating that several potential limitations to microbial growth or activity had been removed. Soil DOC increased when C was added, and when N was added, and

Table 3 – Effects of deepened snow on soil fungal and bacterial quantities (g^{-1} dw soil) immediately upon thawing (Experiment 1), and after incubation with water and factorial combinations of C, N and P (Experiment 2)

		Fungi		Bacteria		Fungal: bacterial mass ratio
		Hyphal length ($\times 10^3$ m)	Mass (mg C)	Counts ($\times 10^9$)	Mass (mg C)	
Experiment 1	Ambient	1.4 (0.3)	1.4 (0.4)	4.43 (0.21)	0.32 (0.02)	4.3 (1.0)
	Snow fence	0.9 (0.2)	1.0 (0.2)	3.30 (0.33)	0.40 (0.08)	3.3 (1.1)
	t-Ratio	1.4	1.2	2.9	1.1	0.7
	d.f.	1,10	1,10	1,10	1,10	1,10
	P	0.2	0.3	0.02	0.3	0.5
Experiment 2	Ambient	5.5 (1.1)	5.8 (0.7)	5.83 (0.32)	0.73 (0.08)	8.8 (1.4)
	Snow fence	3.8 (0.6)	3.6 (0.4)	5.26 (0.27)	0.91 (0.14)	4.4 (0.6)
	t-Ratio	14.7	8.9	1.9	1.2	9.9
	d.f.	1,14	1,14	1,14	1,14	1,14
	P	0.002	0.01	0.2	0.3	0.007

Experiment 2 results are the mean responses (and S.E.) across all control and nutrient-added soils ($n = 6$, Experiment 1; $n = 8$, Experiment 2); d.f.: degrees of freedom.

Table 4 – Summary of statistically significant effects of factorial combinations of C, N and P additions and snow depth treatment on soil biochemical properties (Experiment 2)

Category	Variable	Factor	d.f.	F ratio	P value	Response
Soil solution	DOC	C	25,67	1807.2	<0.0001	Increase
		N	25,67	18.3	<0.0001	Increase
		C × N	25,67	16.6	0.0001	Increase with C increased with N
		C × snow	25,67	12.7	0.0007	Increase with C suppressed with snow
	DON	C	25,67	4.8	0.03	Increase
		N	25,67	184.7	<0.0001	Decrease
		C × N	25,67	4.1	0.05	Decrease with N suppressed with C
		N × snow	25,67	6.2	0.02	Decrease with N increased with snow
	NH_4^+–N	N	25,67	605.8	<0.0001	Increase
		P	25,67	4.2	0.04	Decrease
		Snow	25,67	6.4	0.03	Increase
		C × N	25,67	8.7	0.004	Increase with N suppressed with C
	NO_3^-–N	C	25,67	4.9	0.03	Decrease
		N	25,67	4228.1	<0.0001	Increase
		Snow	25,67	5.8	0.04	Increase
	PO_4^{3-}–P	P	25,66	181.9	<0.0001	Increase
		C × P	25,66	4.6	0.04	Increase with P suppressed with C
		P × snow	25,66	11.7	0.001	Increase with P increased with snow
Microbial activity	CO_2	C	25,67	134.4	<0.0001	Increase
		P × snow	25,67	8.5	0.005	Increase with snow suppressed with P
Microbial pool	MBC	C	25,67	138.6	<0.0001	Increase
		N	25,67	6.6	0.01	Decrease
		P	25,67	6.8	0.01	Decrease
		Snow	25,67	4.7	0.05	Increase
		C × N	25,67	4.5	0.04	Increase with C suppressed with N
		C × snow	25,67	17.7	<0.0001	Increase with C increased with snow
	MBN	N	25,67	208.6	<0.0001	Increase
	MBC:MBN	C	25,67	90.3	<0.0001	Increase
		N	25,67	240.5	<0.0001	Decrease
		P	25,67	4.3	0.04	Decrease
		N × snow	25,67	20.1	<0.0001	Decrease with N suppressed with snow

d.f.: Degrees of freedom.

more so when both C and N were added in combination (Table 4). The significant interaction between the C addition and deepened snow treatments indicates that soils from deepened snow plots had higher DOC concentrations *except* where C was added. Thus the large carbon addition suppressed inherent differences in DOC between ambient and snow fence soils (Table 4). The large N addition increased dissolved total nitrogen (DTN) (data not shown) but significantly reduced dissolved organic N (DON = DTN − (NH_4^+–N + NO_3^-–N)) (Table 4) except when C was added. Furthermore, C additions interacted with N additions to lessen the negative effect of inorganic N on DON concentrations in the soil solution (Table 4).

Of the ~0.7 mg N added as equal proportions of NH_4–N and NO_3–N in each N-addition treatment, ~28% of the ammonium remained in the soil solution at the end of the incubation, while ~80% of the nitrate remained (Fig. 2a and b). Thus, our results indicate that under these non-N-limiting conditions, late winter tundra soil microorganisms preferentially accumulated NH_4–N over NO_3–N. Both NH_4–N and NO_3–N concentrations in the soil solution were altered by the addition of C. Carbon interacted with N additions to reduce NH_4–N concentrations in the soil solution (Fig. 2a, Table 4), and whenever C was added, NO_3–N concentrations were lower relative to when C was not added (Fig. 2b, Table 4). Soil

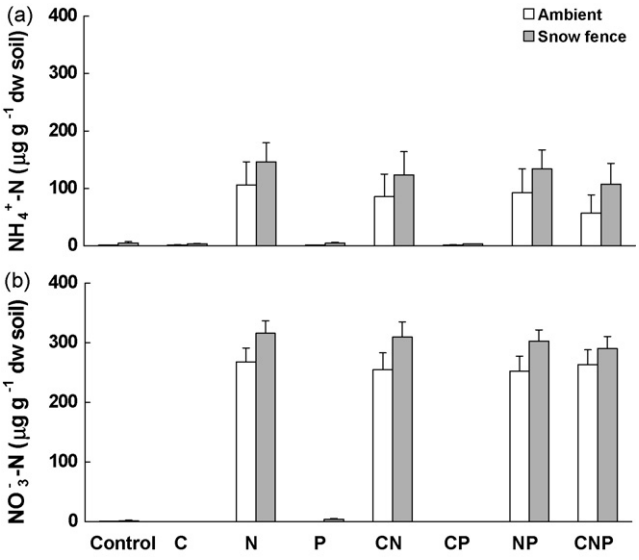

Fig. 2 – (a) Dissolved NH_4^+–N and (b) NO_3^-–N contents in birch hummock soils from ambient and deepened snow plots in late winter that were incubated with factorial combinations of C, N and P additions (n = 6; bars = ±1S.E.).

dissolved inorganic phosphorus (PO_4^{3-}–P) was also affected by the interaction between P and C, with a reduced increase in PO_4^{3-}–P when both were added as opposed to when P was added alone (Table 4).

Respiration from late winter soils responded positively to increased C availability (Table 4) and tended to be higher from the snow fence soils ($F_{25,67} = 3.0$, $P = 0.11$), consistent with our hypothesis that deepened snow exacerbates C-limitation of microbial activity in late winter. The addition of P significantly interacted with snow depth effects on respiration (Table 4), such that CO_2 emissions from snow fence soils were generally higher than from ambient soils except when P was added. As P additions also increased PO_4^{3-}–P more in the snow fence soils (Table 4), soil microbial activity in the deepened snow plots may have been less limited by P availability, resulting in less depletion of the added P.

Although the microbial biomass was not larger in soils from deepened snow plots in late winter (Experiment 1; Tables 2 and 3), the incubation experiment revealed strong physiological differences in the soil microbial community as a result of the snow fence treatment (Experiment 2; Table 4). Deepened snow and C addition resulted in significant increases in MBC that were associated with interactions with each other and with nitrogen (Table 4). MBC was stimulated by C addition more in the deepened snow plots than under the ambient snow, MBC in both soils was slightly reduced by N and P additions, and when C and N were added together, the positive affect of C on MBC was partially suppressed by the negative effect of N (Table 4). As might be expected, MBN was significantly increased by N addition (Fig. 3; Table 4). In addition, we observed larger MBN under the deepened snow treatment in both the *in situ* field measures (Experiment 1) and in the incubation study (Experiment 2—control soils; Fig. 3). Conversely, our MBC:MBN results (Table 4), which probably closely approximate the inverse of the microbial tissue N concentration, demonstrate that microbial N concentrations were particularly and consistently low in soils from beneath ambient snow to which N had not been added (Fig. 3).

Our analysis of all of the samples in the C × N × P data set indicates that fungi dominated MBC in the incubated soils, just as in the *in situ* soils (Table 3). The incubation process and water addition alone (Experiment 2, control) resulted in strong overall increases in fungal mass (×3), fungal hyphal lengths (×3), bacterial mass (×2) and bacterial counts (×1.5), with no

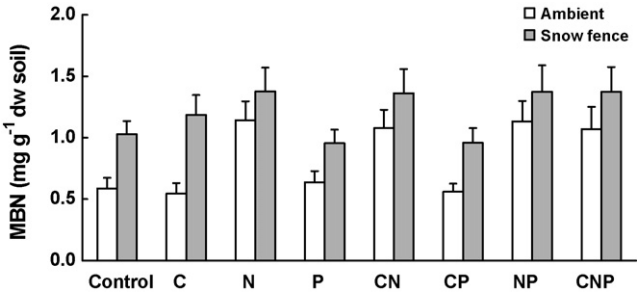

Fig. 3 – Microbial biomass nitrogen contents in birch hummock soils from ambient and deepened snow plots in late winter that were incubated with factorial combinations of C, N and P additions ($n = 6$; bars = ±1S.E.).

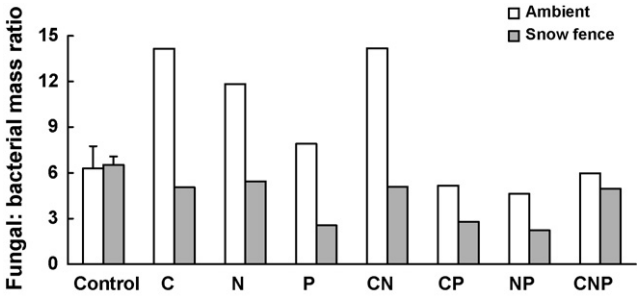

Fig. 4 – Fungal to bacterial mass ratios in composited late winter birch hummock soils from ambient and deepened snow plots that were incubated with factorial combinations of C, N and P additions. Standard error bars derived from the replicate plot data in Experiment 1 have been added to the control columns to indicate probable levels of variability.

overall change in F:B mass of these soils (control bars, Fig. 4). Fungi in the ambient and snow fence soils responded differently to the factorial nutrient additions. Soil fungal hyphal length and mass were both significantly lower (by 31 and 38%, respectively) in soil from under deepened snow, resulting in a marked decline in the overall F:B biomass ratio (Table 3: paired *t*-test across all nutrient addition treatments). The effect of individual nutrient additions on fungi and bacteria cannot be determined as only one composite sample was measured for each nutrient addition. However, assuming that the levels of variability are similar to, or less than, those in Experiment 1 (see derived standard error bars for controls, Fig. 4), we conclude that the F:B mass ratios indicate consistently and substantially smaller responses to almost all combinations of nutrient additions in the snow fence soils as compared to the ambient soils.

4. Discussion

4.1. *Microbial nutrient limitation to growth and activity in late winter*

Our study demonstrates that both the activity and growth of arctic tundra soil microorganisms in late winter is limited by the availability of labile C. Previous studies in an alpine tundra ecosystem concluded that microbial biomass is C-limited because C substrate additions in late winter lead to an increase in respiration (Brooks et al., 2004). The logic behind this conclusion is based upon the historical assumption that soil food webs and microbial activity are energy limited (Richards, 1987) and that macro- and micronutrient fluxes in soil are tied to the availability of C. However, nested food web structures that separate microbial feeding groups to better illustrate nutrient and energy flow (Pokarzhevskii et al., 2003) suggest that proteins and scarce minerals are more likely to limit microbial and macrofaunal growth. Furthermore, theoretical models have suggested that microbial decomposition of organic matter can be limited by exoenzyme production and that although respiration may be C-limited, microbial

growth may actually be N-limited (Schimel and Weintraub, 2003). When C is added and N is limiting growth, microorganisms can continue to respire C (''overflow metabolism''); in this case C is not incorporated into biomass (Bloem et al., 1994; Schimel and Weintraub, 2003). Therefore, soil microorganisms may appear C-limited for activity (ΔCO_2), but may not be C-limited for growth (ΔMBC). In our study we saw an increase in both respired C and MBC with labile C addition (Table 4), indicating that both microbial activity and growth were restricted by available labile C in this arctic tundra soil. Since these responses were larger in soils from beneath the snow fence, our first hypothesis that inherent late winter C-limitation of both activity and growth is exacerbated under deeper snow is supported.

Late winter C-limitation of the microbial biomass is consistent with alpine tundra research (Lipson et al., 2000; Brooks et al., 2004) and has lead to the hypothesis that depleted available organic pools in late winter cause mortality and lysis of soil microbes (Lipson et al., 2000), resulting in a release of cytoplasmic soluble nutrients into the soil solution at spring thaw. Our data indicating strong C-limitation of activity and growth in arctic tundra support the fundamental basis of this idea. Furthermore, our results suggest that in years of deep snow (natural interannual variation), or in areas where snow depth is predicted to increase with climate change, C-limitation will be even more severe, thereby potentially altering the timing or magnitude of associated spring thaw flushes.

N accumulation in the microbial biomass was more strongly limited by N availability in ambient soils than in snow fence soils in late winter. This was evident in the lower MBN and higher MBC:MBN in soils under ambient snow in Experiment 1 (Table 2), as well as the larger relative increase in MBN in those soils when N was added in Experiment 2 (Fig. 3). Colder, less stable soil temperatures throughout the fall and winter may have reduced either or both early winter N mineralization or late winter N immobilization, resulting in a stronger N-limitation for soil microorganisms by late winter under ambient snow. In addition, the activity of the soil microorganisms under ambient snow was more limited by P (Table 4). Increased microbial N immobilization and decreased P-limitation to activity under deeper snow, combined with increased C-limitation by late winter, may imply that this microbial biomass had access to greater pools of high C:N substrate earlier in the winter, which then steadily declined as organic matter decomposition was less restricted by cold soil temperatures over winter. This increased transfer of N to the microbial pool may in turn increase the spring release of N into the soil solution and may alter spring N availability to plants (Grogan and Jonasson, 2003).

In both ambient and deepened snow soils we found that the availability of DOC and DON in the extractable soil solution depended upon the positive interaction between C and N additions. The addition of both C and N increased DOC or DON in the soil more than when C or N were added alone (Table 4), suggesting that despite the relatively greater importance of C to microbes under deepened snow, C and N co-limit organic matter decomposition in these tundra soils.

4.2. *The effect of deepened snow on late winter soil nutrient pools*

Dissolved inorganic N concentrations in the soil did not change with deeper snow (Table 2). Previous results in arctic soils have found increased N mineralization in winter under much deeper snow (Schimel et al., 2004), and soil solution N pools were high when soil microorganisms were C-limited under very deep snow in an alpine system (Brooks and Williams, 1999). Instead, we saw a strong trend towards decreased dissolved organic C and N in snow fence soils, and a significant increase in the microbial storage of N. The decrease in total N pools in the soil solution of the snow fence was tenfold lower than the increase in microbial net N immobilization (increase in MBN) for *in situ* soils (Experiment 1) (Table 2), again indicating that deeper snow enhanced the release of nutrients as a result of soil organic matter decomposition followed by strong microbial immobilization prior to late winter. Although our data demonstrate microbial growth was limited by shortages in labile C over winter, especially under deeper snow, this limitation was not strong enough to stimulate net N mineralization in either snow treatment at that time. Unlike subarctic and other arctic systems (Grogan and Jonasson, 2003; Schimel et al., 2004), net N immobilization is occurring in late winter in these tundra soils.

Inherent soil biochemical differences due to the deepened snow treatment influenced the expected increases in soluble nutrients with corresponding nutrient additions. For example, snow fence soils had higher inherent DOC levels (i.e. where C was not added). Snow fence soils also had higher NH_4–N and NO_3–N levels, and a greater concentration of PO_4^{3-}–P in the P addition treatment (Table 4), suggesting that microbial PO_4^{3-}–P, NH_4–N and NO_3–N uptake was not as P- or N-limited in these soils relative to the ambient soils.

4.3. *The effect of deepened snow on the late winter microbial community*

Deepened snow over winter resulted in a change to the soil microbial community, by increasing the overall concentration of N in the microbial biomass, by reducing the number of bacteria, and by altering the physiological response of fungi to available nutrients. Supporting our third hypothesis, the microbial biomass under both snow depths and in both experiments was consistently dominated by fungi. Fungal rather than bacterial metabolism may be more likely to become C-limited in late winter, as a result of the threefold larger C investment per unit volume in fungal cell structure (see Section 2). However, this is assuming equal C-use efficiencies and constant growth, both of which are unlikely, especially in winter. Although bacterial lysis from freeze–thaw events has been recorded (Skogland et al., 1988) and it has been suggested that fungi may be more stable under temperature fluctuations (Sharma et al., 2006), it is unclear to what degree cold-tolerant bacteria and fungi are susceptible to freeze–thaw cycles, or if fungi or bacteria are preferentially selected during macrofaunal feeding in spring. Both bacterial and fungal-feeding macrofaunal species appear to respond positively to spring freeze–thaw cycles (Sulkava and Huhta, 2003; Sjursen

et al., 2005). There is a growing amount of information on the physiological adaptations of pyschrophilic microorganisms that enable them to be active and proliferate in cold conditions (Robinson, 2001; D'Amico et al., 2006; Walker et al., 2006). However, there is still little information on the ecological implications of these adaptations, their relative distributions amongst fungi and bacteria, and therefore the biological significance of changes in F:B mass ratios.

We expected warmer soils under deeper snow to result in an increase in bacterial abundance, assuming higher relative susceptibility to a dry, frozen environment by bacteria than fungi. Instead, our microscopy results indicate that deeper snow reduced bacterial abundance *in situ*, but had no significant effect on our estimates of bacterial mass (Experiment 1; Table 3). Insignificant differences in bacterial mass were a result of the contrasting effects of reduced cell counts and increased cell volume under deepened snow, suggesting that the dominant bacterial populations differed between soils from beneath ambient and deepened snow. Changes in bacterial abundances and potentially in bacterial physiologies may alter spring biochemical cycling if release from the bacterial cytoplasm is an important component of the spring nutrient pulse (Schimel et al., 2007; Schmidt et al., 2007).

In our second experiment we incubated soils from the two snow treatments at 0–15 °C to investigate the potential physiological differences between the microbial communities during the early stages of spring thaw. In comparison to the *in situ* measurements of ambient and snow fence soils (Experiment 1, Table 3) the incubation process and water addition alone (Experiment 2, control) resulted in strong overall increases in fungal mass, fungal hyphal lengths, bacterial mass and bacterial counts, with no overall change in F:B mass of these soils (control bars, Fig. 4). These results indicate that both bacterial and fungal components of the microbial mass responded in similar positive ways to the incubation conditions. Furthermore, they suggest that if any thaw-related mortality occurred during the establishment of the incubations, this was more than compensated for by the enhanced growth over the 45 h incubation period. Both fungal and bacterial mass responded to the C, N and P additions, but in general a greater response was displayed by fungi from the ambient soils (Table 3), leading to consistently larger fungal:bacterial mass ratios in these soils (Fig. 4, Table 3). This dataset therefore suggests that fungal nutrient limitations to growth were largest in soils that were colder over the winter. In addition, the fungi and bacteria in these two soils responded differently to the C, N and P additions, strongly suggesting that deepened snow altered the soil microbial community in ways that affected its physiological functioning.

Despite increases in bacterial and fungal mass with incubation, we saw a decrease in the MBC with incubation and water addition, and a small decrease or no change in MBN. There was no correlation between MBC and bacterial or fungal mass in our data, and although the microbial mass was primarily fungal, there was a negative correlation between MBC and fungal hyphal lengths. In general, the MBC as estimated with chloroform fumigation was greater than the microbial C mass estimated with epifluoresence (Experiment 1–9×; Experiment 2–2×). Similar or greater mismatches between epifluoresence and chloroform-fumigation methods have been recorded (Bloem et al., 1994; Frey et al., 1999), and may be in part due to problems with masking while performing microscopic counts, organic matter susceptibility to chloroform during fumigation, preferential susceptibility by some microbial groups to chloroform fumigation or overestimation in k_N and k_C factors. Both methods are probably including dead cells with intact membranes and therefore will differ from methods that target active or live organisms only. Although these dead cells are not contributing directly to soil activity, they are an important reservoir of plant-available nutrients.

Our results suggest that arctic tundra soils have a different microbial community structure than alpine tundra soils. The mean total fungal hyphal length in the *in situ* measures (Experiment 1) of our study were approximately five times longer (~1 km g^{-1} soil; Table 3) than total hyphal lengths recorded in alpine winter soils (192.8 m g^{-1} soil; (Lipson et al., 2002)), and much longer than average summer total hyphal lengths in central Siberian tundra (21 m g^{-1} soil; Schmidt and Bolter, 2002). Bacterial counts were similar although slightly less than found in winter alpine tundra soils (Lipson et al., 2002). Winter total fungal to bacterial C mass ratios in alpine soils under snow were 1.6 (Lipson et al., 2002) as compared to 4.3 in our study (Experiment 1; Table 3). In comparative terms, our study clearly demonstrates the larger wintertime dominance of fungi over bacteria in this common Canadian arctic ecosystem compared to the alpine tundra in Colorado, despite the shared limitation by available C.

This study illustrates the potential for arctic tundra soil microbial communities to succumb to low levels of labile C in late winter. This is important if microbial C-limitation is a mechanism for microbial death and nutrient release in spring (Schmidt and Lipson, 2004; Schmidt et al., 2007). Furthermore, this limitation increased in soil from under deepened snow. In addition to this different microbial response to C addition, we found *in situ* higher amounts of N stored in the microbial biomass under deepened snow. Regardless of mechanisms controlling microbial cytoplasmic release at thaw, this increased pool may represent a larger potential pulse of N for tundra plants at spring, and may therefore be instrumental for plant community shifts under future climate change predictions.

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