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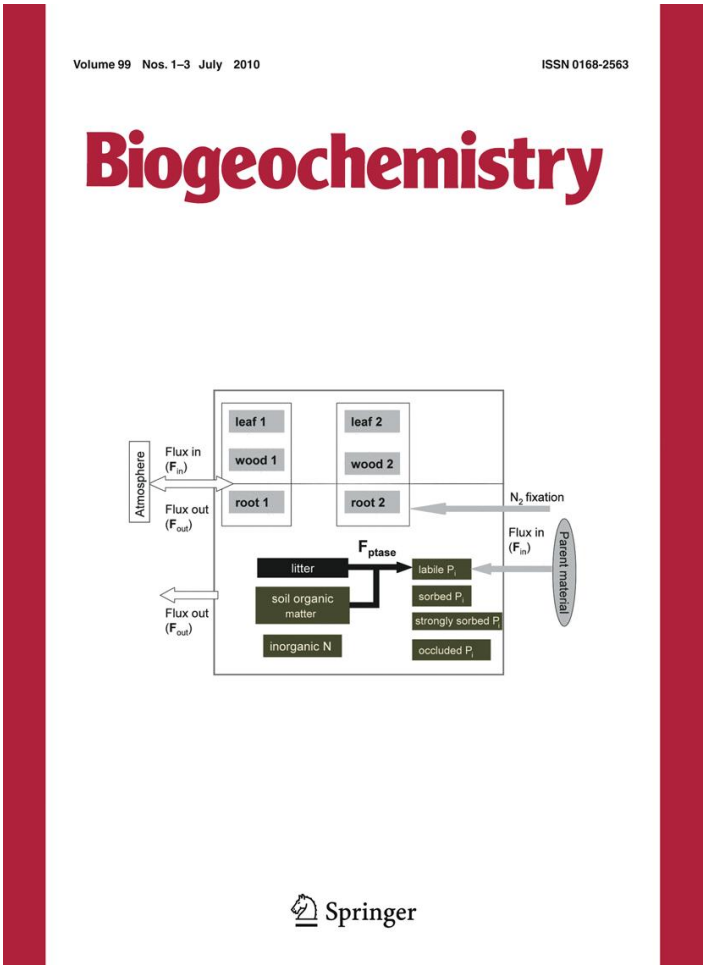

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Soil biogeochemistry during the early spring in low arctic mesic tundra and the impacts of deepened snow and enhanced nitrogen availability

Kate M. Buckeridge · Yan-Ping Cen · David B. Layzell · Paul Grogan

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Abstract Air temperature freeze–thaw cycles often occur during the early spring period directly after snowmelt and before budbreak in low arctic tundra. This early spring period may be associated with nitrogen (N) and carbon (C) loss from soils as leachate or as trace gases, due to the detrimental impact of soil freeze–thaw cycles and a developing active layer on soil microorganisms. We measured soil and microbial pools of C and N in early spring during a period of fluctuating air temperature (ranging from −4 to +10°C) and in midsummer, in low arctic birch hummock tundra. In addition we measured N_2O, CH_4 and CO_2 production in the early spring. All of these biogeochemical variables were also measured in long-term snowfence (deepened snow) and N-addition plots to characterize climate-change related controls on these variables. Microbial and soil solution pools of C and N, and trace gas production varied among the five early spring sample dates, but only marginally and no more than among sample dates in midsummer. N-addition greatly elevated N_2O fluxes, indicating that although denitrifiers were present their activity during early spring was strongly limited by N-availability, but otherwise trace gas production was very low in early spring. The later thaw, warmer winter and colder spring soil temperatures resulting from deepened snow did not significantly alter N pools or rates in early spring. Together, our results indicate strong stability in microbial and soil solution C and N pool sizes in the early spring period just after snowmelt when soil temperatures are close to 0°C (−1.5 to +5°C). A review of annual temperature records from this and other sites suggests that soil freeze–thaw cycles are probably infrequent in mesic tundra in early spring. We suggest that future studies concerned with temperature controls on soil and microbial biogeochemistry should focus not on soil freeze–thaw cycles per se, but on the rapid and often stepped increases in soil temperature that occur under the thawing snowpack.

Keywords Freeze–thaw cycles · Arctic birch hummock tundra · Denitrification · Microbial biomass · Spring thaw · Nitrogen

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Introduction

Early spring is assumed to be a dynamic season for soil biogeochemistry in arctic tundra because of rapid snow melt and high-amplitude fluctuations in air temperature. Soil that was frozen and therefore relatively dry during winter is subjected to rapid large influxes of snowmelt water as well as repeated freezing air temperatures associated with diurnal cycles and weather fronts at this time. Soil temperatures that had been decoupled from fluctuating air temperatures under the fall and winter snowpack and in saturated spring soils (Olsson et al. 2003) become more responsive during the hours to days after snowmelt and as the surface soils drain. Numerous laboratory incubation studies have tested the effects of soil freeze–thaw cycles on microbial biomass and soil physical structure, to understand the mechanistic effects of this soil disturbance and to generate ecological and environmental predictions as to how this type of disturbance influences ecosystem functioning. These studies demonstrate that soil freeze–thaw cycles can clearly be detrimental to both the soil microbial biomass and the soil physical structure (Schimel and Clein 1996; Herrmann and Witter 2002; Larsen et al. 2002; Six et al. 2004). Those microbes that survive such soil freeze–thaw cycles are believed to increase in activity and biomass in response to the thaw-related flush of nutrients released from lysed microbes and physically disrupted soil organic matter (Schimel and Clein 1996; Sharma et al. 2006). Broadly, several studies have suggested that this annual stress to the soil and the soil microbial biomass may produce an annually significant flush of nutrients (Lipson et al. 1999) that could be taken up by roots of certain plant species, thus determining plant community structure and composition (Chapin 1980; Kreyling et al. 2008) or be lost from the system as leachate or gas, thus maintaining ecosystem-wide nutrient limitations (Vitousek et al. 1998).

Arctic tundra soil microbial biomass and organic soil structure may be less sensitive to air temperature freeze–thaw cycles than in temperate agricultural and forest soils (Lipson and Monson 1998; Lipson et al. 2000; Grogan et al. 2004; Walker et al. 2006; Henry 2007; Matzner and Borken 2008). For example, when the amplitude and/or duration of manipulated freeze–thaw cycles in laboratory studies were modified (i.e. reduced in severity) to better reflect realistic in situ tundra field conditions, the ecosystem effects of these spring temperature fluctuations were generally small (Lipson and Monson 1998; Sulkava and Huhta 2003; Grogan et al. 2004). Accordingly, there is now some doubt as to the significance of springtime air temperature freeze–thaw cycles on tundra ecosystems. Therefore, in situ field studies of soil microbial biomass and soil biogeochemistry during early spring are required.

N_2O is an important greenhouse gas (IPCC 2007), and cold season N_2O fluxes—particularly N_2O production during spring thaw—can be a large component of annual trace gas budgets in alpine tundra, grasslands, agricultural fields, deciduous forest and steppe (Brooks et al. 1997; Kammann et al. 1998; Teepe et al. 2000; Groffman et al. 2006; Holst et al. 2008). In addition, snow melt and rapid soil water thawing result in low O_2 availability and the facultative use of NO_3 as the terminal electron acceptor in electron transport phosphorylation for many microbial species (Zumft 1997). Together, these conditions are predicted to promote denitrification (Firestone and Davidson 1989) and likely explain the relatively high rates of N_2O production reported from temperate soils in spring (Teepe et al. 2000). To the best of our knowledge, N_2O production has not been investigated in mesic low arctic tundra soils during spring, when environmental conditions might be most favourable for denitrification. Although bursts of N_2O have been reported at thaw in alpine tundra in Colorado (Brooks et al. 1997), spring soil biogeochemistry is not comparable between this site and arctic tundra because high rates of N deposition in the snowpack over the Rockies result in large NO_3 inputs into these soils at thaw (Brooks et al. 1998). Instead, arctic tundra soils have very low growing season pools of nitrate and low rates of net nitrification (Giblin et al. 1991; Nadelhoffer et al. 1992) suggesting that although environmental conditions may be favourable for N_2O production at thaw, low NO_3 availability may restrict denitrification.

Environmental and biogeochemical conditions during spring are expected to alter in the low Arctic as a consequence of climate change (Serreze et al. 2000; ACIA 2005; IPCC 2007). Several lines of evidence suggest that nutrient availability will be enhanced (Shaver et al. 1992; Shaver et al. 2000), that snow will be deeper (ACIA 2005) and that snowmelt will be earlier (Chapin et al. 2005). Snow depth in

alpine tundra and temperate forests affects the timing of soil thaw and the patterns and magnitude of soil biogeochemistry and trace gas release (Brooks and Williams 1999; Groffman et al. 2006), at a scale that is relevant to annual ecosystem C and N budgets (Brooks et al. 1997). Here, in addition to characterizing in situ nutrient and trace gas flushes in the field in early spring, we used snowfence and N fertilization manipulations to investigate the influences of deepened snow and increased nutrient availability on these biogeochemical processes in early spring. We used our data to test the following hypotheses in a mesic birch hummock tundra ecosystem that extends across the Canadian Low Arctic and the Eurasian Southern Tundra (Bliss and Matveyeva 1992):

1. Soil biogeochemistry during the early spring period following snowmelt is characterized by nutrient peaks in the soil solution or as gaseous losses;
2. Soil biogeochemical pools are larger and more variable in early spring just after thaw than in the growing season;
3. N_2O production in mesic soils during the cold wet conditions of early spring is limited by inorganic N availability—when N is not limiting (i.e. in a N-fertilized system), we predict a large pulse of N_2O from thawing tundra soils;
4. Deeper snow in early spring enhances spring nutrient peaks in the soil solution.

Methods

Site description and experimental treatment

This study was conducted in the early spring (early June) of 2006 in a mesic birch hummock ecosystem at the Tundra Ecological Research Station (TERS) at Daring Lake, Northwest Territories, Canada (64°50′ N, 111°38′ W). Daring Lake is located 300 km northeast of Yellowknife, in the Coppermine River watershed. The hummock ecosystem at this site is located in a valley, midway along a catena. The soils and vegetation along this moisture gradient have a circumpolar distribution and this particular site has been previously described (Nobrega and Grogan 2007, 2008; Buckeridge and Grogan 2008; Lafleur and Humphreys 2008). The soils are classified as Orthic Dystric Turbic Cryosols (Soil Classification Working Group 1998) and consist of an organic layer ~3–20 cm deep above a silt-sand mineral layer. The ecosystem is characterized by hummocks 10–30 cm high and deciduous dwarf birch (*Betula glandulosa*) shrubs that are 10–40 cm tall and attain ~10–30% of the areal coverage of the hummocks, interhummocks (mid-elevation), and hollows. The remaining cover is a mixture of mostly ericaceous shrubs (bog rosemary (*Andromeda polifolia* L.), mountain cranberry (*Vaccinium vitis-idaea* L.), bog blueberry (*V. uliginosum* L.), and labrador tea (*Ledum decumbens* (Ait.))), and sedges, mosses, lichens, and cloudberry (*Rubus chamaemorus* L.).

Climate records from the Daring Lake weather station (1996-2008; Bob Reid, Indian and Northern Affairs Canada, unpublished data) indicate mean daily air temperatures as low as −38°C in winter and as high as 15°C in summer, and mean daily soil temperatures at 5 cm depth as low as −26°C in winter and as high as 12°C in summer. Maximum snow depth in exposed areas averages 37 cm (range: 15–59 cm) by late winter, followed by a snow melt period averaging 15 days (range: 5–28 days) the length of which is partially explained by late winter snow depth ($R^2 = 0.5$). The period following snowmelt—the focus of this experiment—is characterized by air temperature freeze–thaw cycles for approximately 3 weeks, after which air temperatures rise well above zero and plant leaf-out begins. Therefore, our study duration is equivalent to the second half of the 'Thaw' period as defined by Olsson et al. (2003). The snow-free season then lasts ~150 days (typically early June to late September) with an average summer rainfall of 141 mm.

A snow experimental treatment was established in 2004 to increase the depth and duration of snow cover within birch hummock tundra vegetation. Snowfences (1.2 m tall and 15 m long; $n = 5$) created snowdrifts of 8 and 15 m on either side (south- and north-facing, respectively) with a consistent maximum depth of 1 m at the peak of the drift by late winter, stabilizing and increasing 2005–2006 winter soil minima from −16°C to −8°C at 5 cm depth (Buckeridge and Grogan 2008). In addition, the snowfences extended snow cover on the ground in the spring by 1–2 weeks. Control sites (unfenced, 15 m long; $n = 5$) were established parallel to the fences in similar vegetation and offset by more than 30 m to

ensure clear separation from the snowfence drift areas. Nitrogen (N) addition plots (10 g NH_4NO_3-N m^{-2} y^{-1} each summer; 35 m^2; $n = 5$) were established in 2004 within birch hummock vegetation in the same valley (~200 m away), such that these plots had a total of 20 g of N fertilizer added before this study occurred.

Soil temperatures over the spring of 2006 were measured every hour in inter-hummock spaces at 5 cm depth ($n = 12$ each) at control and snowfence sites using CR 10× data loggers (Campbell Scientific, Logan, UT) and thermocouple probes. On each soil sampling day (details below), we measured soil temperatures at 2 and 5 cm depth with hand-held temperature probe, active layer depths and volumetric soil moisture (liquid water) to ~5 cm depth with a hand-held dielectric probe ($n = 5$) (Hydrosense, Campell Scientific). Soil water content was also measured gravimetrically on each collected soil sample (i.e. total water content as liquid and ice) at five random locations next to each gas sampling collar ($n = 5$).

Sampling protocol and sample processing

Soil samples were collected 5 times in the early spring (May 31st and June 2nd, 4th, 7th and 9th) in control, snowfence and N-addition plots, and on three additional days during the growing season (June 26th, and July 10th and 26th) in control plots to compare variability of soil and microbial pools between seasons. We cut out samples (~5 × 4 × 10 cm) of thawed and underlying frozen organic soil from interhummock areas down to the contact with the mineral layer (or thawed soil was sampled to the frozen layer on May 31st) and averaged ~10 cm in depth. Soil sample volume was recorded to calculate bulk density and the aboveground plant material and roots were removed prior to homogenizing the soil. A sub-sample was weighed to calculate gravimetric moisture content and then oven-dried (65°C until a constant mass), and 3 further sub-samples (10 g fresh mass of soil each) were removed for: (1) extraction with water (50 ml) to measure soil solution C and N contents; (2) extraction with a concentrated salt solution (50 ml of 0.5 M K_2SO_4) for salt-extractable C and N contents; and (3) chloroform-fumigation direct-extraction (CFE) (Brookes et al. 1985) for chloroform-susceptible C and N contents for calculating microbial biomass C and N contents. Fumigation lasted 24 h in a darkened vacuum desiccator jar at ~15°C. All extract samples 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.

Gas samples were collected 3–4 times over a ~2-h period from static chambers in the mid-afternoon (close to the daily soil temperature peak) on 4 days in the early spring (June 2nd, 4th, 7th and 9th) from each of the control, snowfence and N-addition plots. We sampled the plots in random order on each date to avoid confounding effects of time of day. PVC collars (29.8 cm internal diameter, 12 cm high) that were beveled at one end had been inserted (~2 cm) into the organic soil on May 30th to enclose patches of interhummock vegetation. The upper rim of each collar contained a groove into which a machined PVC lid with a downward-pointing tongue at the outer circumference fitted snugly. Water was poured into the groove prior to fitting each lid to ensure a gas-tight seal. Height above the vegetation was recorded for each chamber (~3.5 l) to correct the flux rates for differences in headspace volume among plots. A hole in the centre of each lid was attached by gas-tight fittings to a 3-way stopcock to facilitate headspace gas sampling using a syringe and needle (50 ml, Benton Dickson). The headspace gas was mixed prior to sampling by slowly withdrawing (40 s) and then reinjecting the full syringe volume four times prior to removing a sample (20 ml) and transferring it to a glass vial (12 ml, Wheaton). Vials had been sealed with a thick butyl rubber septum and pre-evacuated in Kingston prior to transport into the field (to 0.1 mbar), and pre-evacuated blanks were maintained throughout transport, sampling and storage to test for sample contamination through loss of vacuum (no loss of vacuum occurred). We also tested for linearity in flux rates by collecting successive gas samples from the same plot over longer times (up to 35 total h) and tested repeatability by sampling some plots several times in rapid succession. All gas samples were analyzed for N_2O, CO_2 and CH_4 concentration within 3 months with a gas chromatograph (Model 8610C, SRI Instruments, CA USA) equipped with an electron capture detector (ECD) and a flame ionization detector (FID). N_2O was detected by ECD, whereas the CH_4 and the CO_2 (after being converted to CH_4 in a

methanizer) were detected by FID. Helium gas was used as the carrier gas, while 5% methane in argon was used with the ECD as the make-up gas. Calibration gases were prepared by gas mixing pumps (Wosthoff GmbH, Bochum, Germany) that combined either helium with a pre-calibrated gas containing 1025 ppm N_2O and 963 ppm CH_4 or N_2 with pure CO_2. Rates of soil N_2O, CO_2 and CH_4 emissions from each plot were calculated as the slopes ($r^2 > 0.88$; $P < 0.05$) of the N_2O, CO_2 and CH_4 concentrations in the headspace samples during the $\sim$2 h sampling period.

Biological and chemical analyses

NH_4^+-N and NO_3^--N were determined colourimetrically using automated flow analysis (Bran-Leubbe Autoanalyzer III, Norderstadt, Germany) and the salicylate and the cadmium-reduction sulphanilamide methods, respectively (Mulvaney 1996). C and N contents in the fumigated and non-fumigated extracts were determined by oxidative combustion and infrared (TOC) (Nelson and Sommers 1996) or chemiluminesence (TN) analysis (TOC-TN autoanalyzer, Shimadzu, Kyoto, Japan). Dissolved organic nitrogen (DON) was calculated as the difference between water-extractable TN and (NH_4^+-N + NO_3^--N). Microbial biomass C and N contents (MBC and MBN) were calculated as the difference between fumigated and non-fumigated salt-extractable C and N samples, and no correction factors for fumigation efficiencies were applied to the microbial C or N. All C and N concentrations in the extracts were corrected for the dilution associated with the moisture content of each soil sample. Results are presented as mass of C or N m^{-2} of organic material to 10 cm depth, assuming that organic material is $\geq$10 cm deep for all plots and that the bulk density does not change over that depth interval.

Statistical analyses

The effects of N-addition and deepened snow relative to control were analyzed separately, each with a repeated measures multivariate analysis of variance (RM MANOVA) after transforming data to approach normality. The RM MANOVA determines the effect of sampling date within each treatment with univariate tests. When conditions of sphericity were not met (which falsely inflates the F statistic), the Greenhouse-Geisser estimate was used to reduce the degrees of freedom (von Ende 1993), and thus the degrees of freedom contain decimals in some cases. Spring and summer data for control plots were compared with a one-way ANOVA. The assumption that spring is more variable than summer was tested with O'Brien's ANOVA, which compares sample variances between seasons (O'Brien 1979). Stepwise regression was used to isolate variables that explained a significant amount of variation in patterns of gas flux. All analyses were performed with JMP 7.0 (SAS 2007, Cary, NC) at the $\alpha = 0.05$ level of significance, and all significant results including interactions are reported.

Results

Soil environment

Our study was conducted in the middle of that particular phase of early spring in tundra when snowmelt has recently been completed, leaves of many of the evergreen plant species had not re-greened, and deciduous plants had not yet leafed out. Mean diel soil temperatures rose above 0°C on May 21st (day 141) in control plots and on May 30th (day 150) in snowfence plots (where occasional small patches of snow were still present). However, the temperature loggers in control and snowfence plots indicate dips in soil temperature to 0°C or below (min. −1°C) at 5 cm depth early each morning, with the temperature cycle amplitude at this depth ranging from 1 to 6.5°C just prior to and at the first sampling on May 31st (day 151; Fig. 2a). A cold weather front passed over the region on June 4th, dropping air temperatures below zero (min −4°C) and resulting in more severe soil temperature minima ($\sim$−1°C) in all plots on that day as measured with the data logger (Fig. 1) and with the handheld thermometers at 2 and 5 cm depth ($F_{3,36} = 448.0$; $P = < 0.0001$ and $F_{3,36} = 185.4$; $P = < 0.0001$, respectively). Soil temperature was not measured with thermocouple probes in the N-addition plots, but results from the hand-held thermometers indicate a similar soil temperature during sample times as in the control plots.

The active layer (i.e. soil thaw depth) at the first sampling date was less than half as deep in the

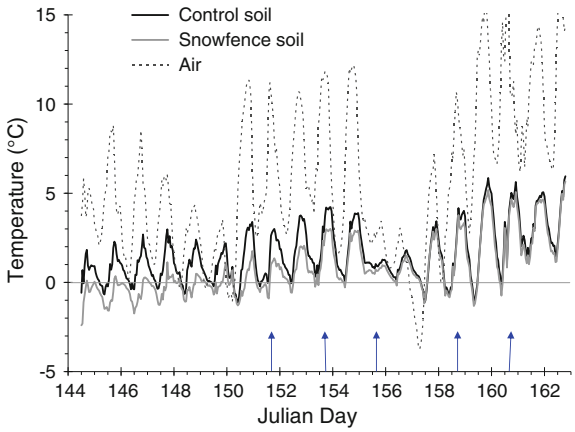

Fig. 1 Hourly soil temperature (°C) at 5 cm depth in control and deepened snow plots and air temperature during late winter and early spring 2006 ($n = 12$ probes (control) or 8 probes (snowfence)). The arrows represent approximate sampling times during this study. Snow melt was complete on day 141 in control plots and day 150 in snowfence plots (see Fig. 5)

snowfence plots compared to controls ($t = 2.4$; $P = 0.006$; Table 1). Soil thaw increased in all plots (Time: Snowfence, $F_{1.5,\ 11.7} = 86.3$; $P < 0.0001$; N-addition, $F_{1.6,\ 12.8} = 39.1$; $P < 0.0001$), although more rapidly in the snowfence plots (Time × Treatment: $F_{1.5,11.7} = 5.1$; $P = 0.04$; Table 1), resulting in similar active layer depths by the end of the study. Soil gravimetric water (i.e. liquid water and ice) was variable among all plots (range: 1.8–7.6 g H_2O/g soil), but fairly constant across the study, typically dropping on June 2nd (day 153) and June 7th (day 158) by ~20% (Time: Snowfence, $F_{4,32} = 2.9$, $P = 0.04$; N-addition, $F_{4,32} = 2.8$; $P = 0.04$; Table 1). However, soil volumetric liquid water contents determined using the hand-held probes did not differ between dates for the control plots during the study period. The mean volumetric water content across all samples measured with probes was similar to, and not lower than, volumetric water calculated from gravimetric soil water and soil bulk density (soil water was 61.2% and 54.6% of the soil matrix, respectively), suggesting that all soil water to 5 cm depth was liquid on all sampling days including on June 4th, immediately after the cold weather front. Soil moisture did not differ significantly between treatments, although there was a trend for the control plots to be drier (Treatment: Snowfence, $F_{1,8} = 3.6$; $P = 0.09$; N-addition, $F_{1,8} = 3.7$; $P = 0.09$; Table 1). Soil bulk density (range: 0.054–0.266 g soil/cm^3) did not differ significantly between treatments or across the sample period.

Soil biogeochemistry and microbial dynamics

The purpose of our study was to investigate the potential impacts of in situ air temperature freeze–thaw cycles on soil biogeochemical dynamics in early spring. Most soil solution and microbial biomass pools and trace gas production rates generally varied significantly but not strongly over the early spring period. For example, although NH_4^+-N did not vary significantly over time in any plot, NO_3^--N fluctuated in control and snowfence plots and was relatively low on the 4th of June (day 155; 0.006 g/m^2 as opposed to 0.010 –0.014 g/m^2) when soil gravimetric water was high and soil temperatures cool (Time: $F_{4,32} = 9.6$, $P < 0.0001$; Fig. 2a and b). Soil DON dropped in the control plots to a low on the 7th of June (day 158), without changing significantly in the N-addition plots or snowfence plots, resulting in significant Time effects (Snowfence: $F_{4,32} = 14.5$, $P < 0.0001$; N-addition: $F_{2,16} = 8.6$, $P = 0.003$) and Time × Treatment effects (Snowfence: $F_{4,32} = 3.8$, $P = 0.01$; N-addition: $F_{2,16} = 10.4$, $P = 0.001$; Fig. 2c). Soil DOC varied significantly over time in the snowfence and control plots and was lower on the 4th and 7th of June (days 155 and 158; Time: $F_{4,32} = 3.1$, $P = 0.03$; Fig. 2d).

N_2O-N and CH_4-C fluxes did not vary significantly over the sample period (Fig. 3a and b). By contrast, microbial respiration (CO_2) was low in all treatments during the cold weather front on June 4th (day 155, Time: Snowfence, $F_{3,24} = 6.7$; P = 0.002; N-addition, $F_{3,24} = 9.1$; $P = 0.0003$; Fig. 3c). Spatial and temporal variation in CO_2 patterns were best explained by a combination of soil temperature at 2 cm depth, active layer depth and MBC:N (log-$CO_2 = (-0.4) + (0.25$ (square root[soil temperature])) + (0.05 (active layer)) + (0.12 (square root [MBC:N])); $R^2 = 0.36$; $P < 0.0001$).

MBC in the snowfence plots was high on the 31st (day 151) and low on the 9th (day 160; Time × Treatment: $F_{4,32} = 4.3$; $P = 0.006$) and was also lower in the N-addition plots on the 9th (Time × Treatment: $F_{4,32} = 4.2$; $P = 0.007$; Fig. 4a). MBN in the snowfence plots was low on the 2nd and the 9th (days 153 and 160), producing a significant Time ($F_{4,32} = 5.6$; $P = 0.002$) and Time × Treatment effect ($F_{4,32} = 2.8$;

Table 1 Active layer depth and soil gravimetric water content in control, deepened snow and N-addition plots in early spring 2006; s.e. is standard error ($n = 5$)

		Julian day									
		151		153		155		158		160	
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Active layer depth (cm)	Control	11	1.3	12	1.3	14	1.5	14	1.7	15	2.2
	Snowfence	5.3	1.1	7.6	0.67	9.7	0.71	11	0.76	13	0.52
	N-added	10	0.55	12	0.83	12	0.91	12	0.86	14	1.1
Gravimetric water content (g/g)	Control	4.2	0.26	3.4	0.43	4.2	0.38	3.7	0.35	3.7	0.20
	Snowfence	5.7	1.0	4.4	0.95	5.5	0.46	4.6	0.48	5.0	0.29
	N-added	4.6	0.40	4.7	0.67	5.3	0.43	4.7	0.51	4.2	0.16

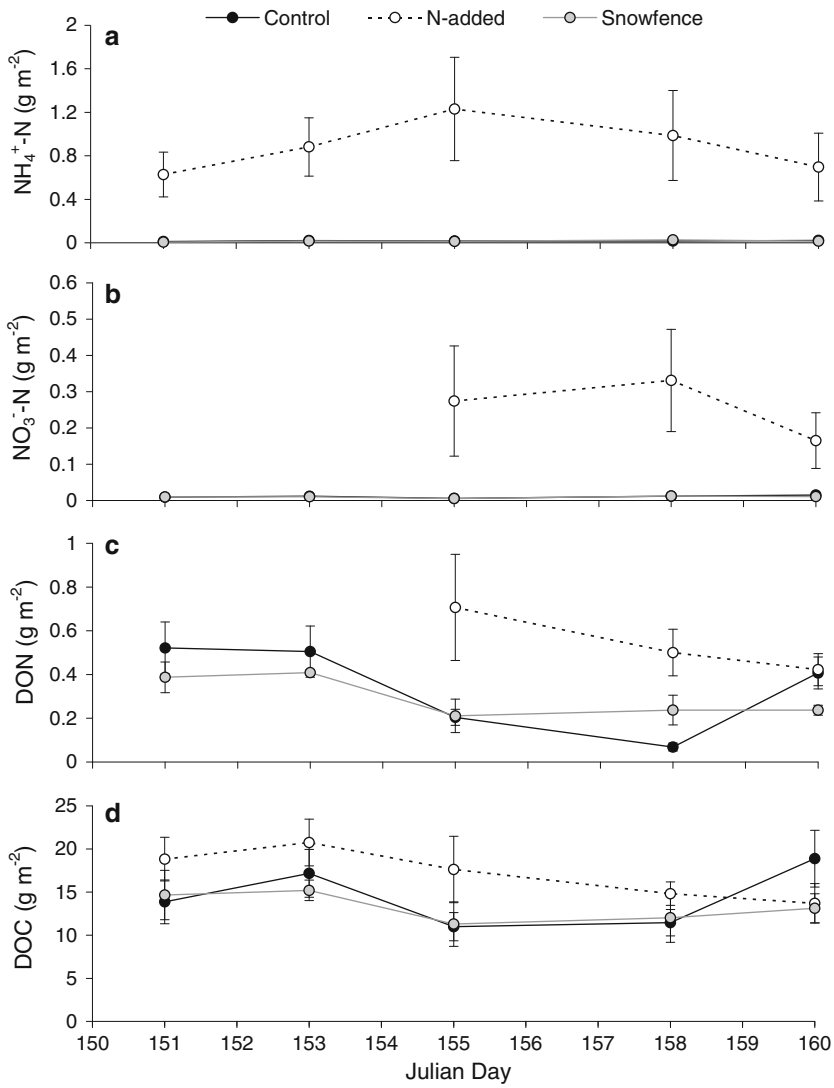

Fig. 2 Water-extractable soil solution ammonium-N (**a**), nitrate–N (**b**), dissolved organic N (**c**), and dissolved organic C (**d**) pools in the top 10 cm of organic soil in control, deepened snow and inorganic N-addition plots in early spring 2006. Bars indicate ± one standard error; $n = 5$. Nitrate (and therefore dissolved organic nitrogen) was not determined in the N-addition plots on days 151 and 153

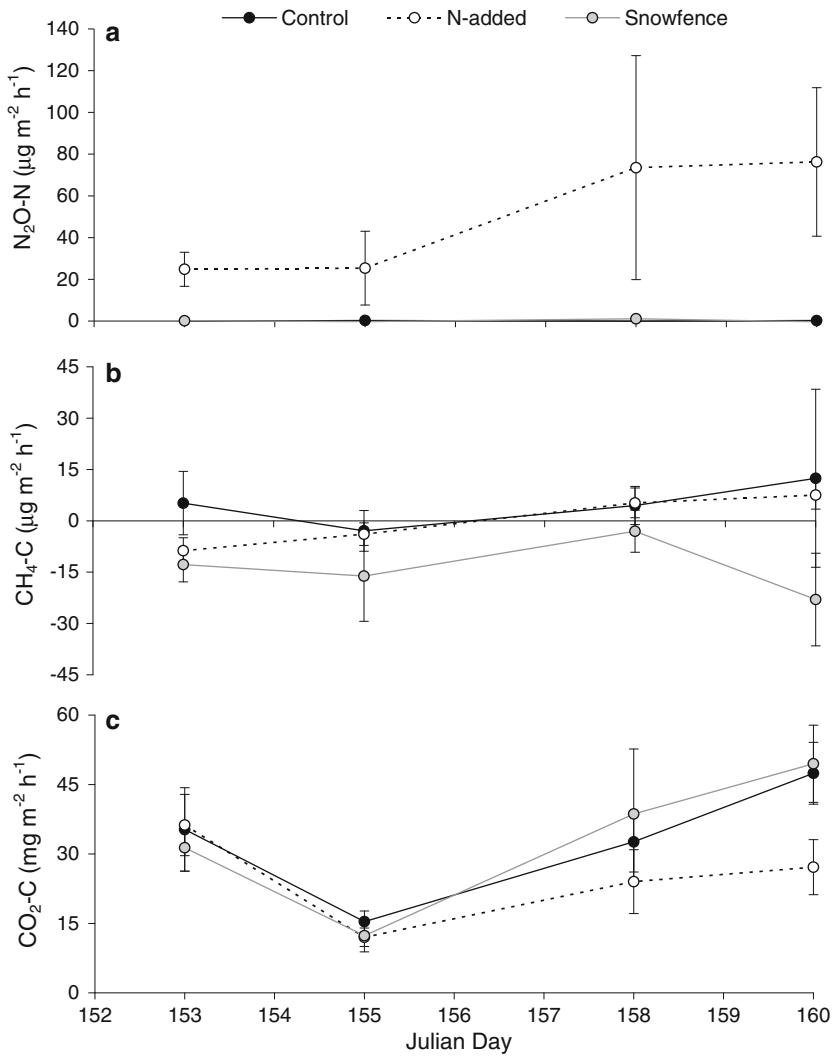

Fig. 3 Nitrous oxide-N (**a**), methane-C (**b**), and carbon dioxide-C (**c**) fluxes in the early spring from control, deepened snow and inorganic N-addition plots in early spring 2006. Error bars indicate ± one standard error; $n = 5$. In contrast to the other biogeochemical variables, gases were not sampled on day 151

$P = 0.04$; Fig. 4b). MBN in the N-addition plots did not vary significantly over the study (Fig. 4b). The MBC:N ratio increased in all plots from the 31st to the 2nd, and then dropped (Time: Snowfence, $F_{4,32} = 18.2$; $P < 0.0001$; N-addition, $F_{4,32} = 4.8$; $P = 0.004$), with a more pronounced rise and fall in the snowfence plots (Time × Treatment: $F_{4,32} = 3.5$; $P = 0.02$; Fig. 4c).

Soil microbial and biogeochemical variables measured on the first, middle and last spring dates and three summer dates were quite similar in both means and variances (Table 2). The exception was soil NH_4^+-N, which differed between seasons, almost doubling in the summer (Table 2). Spring microbial and nutrient pools were generally not significantly more variable than summer pools although there was a strong trend for MBN to be more variable in spring, and soil NH_4^+-N to be more variable in summer (Table 2).

As expected, fertilization with inorganic N in the previous two growing seasons dramatically increased NH_4^+-N, NO_3^--N and DON pools (NH_4^+-N: $F_{1,8} = 39.4$; $P = 0.0002$; NO_3^--N: $F_{1,8} = 10.7$; $P = 0.01$; DON: $F_{1,8} = 23.6$; $P = 0.001$; Fig. 2a–c), but had no significant effect on DOC, microbial respiration or methane fluxes (Figs. 2d, 3b and 3c). N fertilization greatly elevated N_2O emissions from negligible levels in control plots ($F_{1,8} = 27.6$; $P = 0.0008$; Fig. 3a). Furthermore, a significant amount of the large spatial and temporal variation in N_2O production in the control and N-addition plots ($n = 30$) was explained by NH_4^+-N and NO_3^--N

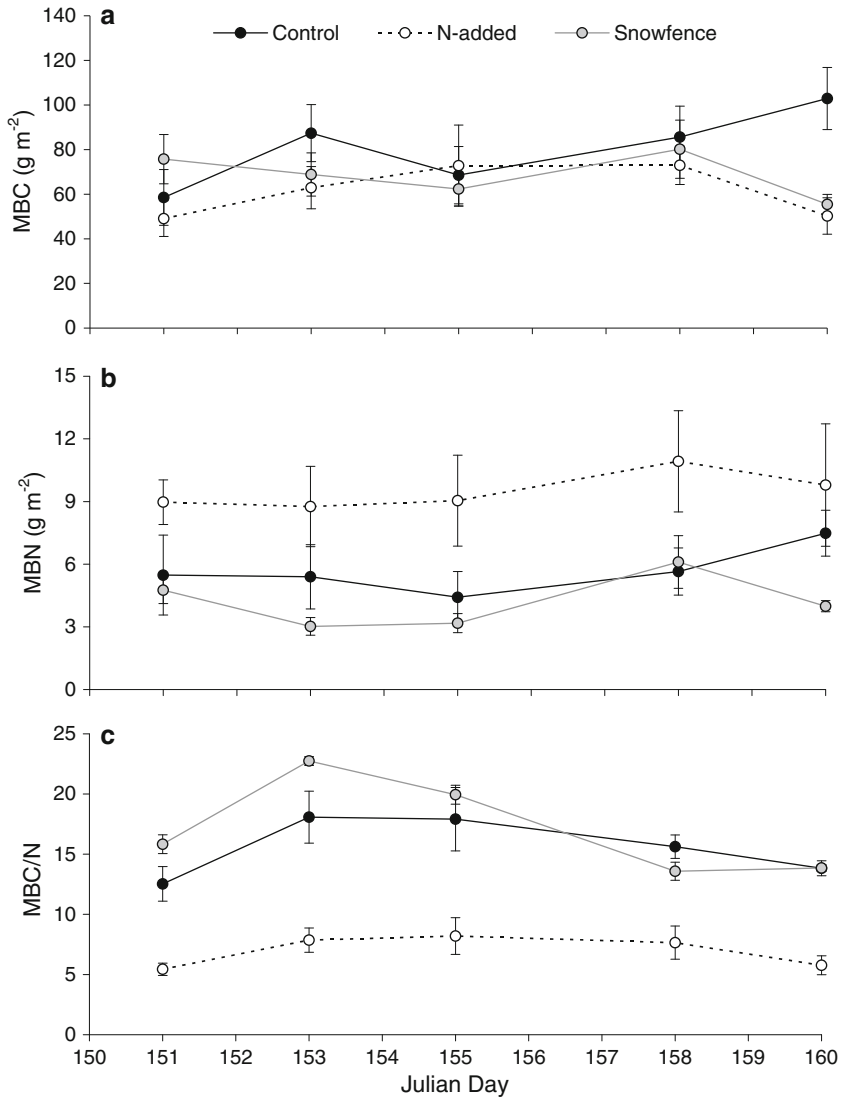

Fig. 4 Microbial biomass carbon (**a**); microbial biomass nitrogen (**b**) and microbial biomass carbon/nitrogen ratio (**c**) in the top 10 cm of organic soil, in control, deepened snow and inorganic N-addition plots in early spring 2006. Error bars indicate ± one standard error; $n = 5$

Table 2 Test of spring vs. summer variability over three sample dates in each season ($n = 15$)

		Spring		Summer		O'Brien's			ANOVA		
		Mean	CV	Mean	CV	df	*F*	*P*	df	*F*	*P*
NH_4^+-N_{salt}	(mg m^{-2})	14	0.91	30	0.44	1, 28	3.0	0.09	1, 28	18	0.0002
DOC_{salt}	(g m^{-2})	7.7	0.43	12	0.64	1, 28	2.1	0.2	1, 28	2.8	0.1
DTN_{salt}	(g m^{-2})	0.67	0.48	1.0	0.75	1, 28	0.70	0.4	1, 28	2.8	0.1
MBC	(g m^{-2})	77	0.44	59	0.37	1, 28	1.8	0.2	1, 28	1.9	0.2
MBN	(g m^{-2})	5.8	0.57	4.4	0.33	1, 28	4.1	0.053	1, 28	0.70	0.4
MBC:N		15	0.30	14	0.26	1, 28	0.22	0.7	1, 28	0.40	0.5

CV is coefficient of variation (standard deviation/mean) where CV < 1 indicates low variance. O'Brien's test is a measure of unequal sample variances; significant results imply that one sample is more variable than another; the ANOVA is used to test for differences between seasonal sample means. DOC_{salt} and DTN_{salt} is 0.5 M K_2SO_4-extracted soil dissolved organic carbon and total nitrogen, respectively; MBC, MBN and MBC:N are microbial biomass carbon, nitrogen and their ratio, respectively; df is degrees of freedom

($r^2 = 0.43$; $P < 0.0001$ and $r^2 = 0.34$; $P = 0.0003$, respectively), suggesting that substrate availability was a critical control on N_2O production. Although MBC was not significantly affected by N addition, MBN approached significance ($F_{1,8} = 3.9$; $P = 0.08$) and was significantly higher than in control plots when all dates were pooled ($F_{1,48} = 12.7$; $P = 0.0009$; Fig. 4a and b). As a result, there was a significant decrease in the MBC:N ratio ($F_{1,8} = 26.6$; $P = 0.0006$; Fig. 4c).

Although the deepened snow resulted in later and therefore more rapidly thawing soils during the study period, there were no significant treatment effects on soil biogeochemistry or microbial biomass, or CO_2 or N_2O production rates. However, there was a significant switch from net methane production in control soils to net methane consumption in soils that had been under deepened snow ($F_{1,8} = 5.5$; $P = 0.05$; Fig. 3b).

Discussion

The impacts of fluctuating environmental conditions in early spring on soil biogeochemistry

Air temperature freeze–thaw cycles did not result in significant soil temperature freeze–thaw cycles in this mesic low arctic tundra site in this year. Soil temperatures declined as low as −1.5°C during the study period, potentially initiating ice nucleation in larger soil pores, especially closer to the surface where temperature minima are likely to be lower. However, high solute concentrations and water advection generally restrict ice formation to much lower soil temperatures (Outcalt et al. 1990; Torrance and Schellekens 2006; Kozlowski 2009). Once these mesic organic soils thawed, there was a strong resistance to refreezing, presumably as a result of the very large latent heat capacity associated with soil water phase changes. Annual temperature records from a nearby weather station (800 m) indicate that the last week of snowmelt is consistently characterized by a "zero-curtain" period (Outcalt et al. 1990) where soil temperatures remained static at 0°C (Fig. 5). After the snow melted and the ground was exposed, there was a 19 d period in 2006—the focus of this experiment—where the mean daily soil temperature at the top of the active layer and just below the soil surface fluctuated near and slightly above 0°C, but did not go below 0°C. This phenomena is similar in other years at our site and appears to be common throughout mesic arctic tundra soils (Outcalt et al. 1990; Hinzman et al. 1991; Olsson et al. 2003). Together these results strongly suggest soil freezing in early spring is infrequent in mesic tundra ecosystems.

The small, naturally occurring temperature fluctuations recorded in the organic soil layer did not result in a detectable spring nutrient pulses in the soil solution at our low arctic tundra site, refuting Hypothesis 1. We observed minor but statistically significant temporal variations in CO_2, DON, MBC and MBC:N in all plots, and in NO_3, DOC and MBN in the deepened snow plots. Furthermore, microbial biomass nitrogen tended to be more variable in spring than summer, however, the variability in all microbial factors was small (Table 2), suggesting that the microbial biomass is fairly constant throughout the spring and summer phases of the snow-free season, refuting Hypothesis 2. Our findings contrast with several laboratory studies indicating that freeze–thaw cycles may disrupt the soil and/or microbial community and produce a flush of CO_2, N_2O and nutrients to the soil solution (Skogland et al. 1988; Schimel and Clein 1996; Herrmann and Witter 2002; Larsen et al. 2002; Sharma et al. 2006). Many of these studies used lower temperature minima and higher frequency cycles than occur naturally in hummock tundra soils on diurnal or weekly time frames (Henry 2007). Several other laboratory investigations have reported moderate or non-existent pulses in some biogeochemical variables in tundra soils that have been cycled through realistic temperature regimes (Lipson and Monson 1998; Sulkava and Huhta 2003; Grogan et al. 2004). In addition, a field study indicated that soil microbial and soluble pools were low and similar on two close dates in early spring in an inundated arctic wet sedge meadow (Edwards et al. 2006) where soil freezing is very unlikely due to the high moisture content. Here, we provide strong in situ field evidence at a relatively high temporal resolution that early spring (i.e. the days immediately after snow melt and soil thaw) is not a period of dynamic C or N pulsing in mesic tundra soils.

Our results suggest that realistic, mild fluctuations around zero degrees in tundra soil are not a source of spring nutrient pulsing as they are not detrimental to

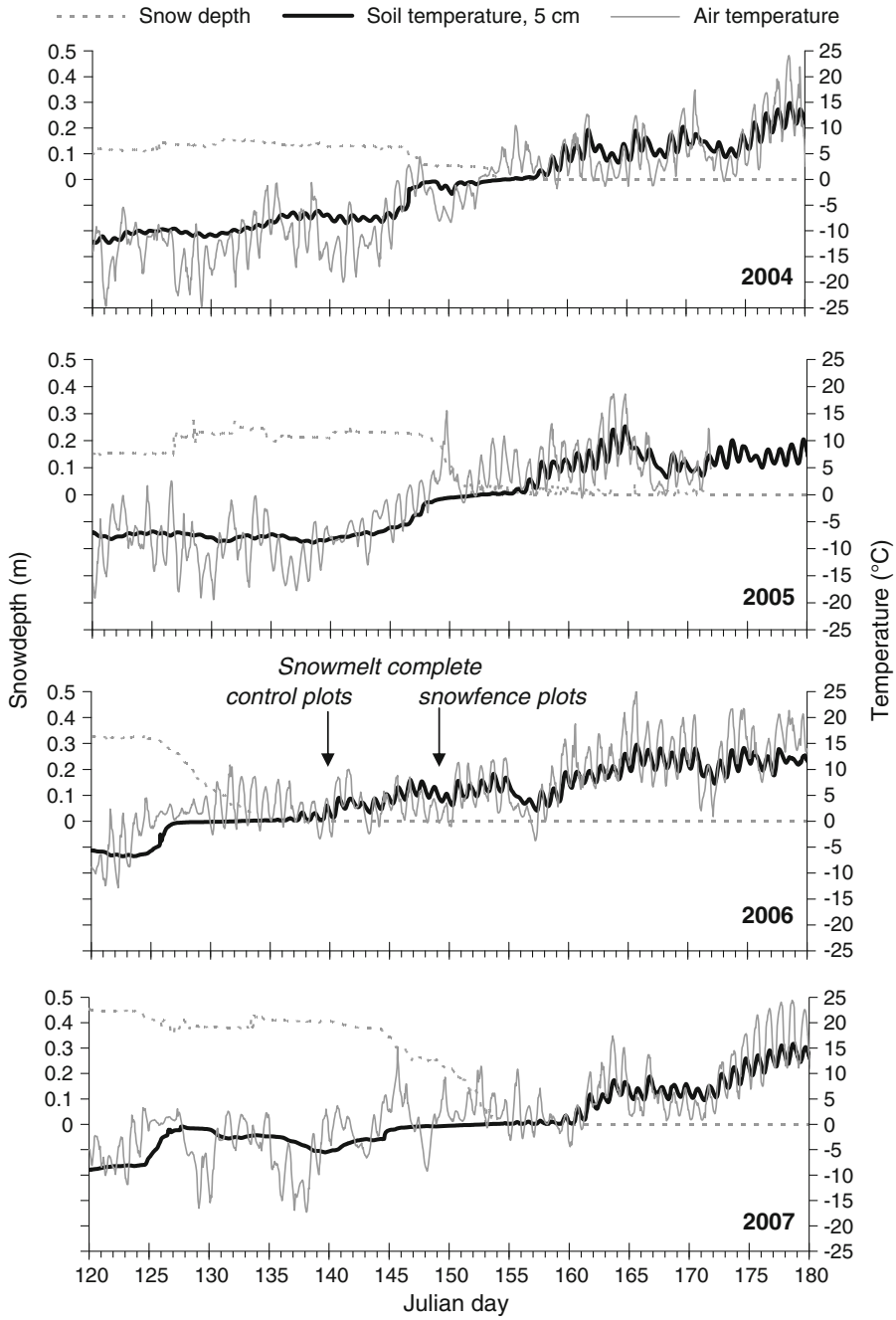

Fig. 5 Hourly soil and air temperatures and snowdepth from the beginning of snowmelt to the start of the frost-free period, from 2004 to 2007 at a weather station in the same valley as our study site. The timing of snowmelt completion in the control (and N-addition) and deepened snow plots in the year of our study (2006) is indicated. Data courtesy of Bob Reid (INAC Water Resources Division)

tundra soil microbes, perhaps due to the large microbial arsenal of osmolytes, antifreeze and ice nucleating proteins (D'Amico et al. 2006; Walker et al. 2006) that act as protectants against environmental fluctuations at spring thaw. The larger fluctuations in air and soil temperatures that occurred after the freezing weather front passed through the region on June 4th may have caused the significant drop in CO_2, soil NO_3^--N and soil NH_4^+-N on that day. However, if temperature changes are an important determinant of nutrient pulses, we suggest that such pulses may occur earlier than in the period measured in this experiment when arctic soils typically, and rapidly, increase in temperature from −20°C to −5°C, within a few hours of the onset of sustained mean diel air temperatures above 0°C

(Hinzman et al. 1991; Olsson et al. 2003; Buckeridge and Grogan 2008). As a result of the strong chemical potential gradients between frozen and thawed water, rapid water movement, changes in soil water film thickness and fluctuating solute concentrations may exist in soils at these lower temperatures (Rivkina et al. 2000; Torrance and Schellekens 2006), at a scale that is critical and stressful to microbial cells (Schimel et al. 2007). If moisture changes are important in determining nutrient pulses (Schimel et al. 2007), then they may occur just after this rapid step up in temperature, when mean diel air temperatures are above zero, and the snow pack begins to thaw (Olsson et al. 2003; Edwards et al. 2006). This step-up occurs ~2 weeks before snow melt and freeze–thaw cycling in hummock tundra soil, and therefore 2 or 4 weeks (snowfence and control plots, respectively) before our first sample date in this study. In summary, although nutrient pulses may well occur in response to temperature and/or moisture changes beneath the snow, our in situ field data strongly suggest that the principal soil biogeochemical pools in this mesic ecosystem are stable once snowmelt is complete in early spring.

The impact of added N on trace gas production, microbial biomass and soil biogeochemistry in early spring

N_2O production and consumption rates were very low at our site, except in plots where substantial inorganic N had been added. These results demonstrate that N_2O production was very strongly limited by the availability of inorganic N substrate in early spring (supporting hypothesis 3), and that fluctuations in soil temperature and moisture were relatively unimportant controls on this process at this time. Low denitrification or N_2O consumption is consistent with other field studies in subarctic or arctic organic soils, at least in the summertime, as is the stimulation of denitrification following inorganic N addition (Chapin 1996; Christensen et al. 1999; Sorensen et al. 2006). Although we cannot say for sure that soil temperature fluctuations did not exacerbate these high rates (because we could not control for this), we saw no significant temporal variation over the study, despite our fairly long sampling periods (several hours in the warmest part of each day and the passage of at least one freezing weather front). Therefore, our results differ from recent measurements that found soil freeze–thaw cycles promoted high rates of denitrification in the Mongolian Steppe (up to 70 μg N_2O-N m^{-2} h^{-1}); although these rates were under snow and dropped substantially after snowmelt, even as soil temperatures continued to fluctuate (~−2 to +7°C) (Holst et al. 2008). Instead, our results indicate large spatial variation in N_2O production that was positively and fairly closely correlated ($r^2 = 0.40$) with the large pools of inorganic N in the soil solution that were the result of N-additions in the previous two summers. Strong positive associations with soil ammonium and nitrate pools suggest that N_2O production may be due to both nitrification and denitrification in the organic soils we investigated. Although N_2O production via denitrification has been documented in peat soils (Aerts 1997), a recent study in tundra soils has identified nitrifiers as the main source of N_2O (Siciliano et al. 2009). The relative importance of these two processes in low arctic tundra soils has not been identified and cannot be tested directly without stable isotopes or acetylene reduction techniques.

N-additions substantially increased soil solution inorganic N, and appeared to stimulate soil decomposition, as indicated by increased pools of dissolved organic N. The increased N availability and N cycling in N-addition plots resulted in a larger incorporation of N into the microbial biomass and a substantially lower microbial C:N ratio, relative to the control plots (Fig. 4). In temperate, alpine and sub-arctic systems soil microbes may act as intermediate retainers of the spring flush of N before plant N uptake begins (Zak et al. 1990; Jaeger et al. 1999; Grogan and Jonasson 2003), theoretically promoting tight internal soil cycling of N. Our results suggest that this microbial mechanism of springtime ecosystem nutrient retention may be important, at least in fertilized tundra.

Nutrient addition beyond the upper limit to microbial N immobilization in early spring may be removed from the system, by facultative denitrifiers, or via physical means as leachate flows to the mineral soil or overland surface flows during spring thaw. For instance, our soil solution results were based on water-extracted values, and thus should indicate the pool of nutrients that could readily flow to aquatic systems during snowmelt run-off when soils are saturated (Hinzman et al. 1991). We added 20 g N m^{-2} over the two years before this study

and documented enhanced N_2O fluxes in the range of 100 μg N_2O-N m^{-2} h^{-1}; however, this is still <1 g N_2O-N m^{-2} $season^{-1}$, assuming comparable N_2O flux rates for all snow-free days. Long-term studies of repeated N-additions in tundra soils have found a lower overall amount of N in fertilized soils after 20 years of fertilization, due to depleted soil N at depth, that is attributed to fertilizer-promoted increases in soil decomposition down the soil profile (Mack et al. 2004) and an unexplained mechanism of N loss. Our early spring results suggest that this N loss in fertilized tundra is unlikely to be explained by N_2O production, and that the missing N may be more likely attributed to N_2 or leachate loss.

The impact of deepened snow on soil biogeochemistry in early spring

Deepened snow resulted in colder soils and rapid active layer development in the soils we sampled, but did not lead to strong differences in measured soil microbial or biogeochemical properties, refuting hypothesis 4. Nevertheless we observed net methane consumption in the snowfence soils, as compared to net methane production in control plots. In general, methane fluxes were very low from the birch hummock tundra plots (-0.07 to +0.1 mg CH_4-C m^{-2} h^{-1}), compared to measures at the same time of year in wet sedge at this research site (mean 1.3 mg CH_4-C m^{-2} h^{-1} on June 10th 2006; P. Grogan, *unpublished data*) or CH_4 pulses during fall freeze in other wetland tundra soils (10–15 mg CH_4-C m^{-2} h^{-1} (Mastepanov et al. 2008)). Together these results suggest that methane fluxes from mesic arctic ecosystems are unlikely to be significant even immediately after snowmelt in early spring when these soils had recently been inundated with snowmelt water.

Deepened snow can significantly increase microbial biomass N at our site (Buckeridge and Grogan 2008), and net N mineralization in Alaskan tundra over winter (Schimel et al. 2004), presumably as a result of warmer soil temperatures (Walker et al. 1999; Schimel et al. 2004; Buckeridge and Grogan 2008). This enhanced winter N mobilization may contribute to plant N requirements in the next growing season or ecosystem losses of N with spring thaw. Yet here we have found no differences in N pools in the soil and microbial biomass between control and snowfence plots, suggesting that any excess N mineralized or immobilized over winter as a result of deepened snow had been acquired by roots or lost as gas or leachate at or prior to snowmelt.

In conclusion, our results suggest that air temperature freeze–thaw cycles in the spring immediately after snowmelt are not detrimental to the soil microbial biomass and are not associated with increased soil solution nutrient pulses. The snowfence manipulation results imply that the warmer winter soil temperatures and later thaw associated with the deepened snow pack did not promote differences in spring soil biogeochemistry. N_2O production did not appear to be a major pathway for N loss from the ecosystem at this time in early spring, as denitrification in these mesic hummock tundra soils was primarily limited by the lack of available inorganic N. Overall, we conclude that biogeochemical cycling in this particular phase of spring, just after snow has melted and the soils have thawed, appears to be much less dynamic than previously believed, at least for birch hummock ecosystems. We suggest that future research on the potential biogeochemical importance of the winter-spring transition in tundra ecosystems should focus not on soil freeze–thaw cycling, but on the initial thaw period when soil temperatures very rapidly rise from winter minima up toward zero and remain stable beneath the melting snowpack.

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