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Soil Biology & Biochemistry 36 (2004) 641–654

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms

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Received 27 September 2002; received in revised form 8 October 2003; accepted 8 December 2003

Abstract

Freeze–thaw fluctuations in soil temperature may be critical events in the annual pattern of nutrient mobilisation that supplies plant growth requirements in some temperate, and most high latitude and high altitude ecosystems. We investigated the effects of two differing freeze–thaw regimes, each of which is realistic of in situ spatial and temporal variation in field conditions, on C and N dynamics in sub-arctic heath tundra mesocosms. In addition, ^{15}N isotopic label was used to follow the partitioning of a labile N pool between major ecosystem components, both during the freeze–thaw treatments phase, and in a subsequent equilibration phase. A single deep freeze treatment phase enhanced dissolved total and labelled N pools in the soil solution at initial thaw, and resulted in reduced pool sizes at the end of the equilibration phase. By contrast, a multiple freeze–thaw cycling treatment directly enhanced the dissolved labelled N pool, but did not significantly affect dissolved total N. Furthermore, both dissolved labelled N and dissolved total N pools were significantly enhanced in the equilibration period following multiple freeze–thaw, the latter due to a marked increase in soil solution NH_4^+. Microbial biomass C was not significantly affected by either of the freezing treatments upon final thaw, but was significantly reduced over the combined treatment and equilibration phases of the multiple freeze–thaw regimes. Furthermore, the treatments had no significant effects on total or labelled N within the microbial biomass over either phase. Total mesocosm CO_2 efflux rates remained closely correlated with soil temperature throughout the experiment in both regimes, suggesting that respiratory flushes associated with treatment-induced microbial cell lysis were negligible. Together, these results indicate that moderate freeze–thaw fluctuations may have minimal influences on microbial biomass pools, but nevertheless can have strong contrasting effects on the amounts, forms, and timing of N and organic C supply into the soil solution. Ecosystem losses via N_2O effluxes were of greatest magnitude immediately upon thawing in both treatments, and were of similar total magnitude to inorganic N leachates in throughflow. Herb leaves, total fine roots, and vascular stems accumulated some ^{15}N label in one or both of the freezing treatments by the end of the experiment. Together, these results indicating very small N losses relative to the magnitudes of internal transfers, suggest tight ecosystem N cycling both during and after freeze–thaw events. Furthermore, our small and subtle effects on microbial and soluble C and N pools relative to previous studies using more severe regimes, suggests that periods of moderate freeze–thaw fluctuations may have only a minor influence on the annual pattern of C and nutrient dynamics in seasonally cold ecosystems.

Keywords: Nitrogen; Freeze–thaw; Carbon; Biomass; Arctic; Alpine; Microbe; ^{15}N isotope; N_2O; Ecosystem; Heath; Winter

1. Introduction

Freeze–thaw fluctuations in soil temperature are common in some temperate, and most high latitude and high altitude ecosystems. Their effects on soil biogeochemical processes are a subject of major ecological interest, because it is often suggested that freeze–thaw events may be a major factor contributing to the microbial release of nutrients in plant available form (Dowding et al., 1981; Schimel et al., 1996; Weih, 1998; Lipson et al., 1999; Grogan and Jonasson, 2003). Furthermore, since plant productivity is often strongly limited by nutrient availability (Vitousek and Howarth, 1991), freeze–thaw effects on soil nutrient transformations may substantially influence the C balance of seasonally cold ecosystems.

Freezing and subsequent thawing of soils often results in an initial flush of microbial respiration

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0038-0717/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2003.12.007

(Burton and Beauchamp, 1994; Schimel and Clein, 1996), an increase in soil solution nutrients (Brooks et al., 1998; Wang and Bettany, 1993), and transient N_2O effluxes (Christensen and Tiedje, 1990; Muller et al., 2002). Laboratory incubation studies indicate that freeze–thaw cycles can lyse a substantial proportion of microbial cells, resulting in C and N releases into the surrounding soil (Ivarson and Sowden, 1970; DeLuca et al., 1992), that may be immobilised by surviving microbes as they consume the enhanced supply of C substrate (Morley et al., 1983; Skogland et al., 1988). These controlled laboratory experimental studies have investigated the responses of small soil volumes to temperature fluctuations with severe soil temperature minima (−7 to −20 °C) over substantial periods (days to months), that may not be characteristic of typical freeze–thaw conditions experienced at many field sites. Other studies in which the temperature regime has been relatively benign (e.g. minimum soil temperatures of −4 to −10 °C for a few hours) often do not reveal significant effects on respiration or soil solution contents (Gasser, 1956; Lipson and Monson, 1998). Thus, the extent and biogeochemical significance of freeze–thaw processes to overall ecosystem nutrient cycling remains unclear, and may vary substantially depending on the precise character of the thermal regime.

Finally, the influence of plants on the overall ecosystem response of biogeochemical cycling to freeze–thaw temperature fluctuations is largely uninvestigated. Most of the above conclusions have been based on studies of soils without vegetation. Soil microbial responses to freeze–thaw are clearly important since soil microbes in high latitude ecosystems generally contain large and active nutrient pools (Jonasson et al., 1999a). Nevertheless, overall ecosystem responses to freeze–thaw events could be strongly influenced by the potential for: (a) plant acquisition of N from the enhanced soil solution pool after freeze–thaw; and (b) root tissue mortality due to freeze–thaw that may result in increased labile C inputs to soil, thereby enhancing microbial immobilisation. Therefore, studies incorporating plants are necessary to fully evaluate freeze–thaw effects on whole ecosystem nutrient and C cycles.

We have exposed sub-arctic heath tundra mesocosms to two different freeze–thaw treatments, both of which are likely to be realistic of field conditions that occur in their place of origin at different times and microsites in early and late winter (Grogan et al., 2001). We measured all major N pools at intervals through the experiment, and also followed the fluxes of an added ^{15}N isotopic label between ecosystem components. Our aim was to investigate if variations in the character of moderate freeze–thaw regimes can have different effects on internal C and N biogeochemical dynamics, as well as on gaseous and leachate N losses. We tested the following hypotheses:

(a) Differing freeze–thaw regimes that are typical of natural variation in thermal conditions in the field can have significantly different effects on C and N dynamics.
(b) Plants can acquire N during, and directly after, periods of freeze–thaw.
(c) Freeze–thaw fluctuations in heath tundra can result in substantial ecosystem N losses via throughflow leachates and N_2O effluxes.

2. Materials and methods

2.1. Site description

This study was conducted on soil–plant mesocosms sampled from a heath ecosystem site (Jonasson et al., 1999b) near Abisko (N 68° 20′ 42″; E 18° 50′ 18″, ~450 m a.s.l.) in North Sweden. The climate is sub-arctic with mean summer and winter temperatures of 10 and −9 °C, respectively, precipitation totalling ~300 mm, and a snow-covered winter season usually lasting from early October to late May (http://www.ans.kiruna.se/ans.htm). The vegetation is a species-rich dwarf shrub community dominated by the evergreen *Cassiope tetragona* (L.), but also containing *Empetrum hermaphroditum* (Hagerup), *Vaccinium uliginosum* (L.), *Betula nana* (L.), occasional herbs (predominantly grass spp.), and significant moss cover (Havström et al., 1993; Michelsen et al., 1996). The site is on an exposed, well-drained slope with a soil organic layer of ~15 cm depth. The organic layer has a bulk density of 0.06 to 0.12 g soil organic matter (SOM) cm^{-3} (mean = 0.08; $n = 24$), and its organic matter content varies from 44 to 95% (mean = 84%; $n = 24$).

2.2. Mesocosm sampling and preparation

On 26 July, 1998, intact mesocosms were removed from randomly selected areas by excavating down to as far as the underlying mineral soil/rock deposits so that the samples (21–24 cm dia, 12–17 cm depth) contained the complete soil organic layer and all rooted vegetation. The mesocosms were placed in pots and transported within 3 d to a gravel bed within the Botanical Garden at The University of Copenhagen, Denmark. After 6 weeks, the pots were moved to growth chambers that maintained the soil temperature at 8 °C (measured at 5 cm depth in organic soil layer) and a photoperiod of 16 h. On 5 Nov, the soil temperature was lowered to 6 °C and the photoperiod was changed to 12 h. On 11 Feb, 1999, the soil temperature and photoperiod were reduced to 4 °C and 6 h, respectively. Throughout this time, de-ionised water (500–600 ml per week) was regularly added to the mesocosms. Although not ideal, this 'storage' period of declining temperature and photoperiod was necessary for logistical reasons. It may, however, have had several advantages in terms of avoiding disturbance

responses from both plants and microbes. Subsequent analyses and comparison with previous studies at the site indicated no adverse biogeochemical effects of the storage on major ecosystem pool sizes. Nevertheless, since other effects cannot be ruled out, some caution should be exercised in assuming that identical responses would occur in the field under natural conditions. Note that this concern does not affect our major conclusions, which are focused on differences in biogeochemical responses to freezing treatments within the experiment.

2.3. ^{15}N isotopic labelling

On 9 April, 1999, ^{15}N was added to each pot in a series of 5 ml injections of a 684 μM solution of 98% enriched $^{15}NH_4Cl$ (Cambridge Isotope Laboratories, MA, USA), resulting in an addition of 1030 μg ^{15}N per pot (equivalent to 1.2–3.8 μg ^{15}N (g SOM)$^{-1}$). A grid was placed on the moss surface to identify a uniform distribution of 19 injection points across the pot. A perforated needle was inserted to a depth 13 cm below the green moss upper surface, and withdrawn slowly at the same time as the syringe plunger was depressed to disperse the labelled solution through the soil. Direct labelling of the surface moss tissue was avoided by withdrawing the needle at a rate that ensured the syringe was emptied as the uppermost perforation in the needle reached within 3 cm of the moss surface. The labelling treatment resulted in a N addition to the inorganic N pool equivalent to 29.8 mg ^{15}N m^{-2}. This value is well below site measurements of 0.5 M K_2SO_4-extractable NH_4-N in the soil solution (0.1–1.0 g N m^{-2}) (Jonasson et al., 1999b; Michelsen et al., 1999) through the growing season. Nitrogen in the form of nitrate is generally a very small component of the inorganic N pool at the site (5 mg NO_3-N m^{-2}) (Michelsen et al., 1999).

2.4. Freezing treatment regimes

On 12 April, 1999, the mesocosms were randomly separated into three groups: four for immediate harvest to determine the initial distribution of the ^{15}N label prior to the treatments; and 10 for each of the two freezing regime treatments (Fig. 1). A single deep freeze treatment was achieved by placing the selected mesocosms in a darkened growth chamber (air temperature −10 to −15 °C). A multiple freeze–thaw cycling treatment was achieved by alternating mesocosms between a darkened growth chamber (air temperature −10 to −15 °C) and a darkened area within an adjacent laboratory (air temperature 20–25 °C) for 1–2 d periods. The mesocosms were exposed to five freeze–thaw cycles over 13 d, after which five mesocosms were removed from each growth chamber and allowed to thaw prior to harvest (3 d later). The air temperature of both growth chambers was then raised to 8 °C to allow an equilibration period (6 d) prior to the final harvest of the remaining mesocosms (25 d after initial labelling), in order

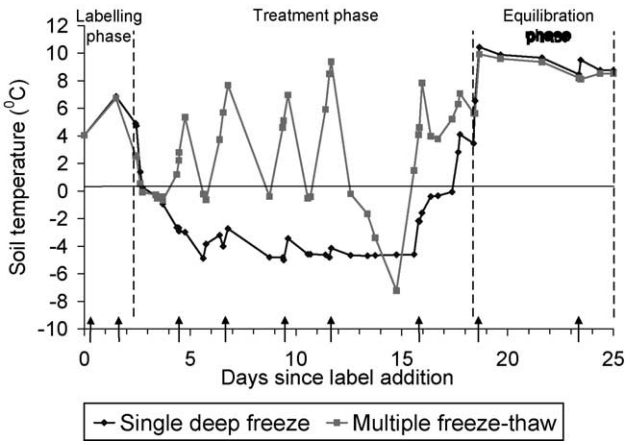

Fig. 1. Mean soil temperatures (5 cm depth) during the initial labeling period, and experimental treatment and equilibration phases of the two freeze–thaw regimes ($n = 5$). The dashed vertical lines indicate the three times when sample mesocosms were harvested. The arrows on the x-axis indicate the gas sampling times.

to identify follow-on effects of each of the treatments. Henceforth, those mesocosms ($n = 5$ in each treatment) that underwent both the freezing regime and subsequent equilibration phases are referred to as 'full-term'.

All mesocosm pots were watered (150 ml de-ionised) 2 d after labelling. Afterwards, no further water was added until the equilibration phase during which the full-term mesocosms received 150 ml (de-ionised) on days 19, 20, 21 and 23 after labelling. Throughout the experiment the bases of each of the full-term mesocosm pots were contained within individual plastic bags to accumulate throughflow leachates. The total volume of leachate drained from each pot was collected at 3, 19 and 24 d after labelling, and filtered (Whatman GF-D) prior to storage (−18 °C).

Soil temperature at 5 cm depth in the F-layer below the transition from green to brown moss tissue in the centre of each of the full-term mesocosms was recorded frequently (including at all gas sampling time points) using hand-held temperature probes.

2.5. Gas measurements

CO_2 and N_2O efflux rates from the full-term mesocosms were measured at intervals of 1–5 d through the treatment and equilibration phases of the experiment (Fig. 1). During the treatment phase, gas efflux rates were measured in the thaw periods of the multiple freeze–thaw cycling treatment, and at simultaneous periods in the single deep freeze treatment.

Respiratory CO_2 release was measured using an infra-red gas analyser (LI-COR 6200, Nebraska, USA) attached to a darkened chamber (5.4 l) fitted with a small circulation fan. The chamber was pressed down tightly over the circumference of each mesocosm pot and allowed to equilibrate for >5 min. Afterwards the chamber pressure equilibration hole was sealed, and where necessary the headspace CO_2 concentration was scrubbed down to 460–500 μl l^{-1}

(ambient), prior to measurement of mean concentrations over six successive 15 s intervals. Blank tests (without soils) using this procedure and apparatus yielded a mean flux of 3 nmol CO_2 m^{-1} s^{-1} (S.E. = 0.001, $n = 6$). We calculated mean flux per sample area at ambient CO_2 using a regression of headspace concentration over time for each mesocosm. Fluxes were corrected for slight differences in headspace volume and surface area between mesocosm pots.

N_2O effluxes from the full-term mesocosms were measured regularly through the experiment using a modification of the static chamber technique in which opened TEDLAR gas sampling bags (Microlab, Hoejbjerg, Denmark) were sealed onto the outside of each pot by taut elastic bands during flux measurement periods. Headspace gas was mixed by pumping a syringe (50 ml) in and out three times prior to withdrawing samples (40 ml), and transferring them to evacuated glass ampoule bottles. The headspace concentration was sampled three times during each flux measurement: initially, after 1–2 h and after 2–4 h. An additional sample (150 ml) was withdrawn at the final sampling time for ^{15}N isotopic analysis. N_2O concentrations were analysed by gas chromatography (Shimadzu). Mesocosm headspace N_2O concentrations between consecutive measurements on individual pots varied substantially (and apparently randomly), resulting in many non-significant regressions for the three sampling points. In order to be consistent, all flux data presented here were calculated as the change in concentration between the initial and final sampling times. Cumulative N_2O effluxes during the treatment and equilibration phases were estimated by interpolating between sample points, and assuming that the soils were inactive for half of each freeze–thaw cycle in the multiple freeze–thaw treatment.

2.6. Mesocosm harvests

Harvests were conducted on days 3, 19, and 25 after initial labelling. All above-ground tissue including the green moss layer was clipped and sorted into the following categories: evergreen leaves; deciduous leaves; herb leaves; vascular above-ground stems (all species combined); mosses; litter (senesced and standing dead leaves, and stems); and lichens. Stems of *C. tetragona* were separated into live and dead shoots according to the status of attached leaves. The below-ground part of each mesocosm was divided vertically into three equal sections that were treated separately in all subsequent sorting, and in the biological and chemical analyses. Each section was cut up into small pieces (10–20 cm^3), that were randomly sub-sampled (ca. 2–4 per section) and sorted into the following categories: living fine roots (< 1.5 mm dia, soft, light-coloured, turgid tissue); living coarse roots (> 1.5 mm dia, including woody roots and rhizomes); and soil (20–40 g fresh mass). The latter fraction was used for determination of microbial biomass C (MBC) and nutrient contents, total soil ^{15}N contents, sub-sample moisture contents, and SOM content. Fine and coarse root samples were washed in de-ionised water prior to rinsing in a 0.5 mM K_2SO_4 solution (1 l) for 2–10 min (Kielland, 1997) in order to remove adhering ^{15}N on external surface tissue. All sorting of soil fractions was completed within 24 h after sampling. All plant and soil material was dried at 70 °C in fan-assisted ovens for a minimum of 3 d. SOM was determined by loss-on-ignition (550 °C for 6 h) of oven-dry soil.

2.7. Biological and chemical analyses

Soil MBC, microbial biomass N (MBN) and P (MBP) contents were determined by the chloroform-fumigation direct-extraction technique (Brookes et al., 1985) using 10 g fresh mass of soil and 50 ml 0.5 M K_2SO_4 for each extraction. Fumigation was with ethanol-free chloroform (25 ml) for 24 h at ~21 °C in a darkened desiccation jar.

Non-fumigated samples were extracted immediately after sorting was completed. All samples were shaken with extractants for 1 h, and then let stand for a further 1 h before filtering (Whatman GF-D). Blanks without sample were included to detect contamination during extraction and filtration. NH_4-N and NO_3-N concentrations in the non-fumigated extracts (i.e. the soil solution inorganic components), and in the leachates were determined colorimetrically by the indophenol and cadmium reduction-sulphanilamide methods, respectively (Allen, 1989). C concentrations in the fumigated and non-fumigated extracts were determined with a Total Organic Carbon analyser (Shimadzu). Total N and P within the extracts (excluding nitrate) was determined by Kjeldahl digestion (20 ml) and colorimetric analyses (Kedrowski, 1983). Dissolved total N (DTN) is reported as the total N content of the digested non-fumigated extracts (i.e. soluble organic plus NH_4-N). MBC, MBN and MBP values were calculated as the differences between fumigated and non-fumigated extracts (Brookes et al., 1985; Vance et al., 1987), using correction factors of 0.35 (k_C) 0.40 (k_N), and 0.40 (k_P), respectively (Jonasson et al., 1996), to account for microbial tissue C, N and P that is not released by exposure to chloroform.

2.8. ^{15}N isotopic analyses

Microbial ^{15}N enhancement by the added label was determined using the acid trap diffusion technique (Stark and Hart, 1996) on the digested extracts. Teflon traps containing two washed and acidified filter paper discs were placed on the surface of the digest solution (51–69 ml) within specimen cups (185 ml). All samples were spiked with 100 μg N (of natural abundance ^{15}N) to raise the total N contents to the optimum required for isotope ratio mass spectrometry (IR-MS) analyses. Afterwards, 12 M NaOH (18 ml) was added to each cup, before quickly sealing and inverting it. The pH of a random selection of cups was tested to ensure that sufficient alkali had been added to raise the pH > 13. Diffusion was allowed for 8 d, during which time

each cup was gently agitated 4–5 times at regular intervals. Afterwards, the filter papers were removed and dried in a desiccator jar. Diffused and pipetted standards (1.6297 atom% ^{15}N of total N) were included in each sample batch (Stark and Hart, 1996).

Total N contents of the plant and soil samples, and ^{15}N contents of the plants, soils and filter paper traps were determined on a CE 1110 elemental analyser coupled in continuous flow-mode to a Finnigan MAT Delta PLUS IR-MS. Isotopic enhancement of the microbial, soil solution, and total SOM pools arising from the injected ^{15}N label were calculated from the enrichment values for each component as: $\{\text{atom\%}^{15}N_{\text{measured}} - \text{atom\%}^{15}N_{\text{natural abundance}}\} \times$ total N pool size. Mean natural abundance $_{15}N$ isotope atom percentage was 0.3660% for sorted soil (SE = 0.0002; $n = 6$), and 0.3695% (SE = 0.0008; $n = 11$) for the fumigated and non-fumigated digested extracts in measurements of random soil samples collected adjacent to a nearby experiment (Grogan and Jonasson, 2003). ^{15}N diffusion blank correction of the extract diffusions was based on isotope dilution (Stark and Hart, 1996), after correcting for the isotopic pool dilution associated with the N spike addition to non-fumigated samples (see above). ^{15}N recovery of the pipetted and diffused standards (50–350 μg N) was: $^{15}N_{\text{measured}} = 0.94 \times (^{15}N_{\text{added}}) + 18.74$, $r^2 = 0.99$; $^{15}N_{\text{measured}} = 0.76 \times (^{15}N_{\text{added}}) + 12.34$, $r^2 = 0.92$, respectively. We used the colorimetrically determined digest N concentrations and the diffusion-determined ^{15}N atom% data to calculate ^{15}N enrichment in all digested extracts. Incomplete total N recovery during diffusion does not affect the accuracy of ^{15}N enrichment determinations provided that the isotope dilution approach to blank correction is used (Stark and Hart, 1996). ^{15}N label enhancement of the microbial isotopic N pool was calculated using the same k_N factor as for total MBN (see above). This approach assumes that the incorporated ^{15}N label pool within microbes was in a form that was equally resistant to chloroform fumigation and extraction as the total microbial N pool. If the incorporated label was less resistant, then our results likely over-estimate label enhancement of the microbial biomass. Nevertheless, since the same k_N factor was applied to all MBN determinations, the proportion of label initially taken up that was subsequently released is unaffected by the actual value used for k_N, provided that the k_N value itself does not change in either treatment through the course of the experiment.

The ^{15}N content of emitted N_2O was measured by flushing the sample bottles with He and cryogenically trapping N_2O prior to separation by GC followed by IR-MS analysis (Stevens et al., 1993).

Vascular plant uptake of added ^{15}N label was estimated by assuming that the mean initial harvest values indicate natural abundance ^{15}N concentrations for each category.

2.9. Statistical analyses

Our data were analysed as concentrations (g SOM^{-1}) in order to investigate treatment influences on biogeochemical processes within the mesocosms, and as pools (g $mesocosm^{-1}$) in order to evaluate overall partitioning of added label between ecosystem components. We tested for significant effects of sampling time (i.e. harvest) on mesocosm soil microbial, solution, leachate, gas efflux and plant atom% ^{15}N enrichment measures in each of the regimes separately, using 1 factor analyses of variance (ANOVA). Mean values of the microbial and soil solution data for the three below-ground sub-sections of each harvested mesocosm were used in these analyses. Since the freezing regimes were achieved using a single modified growth chamber for each treatment, pseudoreplication (Hurlbert, 1984) precludes direct tests of differences between the regimes. Sampling time was treated as a categorical variable in all analyses. Significant differences between means were identified using Fisher's Least Significant Difference (LSD) test. ^{15}N recovery percentage data were arcsine-square root transformed prior to statistical analysis (Zar, 1996). In all data analyses, every statistically significant effect and interaction ($P < 0.05$) is reported in the text.

3. Results

3.1. Environmental variables

The differing character of the two freeze–thaw regimes associated with the treatment phase are indicated by the soil temperatures at 5 cm depth in the centre of each mesocosm (Fig. 1). Note that since cooling and warming of the mesocosms were initiated from the exterior surfaces of each pot, the amplitude of temperatures experienced around the edges, and at the top and bottom of these pots is likely to be larger than presented here. The single deep-freeze treatment was characterised by rapid initial cooling to −4 °C, and then a relatively constant thermal environment for ~10 d, followed by rapid thawing. By contrast, the multiple freeze–thaw treatment consisted of a series of four short (~2 d) cycles of freezing (to just below 0 °C) and immediate thawing to 6–9 °C, followed by a more severe freeze to −7 °C before final thawing. Gravimetric soil moisture contents in the single deep freeze regime were not statistically different between sampling times (range 3.98–4.63 g H_2O per g soil dry $mass^{-1}$), suggesting that the mesocosms were close to field capacity throughout. By contrast, soil moisture contents were significantly lower at the end of the multiple freeze–thaw cycling phase ($F_{2,11} = 19.00$; $P < 0.001$; mean = 2.78 g H_2O g soil dry $mass^{-1}$), but had returned close to initial levels (mean = 4.50 g H_2O per g soil dry $mass^{-1}$) by the end of the experiment due to regular watering in the equilibration

phase. The lower soil moisture contents in the mesocosms sampled at the second harvest may be partly attributed to significantly lower SOM concentrations ($F_{2,11} = 5.12$; $P = 0.027$; mean = 79%, in contrast to 88% in the other harvests).

3.2. Ecosystem organic matter and nitrogen pools

As expected, the organic matter and N pools were dominated by the SOM component (Table 1). The MBC content was ~15% of the total plant C, whereas N within the microbial biomass was ~1.17 times total plant N. The plant biomass components and soil microbial biomass and nutrient pool data broadly corresponded with previous estimates at this site (Jonasson et al., 1999a,b), strongly suggesting that the storage phase prior to the experiment did not adversely affect the size of major ecosystem component pools.

Overall, the major importance of below-ground plant tissues to C cycling in this ecosystem was indicated by the relatively large biomasses of coarse and fine roots (Table 1), resulting in a vascular plant root-to-shoot ratio of ~7:1, and a root-to-microbial biomass ratio of ~5:1. Similarly, the N pool in roots exceeded that in vascular shoots (by ~4:1), although in this case the strong below-ground control on ecosystem cycling was dominated by the soil microbial N pool, which exceeded the root N pool by a factor of ~2.

3.3. Microbial and soil solution carbon and nutrient pools

The single deep freeze treatment had no significant effect on MBC (Fig. 2a). By contrast, the multiple freeze–thaw regime significantly reduced MBC over the full experiment (Fig. 2a. $F_{2,11} = 4.00$; $P = 0.049$; LSD = 3.2). This reduction in MBC was associated with a significant increase in dissolved organic C (DOC) in the same treatment over the equilibration phase of the experiment (Fig. 2b. $F_{2,11} = 8.87$; $P = 0.005$; LSD = 0.37). Neither MBN (Fig. 2c) nor MBP (data not shown) were significantly affected by either treatment.

Dissolved total nitrogen (DTN) in the soil solution was significantly enhanced immediately at the end of the single deep freeze treatment, and then significantly decreased over the equilibration phase (Fig. 3a. $F_{2,11} = 7.45$; $P = 0.009$; LSD = 81.3). Dissolved organic N (DON) was significantly altered by the single deep freeze treatment in an identical manner (Fig. 3b. $F_{2,11} = 5.11$; $P = 0.027$; LSD = 69.3). It made up the largest part of DTN immediately before, and at the end, of the freezing treatments. However, the proportion decreased during the experiment, so that the proportions of organic and NH_4-N were close to equal by the end of the experiment (Fig. 3b and c). The NH_4-N content was strongly enhanced by the single deep freeze treatment but changed little during the equilibrium phase (Fig. 3c. $F_{2,11} = 4.92$; $P = 0.030$; LSD = 45.1). These results suggest that some of the organic N within the soil solution may have been

Table 1
Mass, and C and N pools in plant and soil components in a sub-arctic heath tundra ecosystem

Pool	Component	Category	Mass (g dw m^{-2})	C pool (g C m^{-2})	N pool (g N m^{-2})
Plant: above-ground					
	Vascular	Evergreen leaves	72.1 (11.4)	31.7 (5.0)	0.8 (0.01)
		Deciduous leaves	1.5 (0.4)	0.7 (0.02)	0.1 (0.01)
		Herb leaves	9.5 (1.2)	4.2 (0.5)	0.2 (0.02)
		Stems (total)	138.8 (17.7)	61.0 (7.8)	1.3 (0.02)
	Total vascular		221.9 (25.8)	97.7 (11.3)	2.3 (0.03)
	Non-vascular	Mosses	292.3 (34.7)	128.6 (15.3)	2.6 (0.04)
		Lichens	8.6 (2.5)	3.8 (1.1)	0.06 (0.02)
	Total vascular plus non-vascular		522.8 (41.4)	230.0 (18.2)	5.0 (0.4)
Plant: below-ground					
		Coarse roots/rhizomes	1015 (129.6)	446.6 (57.0)	5.9 (0.9)
		Fine roots	448.7 (48.6)	197.4 (21.4)	3.9 (0.04)
	Total roots		1464 (165.7)	644.0 (72.9)	9.8 (1.1)
	Total vascular plant		1686 (169.3)	741.7 (74.5)	12.1 (1.1)
	Total plant		1987 (172.1)	874.0 (75.7)	14.7 (1.2)
	Surface litter and standing dead		493.3 (44.9)	261.5 (23.8)	4.5 (0.6)
	Soil organic matter		11630 (541)	6164 (280)	206.5 (15.2)
	Soil microbes		306.5 (19.0)	134.9 (8.4)	17.2 (1.3)
	Soil solution (organic)			17.1 (0.9)	1.1 (0.01)
	Soil solution (inorganic)				0.8 (0.01)

Parentheses indicate standard errors ($n = 24$). Mass and associated C and N data, and soil solution data for each category are the mean values of all mesocosms (mean soil depth = 15 cm; SE = 0.23). Total C pools were computed assuming a C concentration of 44% for plant and microbial tissues (Paul and Clark, 1996), and 53% for litter and SOM (Howard and Howard, 1990). SOM mass, C and N pools were calculated by subtracting microbial and soil solution components from the total amounts measured within sorted soil samples.

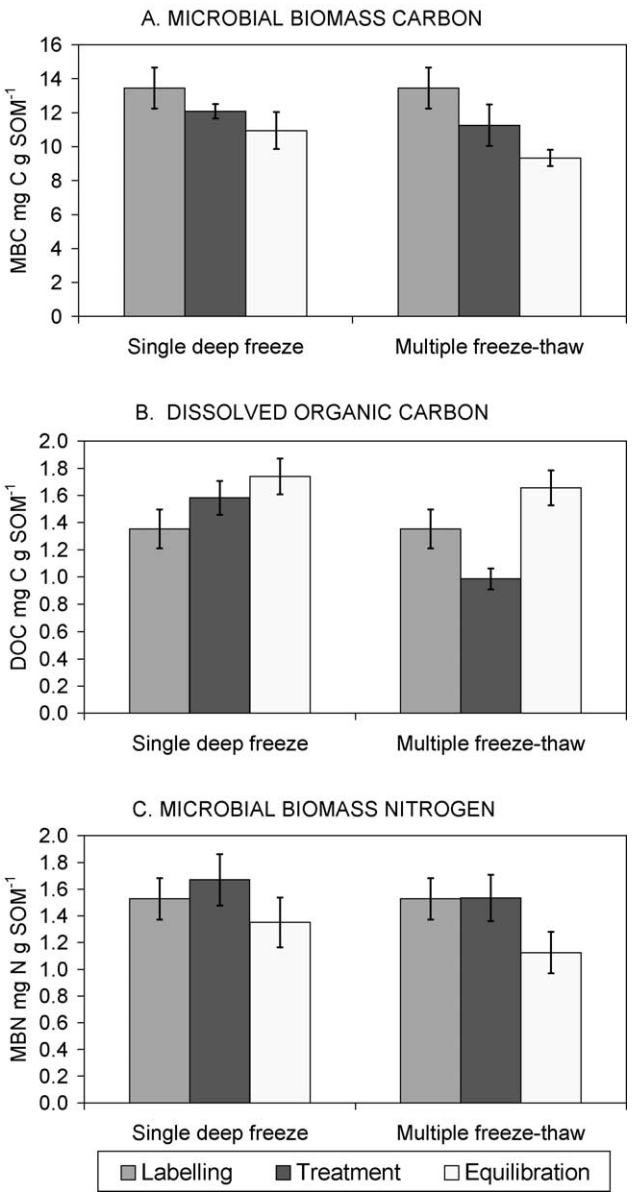

Fig. 2. The time sequences of (a) microbial biomass C; (b) dissolved organic C and (c) microbial biomass N contents in each treatment regime (bars = S.E.s; $n = 4–5$).

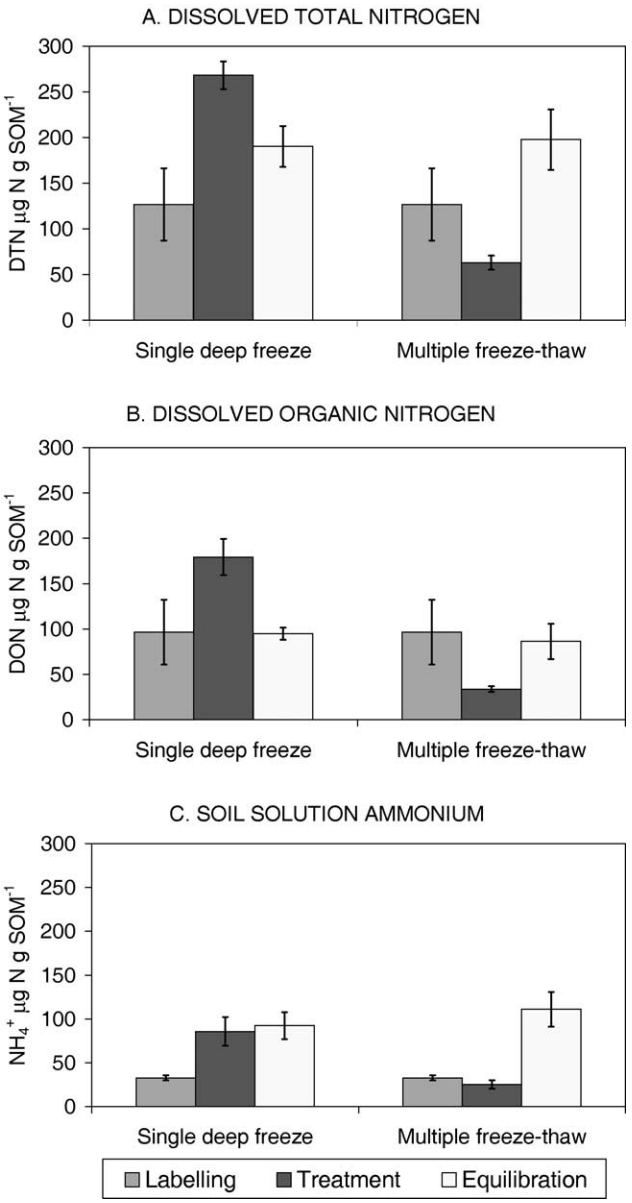

Fig. 3. The time sequences of (a) dissolved total N, and (b) its organic N and (c) NH_4^+-N components, in the soil solution of each treatment regime (bars = S.E.s; $n = 4–5$). Nitrate contents were minor, ranging from 0.4 to 4.1 µg N g SOM^{-1}.

decomposed and taken up by plants, or lost as throughflow leachates or gases. By contrast, DTN was significantly enhanced during the equilibration phase of the multiple freeze–thaw treatment (Fig. 3a. $F_{2,11} = 5.95$; $P = 0.018$; LSD = 91.2). This effect was due to a ~4-fold increase in soil solution NH_4-N during the equilibration phase (Fig. 3c. $F_{2,11} = 14.27$; $P < 0.001$; LSD = 41.0). Nitrate contents in the soil solution throughout the experiment were negligible by comparison (0.4–4 µg N g SOM^{-1}) (data not shown). Thus, the single deep freeze treatment immediately increased both organic and inorganic N components of the soil solution, while the multiple freeze–thaw resulted in a sharp increase in inorganic N over the equilibration phase (Fig. 3). These results indicate strong contrasting effects of the freezing regimes over time, and suggest that the multiple freeze–thaw cycles in particular, induced important follow-on effects that enhanced soil NH_4-N and DOC pools in the post-treatment equilibration phase.

3.4. Leachate inorganic nitrogen pools

The volumes of throughflow leachate collected from the full-term pots on days 3, 19 and 24 after labelling were substantially lower in those from the multiple freeze–thaw treatment compared to the single deep freeze treatment (Table 2). Since this difference occurred even in the initial labelling phase (i.e. pre-treatment), it is most likely an

Table 2
Throughflow leachate volume, nitrate and ammonium concentrations and pools from the full-term experimental mesocosms over the three phases of the experiment

Leachate variable	Sampling interval (days since labelling)					
	Single deep freeze			Multiple freeze–thaw		
	1–3	3–19	19–24	1–3	3–19	19–24
Volume (ml)	191.4^{a} (44.5)	136.2^{a} (18.4)	152.4^{a} (25.3)	125^{x} (22.5)	72.2^{x} (9.0)	120.8^{x} (19.1)
NH_4-N conc.(mg l^{-1})	0.03^{a} (0.01)	0.4^{b} (0.1)	0.4^{b} (0.1)	0.1^{x} (0.1)	0.4^{x} (0.2)	0.4^{x} (0.2)
NH_4-N pool (μg)	6.8^{a} (3.2)	50.4^{ab} (12.7)	67^{b} (24.6)	13.5^{x} (9.5)	28.5^{x} (16.3)	51.8^{x} (21.5)
NO_3-N conc.(mg l^{-1})	3.5^{a} (1.2)	1.3^{a} (0.8)	1.0^{a} (5.4)	2.1^{x} (0.8)	0.7^{y} (0.2)	0.2^{y} (0.1)
NO_3-N pool (μg)	836.9^{a} (358)	169.7^{a} (85)	142.7^{a} (43)	219.9^{x} (76.1)	47.7^{y} (16.5)	22.9^{y} (9.5)

Parentheses indicate standard errors ($n = 5$ mesocosms). Means within each treatment row that differ in superscript letter indicate statistically significant differences between experimental phases ($P < 0.05$).

artefact due to chance differences in soil hydrological characteristics between pots assigned to the two freezing regimes. Nevertheless, we found no corresponding significant differences in SOM concentration, soil bulk density, or any of the plant biomass components between the full-term mesocosms. In any event, we focus here on effects of sampling time within each treatment on leachate N concentrations and pools.

As with extractable soil solution NH_4-N (Fig. 3c), throughflow leachate NH_4-N concentrations were significantly increased during the single deep freeze treatment, and remained high over the equilibration phase (Table 2. Sampling time: $F_{2,12} = 6.69$; $P = 0.011$; LSD = 0.25). Accordingly, the total leachate NH_4-N pool during the equilibration phase was significantly enhanced (Sampling time: $F_{2,12} = 3.72$; $P = 0.055$; LSD = 49.7) to a mean value ~10-fold higher than that in the initial pre-treatment phase. These results are consistent with the hypothesis that part of the significant reduction in DON in the single deep freeze treatment over the equilibration phase was due to its decomposition and subsequent N losses as leachate NH_4-N. By contrast, the significant increase in extractable soil solution NH_4-N during the equilibration phase in the multiple freeze–thaw treatment (Fig. 3c) did not result in a corresponding significant increase in throughflow leachate NH_4-N concentrations or pools (Table 2).

Nitrate concentrations and pools within throughflow leachate were not significantly altered by the single deep freeze treatment (Table 2). By contrast, leachate NO_3-N concentrations were significantly reduced during the multiple freeze–thaw treatment phase, and remained low over the equilibration phase (Table 2. Sampling time: $F_{2,12} = 4.48$; $P = 0.035$; LSD = 1.41), resulting in a mean leachate NO_3-N pool reduction to ~1/10th that during the pre-treatment phase (Sampling time: $F_{2,12} = 5.62$; $P = 0.019$; LSD = 140). Together, these data indicate that the two freezing regimes led to marked increases in the proportion of NH_4-N to NO_3-N within throughflow leachates. The results suggest that the reasons for these increases differed between treatments: NH_4^+ production was enhanced in the single deep freeze treatment (Fig. 3c) resulting in enhanced throughflow losses; whilst nitrification may have been inhibited during the equilibrium phase of the multiple freeze–thaw treatment.

3.5. Partitioning of ^{15}N label in microbial and soil solution pools

MBN pool enhancement with ^{15}N label ($MB^{15}N$) was not significantly altered in either treatment (Fig. 4a), consistent with the MBN pool results (Fig. 2c). The patterns of ^{15}N enhancement of the dissolved total N pool ($DT^{15}N$), however, closely mimic the DTN pool (Fig. 4b. Single deep freeze: $F_{2,11} = 3.63$; $P = 0.062$; LSD = 0.067. Multiple freeze–thaw: $F_{2,10} = 7.52$; $P = 0.010$; LSD = 0.018).

3.6. Gas efflux rates

Respiratory CO_2 efflux rates in the single deep freeze treatment at each of the sampling times during the freezing treatment phase were consistently low, and increased ~9-fold immediately after thawing (Fig. 5a). By contrast, and as would be expected from the soil temperature differences (Fig. 1), respiration rates during the corresponding thawed periods of the multiple freeze–thaw treatment were generally high. Note that the low efflux rate 5 d after labelling (Fig. 5a) is most likely due to the relatively low soil temperature at that flux sampling time (Fig. 1; 2.8 °C compared with soil temperatures of 4.6–8.5 °C at subsequent sampling times). Thus, the CO_2 efflux rates in the multiple freeze–thaw regime were remarkably consistent both within the treatment phase, and between the treatment and equilibration phases. Furthermore, the efflux rates were very similar in the multiple freeze–thaw and single deep freeze treatments during the equilibration phase. Overall, the CO_2 efflux rates and soil temperatures were very closely correlated (Ln efflux = 0.21(temperature)−0.75; $r^2 = 0.79$, $P < 0.001$, $n = 70$), strongly suggesting that soil temperature closely regulated total ecosystem respiration throughout this experiment, and that indirect effects such as

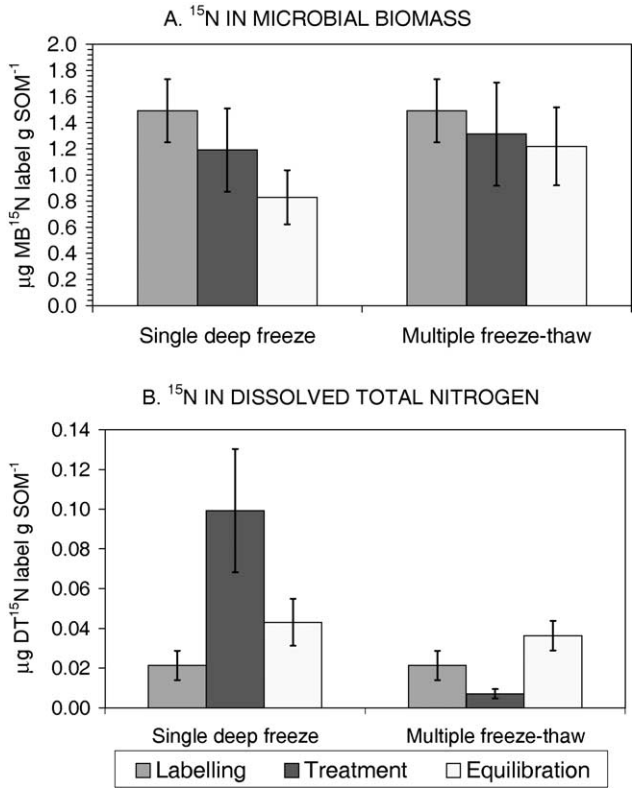

Fig. 4. The time sequences of ^{15}N label enhancement of the (a) microbial biomass and (b) dissolved total N pools in each treatment regime (bars = S.E.s; $n = 4–5$). Note the different scales.

differences in soil moisture content between the freeze–thaw regimes had negligible influence on biological activity.

The N_2O fluxes for each regime were highly variable among mesocosms, and among sampling times (Fig. 5b). Nevertheless, some clear patterns emerged. N_2O efflux in the single deep freeze treatment was characterised by a sudden strong increase immediately after thawing, whereas N_2O efflux in the multiple freeze–thaw treatment was largely confined to the thawing points within the treatment (Fig. 5b). These efflux patterns closely matched the pattern of increases in the levels of atom% ^{15}N enrichment (i.e. the ratio of label ^{15}N plus natural abundance ^{15}N to ^{14}N) of the N_2O (Fig. 5c), strongly suggesting that the added ^{15}N label provided an important substrate for N_2O production in each regime.

3.7. Plant ^{15}N atom% enrichment

Significant increases in total ^{15}N atom% enrichment of the plant community were observed in only three of the harvested categories. Leaves of herbaceous plants (predominantly grasses) were significantly enriched over the full experiment (Fig. 6a) in both single deep freeze ($F_{2,11} = 3.64$; $P = 0.06$; LSD = 0.021), and multiple freeze–thaw treatments ($F_{2,11} = 5.51$; $P = 0.02$; LSD = 0.027). In addition, there was a significant increase in ^{15}N atom% enrichment of all fine roots combined

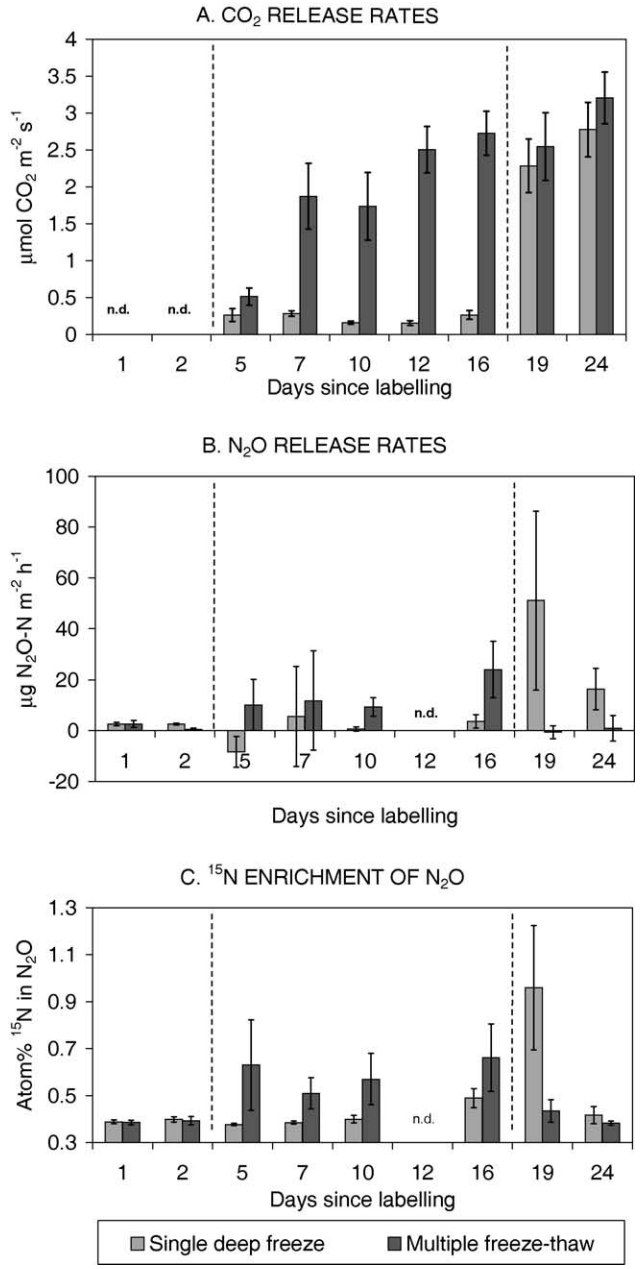

Fig. 5. The (a) CO_2 and (b) N_2O efflux rates, and (c) atom % ^{15}N enrichment of N_2O during each treatment regime (bars = S.E.s; $n = 5$). The vertical dashed lines separate labelling, freezing treatment, and equilibration phases (n.d., not determined).

following the multiple freeze–thaw treatment (Fig. 6b. $F_{2,11} = 3.90$; $P = 0.05$; LSD = 0.021). Finally, there was also a significant but relatively small increase in ^{15}N enrichment of stems of all vascular species combined in the single deep freeze treatment (Fig. 6c. $F_{2,11} = 4.02$; $P = 0.05$; LSD = 0.003).

3.8. Ecosystem partitioning of added ^{15}N label

Data on the recovery and ecosystem partitioning of added label are presented per mesocosm pot (Table 3).

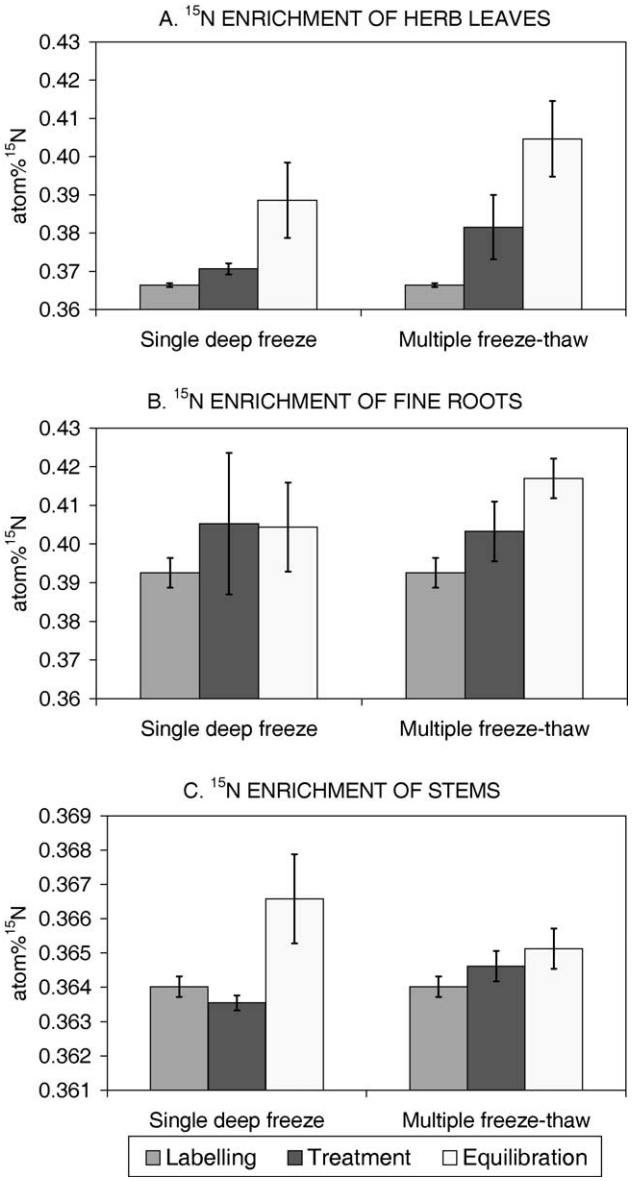

Fig. 6. The time sequences of total atom percent ^{15}N in (a) herb leaves; (b) fine roots of all species combined, and (c) stems of all vascular species combined in each treatment regime (bars = S.E.s; $n = 4–5$). Note the reduced scale for the stem data.

Of the 1030 μg ^{15}N added to each pot, microbial uptake dominated ecosystem partitioning in the initial labelling phase, accumulating ~64% of the total amount added. By the end of the experiment, ^{15}N label enhancement of the microbial biomass had declined to 35 and 53% in the single deep freeze and multiple freeze–thaw treatments, respectively (Table 3). The overall percentages of added ^{15}N label that were recovered within the microbial biomass of each mesocosm were not statistically different through the experiment in either regime (data not shown). By contrast, ^{15}N label fixation in the soil was low initially (~11%), but became the dominant pool in both treatments by the end of the experiment (Table 3). Plant acquisition of label, as well as soil solution label enhancement were minor relative to

Table 3
The distribution of ^{15}N label amongst measured ecosystem components in the mesocosms at the end of the experiment (μg ^{15}N label per pot)

Component	Single deep freeze	Multiple freeze–thaw
Soil microbes	364 (54)	545 (139)
Dissolved total	19 (4)	17 (4)
Plants	13 (15)	45 (9)
Nitrous oxide	2	1
Soil fixation	434 (110)	836 (119)
Total	831 (114)	1450 (252)

Also included are estimates of mean total ^{15}N label loss as N_2O-N over the experiment (see Section 2 for details of calculation). Soil fixation of label was calculated by subtracting soil microbial and dissolved components from total ^{15}N label enhancement of the sorted soil fraction. Parentheses indicate standard errors ($n = 4–5$). Total label addition to each mesocosm was 1030 μg ^{15}N. Potential label losses via other gaseous forms or throughflow leachates were not determined.

the above pools, and estimated total N_2O label losses were negligible (Table 3). Thus, initial label partitioning, and subseqent re-distribution within the mesocosms was governed primarily by interactions between microbial processes and soil physico-chemical effects.

4. Discussion

4.1. The significance of differing freeze–thaw regimes to tundra soil biogeochemistry

In this study, we exposed tundra mesocosms to two differing freeze–thaw regimes each of which was realistic of field conditions that might be experienced at times during the beginning and end of the winter season in their site of origin (Grogan et al., 2001), and also at many other high latitude (Grogan and Chapin, 1999), high altitude (Lipson and Monson, 1998) and temperate sites (Taylor and Parkinson, 1988). Our results demonstrate that differing freeze–thaw regimes can have substantially different effects on below-ground nutrient and carbon pool dynamics. For example, only the multiple freeze–thaw treatment resulted in a significant decrease in MBC, and a significant increase in DOC (Fig. 2a and b). Furthermore, the two treatments had strongly contrasting effects on dissolved total N (Fig. 3a) and ^{15}N pools in the soil solution (Fig. 4b), as well as throughflow leachate variables (Table 2) during the experiment. These results indicate that differences in the character of freeze–thaw regimes, that emulate likely natural spatial and temporal variation in field conditions at the site of origin, can have markedly different effects on microbial and soluble C and N and pool dynamics.

4.2. The character of the freezing regimes

Several aspects of the character of the freezing regimes may have contributed to the observed differences in

biogeochemistry. For example, the treatments varied in three major features of freeze–thaw: the number of freeze–thaw cycles; the duration of the severe freeze period; and the absolute minimum temperature (Fig. 1). In addition, there was a significant reduction in soil moisture during the treatment phase of the multiple freeze–thaw treatment that was probably due to evapotranspiration losses during the thaw phases, as well as slightly lower SOM concentrations in the second harvest mesocosms (see Results). In any event, the decline in moisture content does not appear to have influenced either respiration or N_2O production rates (Figs. 5a and b), suggesting that its effect on microbial processes was small. Furthermore, similar declines in soil moisture due to evapotranspiration might also be expected in the field under conditions of multiple freeze–thaw, and therefore, this effect should be considered as a potential interaction with temperature in interpreting the multiple freeze–thaw treatment results. Thus, although the precise factors that resulted in the treatment differences were not elucidated in this experiment, the overall character of the two freezing regimes was realistic of natural field variation in thermal conditions at the site of origin, and probably at a wide range of other seasonally cold locations.

4.3. Freezing regime effects on microbial N turnover

The soil microbial biomass dominated initial uptake of the ^{15}N label (Fig. 4a). Although the data suggest that the single deep freeze treatment may have reduced MB^{15}N over the experiment (Fig. 4a), this trend is associated with high variability that precluded statistical significance. The labelled N pool within the microbial biomass was approximately 0.1% of the microbial total N pool in each treatment (Figs. 2c and 4a), and approximately 30 times larger than the labelled dissolved total N pool (Fig. 4a and b). Thus, even though we did not detect significant treatment effects on ^{15}N label enhancement within the relatively large MBN pool, the significant and contrasting changes within DT^{15}N in both treatments (Fig. 4b) strongly suggest that the freeze–thaw regimes had differing influences on ^{15}N label turnover within the microbial pools.

4.4. Follow-on effects of the freezing regimes

Our results indicate that moderate variations in freeze–thaw regime can have significant and substantially different follow-on effects on biogeochemical processes in recently thawed soils. For example, the significant decline in MBC in the multiple freeze–thaw treatment only occurred over the full course of the experiment, including the equilibration period (Fig. 2a). Likewise, the significant increases in DOC and soil solution NH_4-N in the same treatment occurred over the equilibration phase (Figs. 2b and 3c). By contrast, the significant increases in DTN and DT^{15}N at the end of the single deep freeze treatment phase were followed by significant decreases in these same variables over the equilibration period (Figs. 3a and 4b). Thus, the single deep freeze treatment may have stimulated an initial release of label immediately upon thawing, followed by ongoing microbial mineralisation of label over the post-treatment phase and its transfer to plants, leachates and gaseous losses. In contrast to the single deep freeze mesocosms, the soils in the multiple freeze–thaw treatment were thawed for up to 3 d prior to the second harvest (Fig. 1), implying that some 'equilibration' responses could have occurred and influenced the pool sizes at this sampling point. In any event, the significant increases in DOC, NH_4-N and DT^{15}N in the multiple freeze–thaw treatment (Figs. 2a, 3c and 4b) all occurred during the post-treatment equilibration period after the second harvest. Thus, the multiple freeze–thaw cycling treatment had significant follow-on effects that depleted MBC and MBN pools at least 3 d after the final thaw. These differences in the timing of the treatment effects indicate that variation in freeze–thaw regime can result in substantial temporal heterogeneity in biogeochemical pools in the weeks immediately following final thaw.

4.5. Freeze–thaw effects on microbial lysis and respiration

MBC, MBN and DOC were not significantly affected by the single deep freeze regime, indicating no evidence for treatment-induced microbial cell lysis and cytoplasm release. Nevertheless, there were significant increases in DON, NH_4-N and DT^{15}N immediately at the end of the freezing period that could be attributed to microbial cell lysis, or to physical fragmentation of litter or humus and subsequent microbial N mineralisation. Similarly, the multiple freeze–thaw treatment did not significantly reduce MBC or MBN during the initial thaw period at the end of the second harvest (Figs. 2a and c), and there were no corresponding increases in DOC or soil solution N pools. The CO_2 efflux data also provide no evidence to support the lysis hypothesis in either treatment. Instantaneous respiration rates directly at the time of final thaw in the multiple freeze–thaw cycling treatment (day 16) were similar to earlier and later measures (Fig. 5a). Furthermore, respiration rates were very closely correlated with soil temperature throughout the experiment in both treatments. In addition, total respiration rates from the frozen mesocosms during the single deep freeze treatment closely matched field measurements of mid-winter ecosystem CO_2 production at the site of origin (Grogan et al., 2001). Finally, the measured efflux rates at thaw points during the multiple freeze–thaw treatment phase, and in the equilibration periods for both treatments, were very similar to test measurements of mesocosm CO_2 fluxes made prior to the experiment (Grogan, unpublished data), and to summertime ecosystem respiration rates at the site of origin (Illeris, personal communication). Together, these respiration results indicate that the burst of CO_2 efflux commonly observed in previous studies of soil freezing that is attributed to stimulated microbial attack of the lysed contents of freeze-sensitive

microbes (Skogland et al., 1988; Schimel and Clein, 1996), was not apparent here. Similar conclusions were reached in a study of moderate freeze–thaw regime effects on plant-microbial interactions in alpine tundra (Lipson and Monson, 1998). In contrast to most previous studies, our study and the Lipson and Monson (1998) study included intact vegetation, and, therefore, reports measurements of total ecosystem respiration. Thus, respiratory activity associated with plants (i.e. plant metabolism, and respiration associated with rhizosphere exudates and fresh litter) may have masked any freeze–thaw induced soil CO_2 flushes within total ecosystem respiration. In any event, the absence of immediate freeze–thaw effects on respiration, or on MBC, MBN and MBP pools, together suggest that the microbial communities were relatively resistant to our freeze–thaw regimes, and that microbial cell lysis and cytoplasm release may have been very limited.

4.6. Total ^{15}N recovery among ecosystem components

Our attempt to account for the total ^{15}N added per mesocosm pot was only partly successful (Table 3). Approximately, 20% of the added label was not accounted for in the single deep freeze treatment, whilst the sum of the ^{15}N labeled components measured in the multiple freeze–thaw treatment exceeded the amount added by ~40%. Several factors may have contributed to errors and inaccuracies in our determinations. First, some spatial heterogeneity in initial label distribution is inevitable despite our efforts to minimize this effect by spacing the injections <5 cm apart across the top surface area of each mesocosm. Note that we also attempted to restrict spatial heterogeneity effects by splitting each mesocosm into three separate sections that were sorted and analysed separately prior to computing mean values for each mesocosm. Second, although we went to considerable effort to homogenize the below-ground sections of each mesocosm before sampling (see Section 2), the small proportion of total soil in each mesocosm that was sampled and analysed (1–2%), implies that sample heterogeneity was likely. Third, the determination of ^{15}N label contents in the two largest pools (microbial biomass, and soil fixation) incorporated several assumptions that may not be appropriate. For example, we assumed that the k_N factor for estimating total N in the microbial biomass from the chloroform-labile MBN pool (see Section 2) has the same value for the ^{15}N labeled pool in the microbial biomass. Furthermore, calculation of the amount of label fixed in soil was determined by subtraction of the microbial and dissolved label ^{15}N pools from measures of total label enhancement of the sorted 'root-free' soil fraction. This process has at least three sources of potential error: (a) total ^{15}N is determined on a tiny sub-sample of the total soil mass and computed by multiplying by a factor of $\sim 10^4$, implying obvious sample heterogeneity concerns; (b) computation by subtraction means that any errors associated with estimation of the microbial biomass ^{15}N label pool are transferred to the computed value for the soil fixation label pool; and (c) removal of roots may also remove associated rhizosphere microbes which would ultimately result in an underestimate of total soil label enhancement, and, therefore, of soil label fixation. As a consequence of these various methodological issues (that are common to many ^{15}N labeling studies), it would be very surprising if our attempts to account for all the label added in each treatment were accurate. Nevertheless, these methodological concerns do not detract from our major conclusions on the general pattern of substantial ^{15}N label distribution in the microbial and soil fixation pools, and relatively little accumulation in the soil solution, gaseous and plant component pools in the experiment (Table 3).

4.7. Plant ^{15}N uptake in the treatment regimes

Our results indicate that vascular plants were able to acquire ^{15}N label in both treatment regimes (Fig. 6). These data, in combination with the ecosystem respiration data, strongly suggest that plant functioning in this heath tundra ecosystem was resistant to the two freeze–thaw regimes. Plant ^{15}N uptake was occurred during the equilibration phase of the single deep freeze treatment, and corresponded with a reduction in $DT^{15}N$. By contrast, the data suggest that fine roots (of all species combined) were able to acquire labelled N during the multiple freeze–thaw cycles or immediately after the final thaw, as well as over the equilibration phase. The rapid label acquisition and its translocation to leaves of herbs (predominantly grasses) in this strongly nutrient-limited ecosystem (Jonasson et al., 1999b), suggests that graminoids can optimise N uptake and translocation, and therefore, photosynthetic capacity in response to flushes of available nutrients at spring-thaw. Field studies in an adjacent site (Grogan and Jonasson, 2003) have also demonstrated a particularly strong capacity amongst the graminoid vegetation component in this regard. Nevertheless, the comparable label enhancement concentrations in herb leaves and total fine roots (Fig. 6), and the much larger biomass and N pool of the latter component (Table 1), strongly suggest that most of the ^{15}N label taken up by plants in this ecosystem was accumulated in the roots of non-herbaceous (i.e. shrub) vegetation.

4.8. Freeze–thaw effects on ecosystem N losses

Our results consistently indicated enhanced N_2O release during the thaw stage immediately following soil freezing in both treatments. Furthermore, the very close correlation between patterns of N_2O release and atom% ^{15}N enrichment of N_2O indicate that the added ^{15}N label may have provided an important substrate for N_2O production. Previous studies close to our site indicate that NH_4NO_3 additions (during summer) can result in strong and rapid stimulation of N_2O production (Christensen et al., 1999). Together, these results

suggest that the most labile, active N pools in soil may be the primary sources for N_2O production both in summer and during freeze–thaw cycles.

N_2O effluxes during freeze–thaw cycles could represent a major proportion of the total annual N_2O emissions from certain ecosystems (Smith, 1997). Our estimates suggest that cumulative ecosystem total N losses via N_2O ranged from ~168 to ~94 μg N per mesocosm (single deep freeze and multiple freeze–thaw treatments, respectively). The effects of moderate snowmelt were emulated in our study by adding substantial quantities of water to each set of mesocosms during the equilibration phase, which ultimately resulted in mean gravimetric soil moisture contents of ~4.25 g H_2O g soil dry mass^{-1} (no significant difference between treatments). These saturating amounts, accomplished under through-flow conditions, are likely to emulate the field environment in many freely draining situations, including the mesocosm site of origin. Nevertheless, ecosystem N_2O losses at spring-thaw may be substantially higher under the more extreme saturating soil moisture conditions that occur in permafrost and poorly drained sites.

Throughflow leachate nutrient losses are likely to be of greatest magnitude during the spring freeze–thaw in tundra ecosystems (Dowding et al., 1981; Schimel et al., 1996). Total leachate inorganic losses during the treatment and equilibration phases ranged from ~430 to ~150 μg N per mesocosm (Table 2) in the single deep freeze and multiple freeze–thaw treatments, respectively. Together, these estimates indicate that leachate inorganic N losses and N_2O-N losses during and after the differing freezing regimes were of a similar magnitude overall, and that the magnitude of these losses was extremely small relative to changes within the soil solution N pools (e.g. NH_4-N increased by ~18,000 and ~33,000 μg N per mesocosm during the single deep freeze and multiple freeze–thaw regimes, respectively). Assuming that cumulative N_2 and NH_3 gaseous losses, and organic N leachate losses were of similar magnitude to the N_2O and inorganic leachate losses, these data suggest minimal losses and very efficient internal N cycling in heath tundra ecosystems during and shortly after moderate freeze–thaw regimes of varying character.

4.9. The significance of freeze–thaw cycles to spatial and temporal patterns of nutrient cycling

We investigated the potential importance of particular freeze–thaw regimes to biogeochemical pool dynamics in seasonally cold ecosystems. It has been widely assumed that the freeze–thaw fluctuations experienced by soils in these ecosystems are a major driver of nutrient mobilisation from the microbial biomass, resulting in enhanced plant-available nutrient pools (Dowding et al., 1981). Furthermore, since net mineralisation during summertime is often negligible (Schimel et al., 1996), freeze–thaw events may have a critical influence on annual biogeochemical patterns in both arctic (Grogan and Jonasson, 2003) and alpine ecosystems (Lipson et al., 1999). Previous field research has indicated that substantial N release can occur at spring-thaw in a wide range of seasonally cold ecosystems (Wang and Bettany, 1993; Brooks et al., 1998). Laboratory incubation studies aimed at elucidating mechanisms indicate that freeze–thaw cycles can lyse a substantial proportion of microbial cells, resulting in C and nutrient releases into the surrounding soil (Ivarson and Sowden, 1970; DeLuca et al., 1992), that may be immobilised by surviving microbes as they consume the enhanced supply of C substrate (Morley et al., 1983; Skogland et al., 1988). The results from these controlled laboratory studies may differ from ours because, rather than investigating the responses of small soil volumes to soil temperature fluctuations with severe minima (−7 to −20 °C) over substantial periods (days to months), we applied a relatively moderate freeze–thaw regime to intact plant–soil mesocosms. Small- and large-scale heterogeneity in vegetation cover, soil moisture, soil hydraulic conductivity and solute concentrations are likely to result in strong spatial and temporal variability in the character of freeze–thaw regimes experienced within seasonally cold ecosystems (Edwards and Cresser, 1992). Here, we used relatively moderate freeze–thaw regimes both of which are likely to be realistic of thermal conditions that can occur within microsites at times during the transitions to and from winter at the site of origin (Grogan et al., 2001). Our results indicate relatively small and subtle effects on microbial and soluble C and N pool dynamics compared to most previous studies where more severe freeze–thaw regimes were used. Thus, our results suggest that the precise character of the thermal regime is important in evaluating effects of freeze–thaw, and that periods of moderate freeze–thaw fluctuations may have only minor influences on annual biogeochemical patterns in seasonally cold ecosystems.

Acknowledgements

We thank Lotte Illeris for help in the field, and Camilla Brock, Karina Clemmensen and Suzanne König for their assistance with harvesting the mesocosms. In addition, we thank Gosha Sylvester, Esben Nielsen, and Karna Heinesen for help with the chemical analyses, Sven Danbaek for technical assistance with the growth chambers, Karin Vestberg for conducting most of the N_2O analyses, Claus Bang-Berthelsen and Kristian Sögaard Jensen for assistance with sample preparation, and Merete Brink for the ^{15}N analyses. Furthermore, this research would not have been possible without the excellent facilities and support provided by the team at the Abisko Scientific Research Station, to whom we are very grateful. Sharon Hall and Loretta Johnson kindly provided advice on the ^{15}N diffusion technique. Thanks to Karl Ritz and to two anonymous reviewers for comments on an earlier version of this manuscript. This research was funded by an E.U. Marie Curie

post-doctoral fellowship to P.G., and by grants from the Danish Natural Science Research Council to S.J.

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