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The seasonal pattern of soil microbial community structure in mesic low arctic tundra

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ABSTRACT

Soil microorganisms are critical to carbon and nutrient fluxes in terrestrial ecosystems. Understanding the annual pattern of soil microbial community structure and how it corresponds to soil nutrient availability and plant production is a fundamental first step towards being able to predict impacts of environmental change on ecosystem functioning. We investigated the composition, structure and nutrient stoichiometry of the soil microbial community in mesic arctic tundra on 9 sample dates in 6 months from winter to fall using phospholipid fatty acid analysis (PLFA), quantitative polymerase chain reaction (qPCR), epifluorescent microscopy and chloroform-fumigation–extraction (CFE). PLFA analysis indicates that the winter microbial community was fungal-dominated, cold-adapted and associated with high C, N and P in the soil solution and microbial biomass. The microscopy data suggest that both bacteria and fungi were active and growing in soils between −5 °C and 0 °C. A significant shift occurred in the PLFA data, qPCR patterns, microscopy and microbial biogeochemistry after the thaw period, resulting in a distinct community that persisted through our spring, summer and fall sample dates, despite large changes in plant productivity. This shift was characterised by increasing relative abundances of certain bacteria (especially Gram +ves) as well as a decline in fungal biomass, and corresponded with decreasing C, N and P in the soil solution. The summer period of low substrate availability (plant–microbe competition) was associated with microbial indicators of nutritional stress. Overall, our results indicate that tundra microbial communities are clearly differentiated according to the changes in soil nutrient status and environmental conditions that occur between winter and post-thaw, and that those changes reflect functionally important adaptations to those conditions.

1. Introduction

Soil microorganisms are critical to carbon and nutrient fluxes in terrestrial ecosystems. Microbial processing of soil organic matter is determined directly by environmental conditions, and may be indirectly affected by changes in environmental conditions or substrate availability on the composition and relative abundances of the microbes (i.e. the community structure) – for *some* metabolic pathways at least (Schimel et al., 1995; Rinnan et al., 2007). Although the influences of changes in temperature and moisture on microbial activities have been extensively investigated (McKane et al., 1997; Hobbie et al., 2002; Davidson and Janssens, 2006), we know extraordinarily little about the consequences of environmental change for microbial community structure. Therefore, our ability to predict impacts of perturbations such as climate change on microbial structure and function is greatly hindered (Schimel and Gulledge, 1998). Investigating microbial community change is particularly important in Arctic surface soils, where there are strong fluctuations in annual environmental and biogeochemical conditions (Buckeridge and Grogan, 2010), high ecosystem sensitivity to changes in seasonal climate (ACIA, 2005), and a very large and sensitive soil carbon pool that has the potential to impact global atmospheric carbon pools (Schuur et al., 2008; Tarnocai et al., 2009). To date, there have been very few characterisations of Arctic tundra microbial community structure over time (Wallenstein et al., 2007) and only one (from alpine tundra) that linked an observed seasonal switch between fungi in winter and bacteria in summer (Schadt et al., 2003) to a shift in microbial decomposition from recalcitrant to labile substrates respectively (Lipson and Schmidt, 2004). We are still uncertain if larger fungal

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biomass over winter is the result of enhanced winter fungal growth, or residual (frozen) high fall fungal biomass, or if this seasonal shift occurs in non-alpine tundra ecosystems.

Apart from potential shifts in microbial community composition, seasonal variation of the total microbial biomass and the nutrients stored within that biomass in Arctic soils can drive seasonal patterns of nutrient cycling (Wardle, 1998; Jonasson et al., 1999; Schimel et al., 2007; Schmidt et al., 2007). In addition, the synchronicity – or lack of it – between microbial biomass patterns and plant primary production may have consequences for ecosystem-wide nutrient retention. Several authors have proposed that this is a particular concern during the thaw period at the end of snowmelt (Brooks et al., 1998; Jaeger III et al., 1999; Grogan and Jonasson, 2003), when there is a rapid decline in the microbial biomass C, N and P and plant uptake is negligible (Edwards et al., 2006; Buckeridge and Grogan, 2010). In fact, this period may be the most stressful phase in the entire annual cycle for microbes in the surface soil environment because of the physiological impacts of rapid temperature and severe osmotic pressure changes as wet-up occurs (Skogland et al., 1988; Schimel and Clein, 1996; Schimel et al., 2007; Jefferies et al., 2010). In addition, belowground trophic interactions such as increased mesofaunal predation through enhanced soil pore-space connectivity as soil water melts during the thaw period may also contribute to microbial species or functional group turnover (Wardle, 2002). In this study, we investigated the seasonal pattern of the soil microbial biomass, and community structure as well as principal nutrient pools from winter through fall in a mesic low arctic tundra ecosystem. We deliberately sampled from the pre- to post-thaw period at relatively high frequency compared to previous studies to determine if the dynamic environmental fluctuations at that time result in significant changes in the soil microbial communities.

Soil microbial biomass investigations can be accomplished in many ways, with varying agreement between methods (Balkwill et al., 1988; Zelles et al., 1992; Zogg et al., 1997; Bardgett and McAlister, 1999; Fritze et al., 2000; Grayston et al., 2001; Rogers and Tate, 2001). Discrepancies are common and not surprising because many techniques measure different aspects of the soil microbial community. For instance, variation in biomass (total microbial C), may not agree with variation in activity and growth (gene copy numbers). Both of these results may be clouded by changes in community; biomass may increase even as cell abundances decrease if many smaller bacterial cells are being replaced by larger bacterial cells or fungal hyphae. Increased activity may not be evident as increased gene copy numbers if community shifts occur between microbes with many gene copy numbers per cell to microbes with fewer gene copy numbers per cell. However, a combination of methods can provide a more complete description of the microbial community structure than any single method (Strickland and Rousk, 2010). In this study, we measured soil environmental properties and soil carbon, nitrogen and phosphorus pools over nine dates from winter to fall, and used four different methods (chloroform-fumigation, epifluorescent microscopy, phospholipids fatty acid analysis (PLFA) and quantitative polymerase chain reaction (qPCR) of taxon-specific rRNA) to characterize the soil microbial biomass and microbial community structure. With this study we investigated the following hypotheses in low arctic birch hummock tundra:

1. The soil microbial community structure of mesic arctic tundra displays community shifts, functional group crashes and new growth during the thaw period between winter and early spring.
2. Fungal biomass and 18S ITS gene copies in mesic arctic tundra soils are more abundant than bacterial biomass or 16S gene copies in winter and become less dominant in summer.
3. The pre-thaw and post-thaw microbial communities indicate functional group changes that are consistent with the differing environmental conditions of winter as compared to spring, summer and fall.

2. Methods

2.1. Site description and experimental design

This study was conducted in the winter, spring, summer and fall of 2007 at the Tundra Ecological Research Station (TERS) at Daring Lake, Northwest Territories, Canada (64° 50′N, 111° 38′W), 300 km northeast of Yellowknife, in the Coppermine River watershed. The mesic birch hummock ecosystem of our study is located in a valley, midway along a catena. The climate, soils and vegetation-types 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, 2010; Lafleur and Humphreys, 2008).

2.2. Soil sampling protocol

Daily mean soil temperature was monitored at 5 cm depth over the winter and spring, summer and fall ($n = 4$), using CR 10X dataloggers (Campbell Scientific, Logan, UT). Soil water content was measured gravimetrically (i.e. total water content as ice plus liquid) on each collected soil sample to ~5 cm depth. Snow depth, soil volumetric water content and air temperature were measured as described in Buckeridge and Grogan (2010). All sampling and monitoring occurred in the organic horizon, where the majority of plant rooting and biogeochemical cycling occurs in this ecosystem (Churchland et al., 2010).

Soil samples ($n = 5$) were collected over 9 sample dates over six months, beginning in winter (day 100), when samples were quickly returned to the lab at Queen's University by the next evening and kept just below 0 °C until processing on the following day. Further field samples were collected: in late winter (day 130); in early thaw, at the start (day 139) and end of snowpack thaw (day 147); in late thaw, when soils were just above 0 °C (day 157); in spring (day 169); in late spring, just after the start of bud break of the dominant shrub, *Betula glandulosa* (day 179); in mid-summer (day 192); and in early fall, just after the start of *B. glandulosa* leaf senescence (day 244).

Sampling details have been previously described (Buckeridge and Grogan, 2010). Briefly, sample characteristics such as aboveground vegetation were not pre-selected, but determined by the location of the base of the snowpit. Deep hollows and tall hummocks were avoided and thus interhummock areas were favoured. As thaw approached and vegetation was revealed, this sampling protocol was maintained. Therefore our results incorporate some of the tundra heterogeneity associated with hummocks, interhummocks and hollows. Soil samples were collected from frozen soils by hammering an axe to the depth of the organic layer (average depth 5.3 cm, range 2.9–9 cm), or by cutting out the organic layer with a knife in thawed soils. All samples were stored overnight in plastic bags in a cooler in the field lab then processed the following morning (soils were maintained frozen if collected frozen). Aboveground vegetation and litter was cut off at the moss green-brown transition, soil was chopped (frozen) or crumbled (thawed) into small pieces, large roots were removed, and soils were subsampled for immediate biogeochemical or microbial extraction. We minimized soil handling during soil mixing to limit disturbance of the soil microbial community in the lab at Queen's University and in the field. We were particularly careful to prevent thawing the frozen soils before extraction

instead of during extraction and emphasized consistent, efficient soil processing.

Soil physical characteristics and nutrient status were determined from these soil samples, as part of a study evaluating the impact of deepened snow on soil biogeochemistry (Buckeridge and Grogan, 2010).

2.3. Microbial biomass and community analysis

We used four techniques to quantify components of the soil microbial biomass and community: 1) a community 'fingerprint' as soil phospholipid fatty acid analysis (PLFA); 2) bacterial and cell counts and cell volume (to calculate bacterial C) and fungal hyphal length and hyphal volume (to calculate fungal C), enumerated with epifluorescent microscopy; 3) bacterial and fungal genomic ratios, using qPCR-analysis of bacterial 16S and fungal 18S ITS rRNA, and 4) soil microbial element stoichiometry through chloroform fumigated microbial biomass C, N and P (CFE).

In the field, PLFA subsamples (~5 g) were fresh frozen (−20 °C) and shipped to the University of Saskatchewan for freeze-drying and PLFA analysis. Microscopy subsamples and blanks were blended in a Waring lab blender with 90 mL of filter-sterile water for 5 min at 13,000 rpm then 9 mL of solution were fixed with 1 mL of formalin and stored at 4 °C until slide preparation at Queen's University. We used 0.25 g of soil and MoBio Power Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) to attain soil genomic DNA in the field; we then froze these extracts (−20 °C) and shipped them to the University of Saskatchewan for qPCR analysis. Soil and microbial nutrient contents were determined on fumigated (24 h in a darkened vacuum desiccator jar at ~15 °C) and non-fumigated subsamples (10 g fresh weight soil) extracted in 0.5 M K_2SO_4 (50 mls), using the chloroform-fumigation direct-extraction (CFE) (Brookes et al., 1985). Extracts were made with filter-sterilized (0.2 μm) lake water, and blanks were analyzed for each sample date. All salt-extract subsamples were shaken manually several times for a minimum of 1 h in extractant, filtered through a 1.2 μm pore-size glass fiber filter and frozen at −20 °C until laboratory analysis of C, N and P content.

Microbial biomass C, N and P contents (MBC, MBN and MBP) were calculated as the difference between TOC, TN or PO_4^{3-}—P in fumigated and non-fumigated salt-extracts (Brookes et al., 1982, 1985), and no correction factors for fumigation efficiency were applied to the microbial biomass C, N or P or for incomplete PO_4^{3-}—P recovery from soil to microbial biomass P. PO_4^{3-}—P in extracts was determined colourimetrically, using automated flow analysis (Bran-Leubbe Autoanalyzer III, Norderstedt, Germany) and the ascorbic acid (Kuo, 1996) method. Total dissolved C and N contents in the fumigated and non-fumigated salt extracts and in the water extracts were determined by oxidative combustion and infrared analysis (TOC) (Nelson and Sommers, 1996) and chemiluminescence analysis (TN) (TOC-TN autoanalyzer, Shimadzu, Kyoto, Japan). All C, N and P concentrations in the soil extracts were corrected for the dilution associated with the moisture content of each soil sample. PLFA analyses were performed using a modified version of the White et al. (1979) method, based on the original method of Bligh and Dyer (1959). Fatty acids were separated on a solid phase extraction column (0.50 g Si; Varian Inc. Mississauga, ON, Canada). Phospholipids were methylated and the resulting fatty acid methyl esters were analyzed using a Hewlett—Packard 5890 Series II gas chromatograph with a 25 m Ultra 2 column (J&W Scientific). Peaks were identified using fatty acid standards and identification software (MIDI Inc., Newark, DE), and quantified based on the addition of methyl nonadecanoate (19:0) as an internal standard. A total of 69 different PLFAs were extracted, and the sum of these total extractable PLFAs was used as an indicator of microbial biomass. From this larger pool, 44 fatty acids were selected (each were required to be > 0.5 mol percent abundance (mol %) of the total biomass on any date) for use in calculating relative abundances of individual fatty acids and biomarkers, and for community ordination analysis. Specific PLFAs were used as biomarkers to quantify the relative abundances (mol%) of particular functional groups within the group of 44 fatty acids, on the basis that they have been isolated from organisms within these functional groups with some specificity (Zelles, 1997, 1999). Fatty acid nomenclature follows Frostegard et al. (1993). Fungal biomass was calculated based on the fatty acid 18:2 ω6,9c, the total bacterial biomass was obtained by summing 14:0, 15:0, *a*15:0, *i*15:0, *i*16:0, 16:1 ω7c, 16:1 ω11c, 10 Me 16:0, 17:0, *a*17:0, cy17:0, *i*17:0, 17:1 ω8c, 10 Me 17:0, 18:0 2OH, 18:1 ω5c, 18:1 ω7c, 10 Me 18:0, 19:1 ω6c and cy19:0 ω8c, and the fungal to bacterial ratios were calculated as the fungal biomarker divided by an average of all bacterial fatty acids (Fraterrigo et al., 2006). Gram-negative bacteria were calculated as the sum of 16:1 ω5c, 16:1 ω11c, cy17:0, 18:0 2OH, 18:1 ω5c, 18:1 ω7c, 18:1 ω9c and cy19:0 ω8c, Gram-positive bacteria as the sum of *i*14:0, *a*15:0, *i*15:0, *a*17:0 and *i*17:0, actinomycetes as the sum of all fatty acids methylated on the tenth C (10 Me 16:0, 10 Me 17:0 and 10 Me 18:0), and protozoa were identified by the polyunsaturated fatty acid 20:4 ω6,9,12,15c (Zelles, 1997, 1999). We used two ratios of PLFA relative abundance as indicators of changes in physiological status of the microbial community, the ratio of cyclopropyl fatty acids to their monoenoic precursors, and the ratio of total saturated to total monosaturated fatty acids (Bossio et al., 1998; Fierer et al., 2003). Increases in these ratios have previously been shown to be indicative of starvation, stationary phase growth and nutrient limitation (Guckert et al., 1986; Kieft et al., 1994).

Epifluorescent microscopy slide preparation was according to Bloem et al. (1995) with some modifications, as described in Buckeridge and Grogan (2008), using the polysaccharide stain fluorescent brightener 28 (FB 28) to stain total fungi (i.e. live plus dead fungal mass), and the nucleic acid stain DTAF to stain total bacteria (i.e. live plus dead bacterial mass). All samples were viewed with an epifluorescent microscope (Nikon E600W), and photographed with a cooled 16-bit digital colour camera (QICAM 1394, QImaging, Burnaby, B.C.). Bacterial cell counts and volume were based upon the average of 10 fields-of-view per slide and fungal hyphal analyses were the average of 20 fields-of-view per slide. Slide images ($n = 450$ for bacteria, 900 for fungi) were randomized across date, replicate and field-of-view with a random number table to determine order of image analysis, and all visual analyses were performed by the same person. Enumeration of bacteria and fungi was done semi-automatically, as previously described (Buckeridge and Grogan, 2008). We calculated bacterial volume/cell and fungal volume/hyphal unit length to monitor morphological shifts that might be due to changes in physiology or species composition.

The ratio of bacterial and fungal abundance was determined by quantifying bacterial and fungal ribosomal gene copy numbers using QuantiTect™ SYBR® Green PCR Master Mix and an ABI 7500 real-time polymerase chain reaction machine (Applied Biosystems, Foster City, CA). Bacterial 16S rRNA copy numbers were estimated by primer set 338f (5′ACTCCTACGGGAGGCAGCAG) and 518r (5′ATTACCGCGGCTGCTGG) (Ovreås et al., 1997). Fungal 18S rRNA ITS copy numbers were estimated by primer set 18S ITS 1f (5′CTTGGTCATTTAGAGGAAGTAA) and 18S ITS 4r (5′CAGGAGACTTGTACACGGTCCAG) (Gardes and Bruns, 1993). PCR amplification used the following program: 92 °C for 2 min; 30 cycles of denaturation at 92 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min; and a single final extension at 72 °C for 6 min. Each 20 μl reaction contained 10 μl of master mix, 10 pmol of the primers, 6 μl sterilized milli-Q water, and 2 μl template DNA. The amplification efficiency of

the genes was between 80% and 100%. Standard curves ($r^2 > 0.995$) were generated by preparing standards from purified PCR product from one of the soil DNA extracts. Only the standard curves linear over five orders of magnitude were selected and standards were run on each individual q-PCR plate. The specificity of the amplified products was examined by melting curve analysis. Amplification inhibition effects were evaluated according to Dumonceaux et al. (2006). Gene copies were presented as copies g^{-1} dw soil, and not corrected by DNA concentration, as we suspect a large proportion of the total genomic DNA was plant root or other DNA (i.e. not just bacterial and fungal DNA), which may have strong seasonal variation.

2.4. Statistical analyses

To test the effect of sampling date on the biogeochemical and microbial data we used repeated measures analyses of variance (RM ANOVA), in JMP 8.0 (SAS, Cary ID); all significant results are reported. We assessed similarity among microbial analytical methods by comparing the data using pairwise correlations (JMP). All data were transformed before these parametric analyses if necessary, as determined by the Shapiro–Wilks test to meet the requirements of normality.

We investigated the influence of sampling date on the fatty acid relative abundance (mol %) profiles using non-metric multidimensional scaling analyses (NMS; Kruskal, 1964) with PC-ORD 5.0 software (McCune and Mefford, 2006). NMS is an iterative multivariate ordination technique that is suited to non-linear data that are not normally distributed (McCune and Grace, 2002). The data were first arcsine-square root transformed to improve the effectiveness of the distance measure (McCune and Grace, 2002) then the ordinations were run using a Sørensen distance measure from a random start and a maximum of 500 iterations in 50 runs with real data. Statistical significance of the solutions was assessed by comparing observed final stress with the final stress in 50 runs of randomized data using a Monte Carlo test (McCune and Grace, 2002). Environmental, microscopy and qPCR variables and fatty acid biomarkers were presented as biplot vectors on the NMS ordination plot and represent those factors that most strongly correlate with the NMS ordination axes (variables with an $r^2 > 0.3$ were included). Statistically significant effects of the sampling date on the PLFA profiles was assessed with a non-parametric multi-response permutation procedure (MRPP; Mielke, 1991) in PC-ORD. MRPP yields a *P*-value to evaluate how likely it is that an observed difference between groups (sampling date) is due to chance, as well as the chance-corrected within-group agreement (A), which describes within-group homogeneity compared to random expectation (McCune and Grace, 2002). 'A' values ranges from 1 (when all items within a group are identical) to 0 (when heterogeneity within groups equals expectation by chance). To investigate which fatty acids have a non-random distribution across the sample dates, we used indicator species analysis (ISA; Dufrene and Legendre, 1997) in PC-ORD, which provides an indicator value for each fatty acid based on its abundance in particular groups and the relative frequency within a group. The significance of the indicator value of a fatty acid is calculated with the Monte Carlo method, generating a *P*-value for the proportion of times that the indicator value from a randomized dataset equals or exceeds that from the actual dataset (McCune and Grace, 2002).

3. Results

3.1. Seasonal soil environment and nutrient availability

The soil environment and nutrient availability for this site are summarized for the dates specific to this investigation (Table 1), and have also been recorded during the same experimental period as part of an earlier study of deepened snow effects (Buckeridge and Grogan 2010). Environmental conditions and soil nutrient pools were variable throughout the year, particularly over the period from winter to spring thaw (Fig. 1; Table 1). Generally, winter soils were cold, dry and relatively high in dissolved inorganic and organic nutrients. Soils during spring thaw were cool, wet and relatively high in both inorganic and organic nutrients. Summer and fall soils were cool to warm, mesic with low nutrient availability.

3.2. Seasonal microbial biogeochemistry

Microbial biomass C and microbial N accumulation (measured with chloroform fumigation) varied significantly during the study (Table 1; $F_{8,31} = 7.6$, $P < 0.0001$ and $F_{8,32} = 2.8$, $P = 0.02$, respectively), declining from winter to late winter, then increasing during thaw before dropping again significantly by spring. Over the summer, MBC and MBN recovered to reach values in fall that were only slightly lower than in the previous winter (Table 1). This seasonal pattern in MBN was muted relative to MBC. By contrast, the pool of phosphorus within the microbial biomass generally decreased through the study, declining to ~1/3 of the winter value by the

Table 1
Environmental and biogeochemical properties at nine sampling times during 2007, in birch hummock tundra at Daring Lake, NWT.

Season	Winter	Late winter	Early thaw	Thaw	Late thaw	Early spring	Bud break	Summer	Fall
DOY	100	130	139	147	157	169	179	192	244
Soil C/N	48 (4.7)	47 (6.7)	40 (2.8)	35 (4.8)	28 (1.4)	35 (4.8)	35 (3.4)	33 (2.6)	29 (2.1)
Soil C	50 (0.26)	49 (0.43)	47 (0.56)	45 (1.6)	44 (1.3)	43 (2.4)	46 (1.7)	47 (0.42)	45 (2.3)
Soil N	1.1 (0.11)	1.1 (0.19)	1.2 (0.077)	1.4 (0.15)	1.6 (0.077)	1.3 (0.18)	1.3 (0.089)	1.4 (0.11)	1.5 (0.060)
Soil pH	3.8 (0.023)	4.1 (0.037)	3.8 (0.11)	3.7 (0.051)	3.8 (0.047)	3.8 (0.064)	3.9 (0.058)	3.9 (0.026)	3.9 (0.090)
DIN	0.83 (0.26)	1.7 (0.55)	1.3 (0.55)	0.47 (0.42)	0.36 (0.10)	0.39 (0.38)	0.052 (0.0078)	0.14 (0.11)	0.28 (0.093)
DIP	1.3 (0.15)	9.6 (4.9)	3.3 (1.3)	1.7 (0.79)	0.50 (0.27)	0.29 (0.11)	0.31 (0.049)	0.075 (0.033)	0.22 (0.023)
DON	25 (1.6)	33 (9.3)	31 (6.1)	13 (1.0)	6.9 (1.7)	5.1 (1.5)	7.0 (1.9)	4.7 (1.9)	16 (1.5)
DOP	16 (1.6)	46 (21)	26 (6.5)	48 (32)	11 (2.2)	5.4 (4.7)	4.8 (1.1)	5.5 (1.0)	5.9 (0.94)
DOC	1.1 (0.074)	2.9 (0.82)	4.3 (1.6)	1.1 (0.13)	0.57 (0.070)	0.30 (0.066)	0.49 (0.081)	0.34 (0.046)	0.56 (0.049)
MBC	7.6 (0.72)	4.9 (0.97)	7.1 (1.5)	5.1 (0.56)	5.0 (0.41)	2.9 (0.12)	4.3 (0.43)	3.8 (0.44)	6.4 (0.50)
MBN	0.36 (0.026)	0.28 (0.055)	0.31 (0.030)	0.30 (0.022)	0.30 (0.0060)	0.19 (0.010)	0.39 (0.041)	0.30 (0.044)	0.33 (0.027)
MBP	0.13 (0.029)	0.081 (0.031)	0.10 (0.014)	0.074 (0.030)	0.040 (0.028)	0.051 (0.023)	0.043 (0.018)	0.034 (0.014)	0.040 (0.022)

Parentheses indicate one standard error ($n = 5$).
Soil C and N are %; DIN, DIP, DON and DOP are μg of dissolved water-extractable inorganic nitrogen (NO_3^-–N + NH_4^+–N), inorganic phosphorus (PO_4^{3-}–P), organic nitrogen and organic phosphorus, DOC is mg of dissolved water-extractable carbon, and MCN, MBN, MBP are mg of $CHCl_3$-fumigated and 0.5 M K_2SO_4-extractable microbial biomass organic carbon, total nitrogen and PO_4^{3-}–P, respectively, per g dry weight soil.

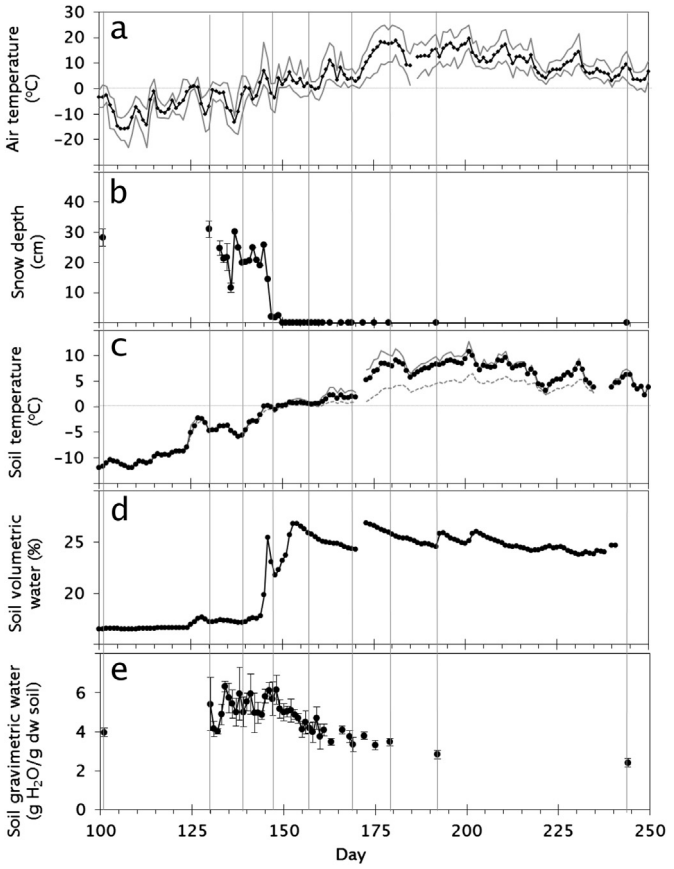

Fig. 1. Daily mean (black line), maximum and minimum air temperatures (a); mean snow depth (cm, $n = 5$) (b); soil temperature at 2 (grey line), 5 (black line with closed circles) and 10 (grey dashed line) cm depth (°C; $n = 2$, 4, and 2) (c); soil volumetric water (%; $n = 2$) (d); and soil gravimetric water (i.e. liquid and frozen g H_2O/g dw soil; $n = 5$) (e) in birch hummock vegetation at Daring Lake, NWT during the winter–fall of 2007. Error bars indicate ± one standard error. Vertical grey lines mark the sample dates (see Table 1 for sampling date titles).

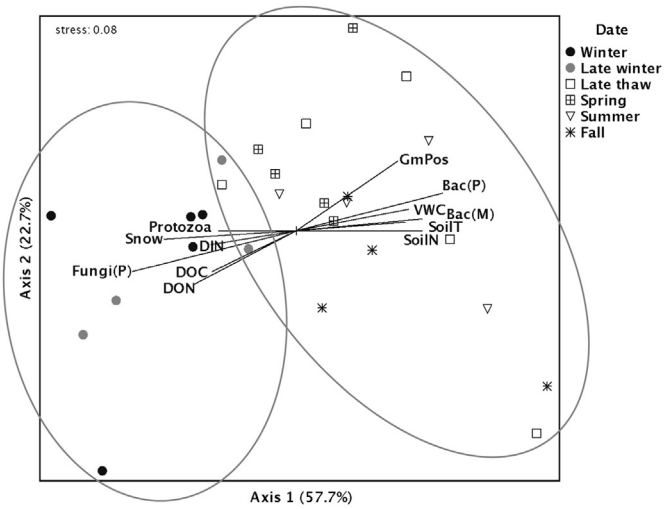

Fig. 2. Non-metric multidimensional scaling (NMS) ordination of PLFAs over six sample dates from winter through to fall (total $n = 27$). Sampling for 'winter' and 'late winter' (left-hand oval) was on Julian days 100 and 130 respectively, and for 'late thaw', 'spring', 'summer', and 'fall' (right-hand oval) was on Julian days 157, 169, 192 and 244 respectively. Ordination data points represent fatty acid profiles from individual sample plots. Lines are biplot vectors that represent those environmental variables or fatty acid biomarkers that most strongly correlate with the NMS ordination axes and only variables with an $r^2 > 0.3$ are represented. 'Protozoa' and 'GmPos' are the relative abundance of fatty acids used to indicate protozoa and Gram-positive bacteria; 'Snow' is snow depth; 'VWC' is volumetric water content; 'SoilT' is soil temperature; 'DIN', 'DOC', 'DON' are water-extractable soil inorganic N, organic C, and organic N, respectively; 'SoilN' is total N in the soil; Fungi(P) and Bac(P) are the relative abundance of the fungal and bacterial biomarkers as estimated with PLFA; Bac(M) are the numbers of bacterial cells as enumerated with epifluorescent microscopy.

succeeding fall, and suggesting substantial microbial uptake of P in the cold season between September and April (Table 1).

3.3. Soil microbial community change at thaw

NMS analysis of the PLFA data indicated a strong shift in the whole community profiles of fatty acid relative abundances between the late winter and late thaw sampling dates (i.e. period during which the snow cover completely melts, and the soil liquid water rises dramatically) (MRPP, $A = 0.13$, $P < 0.0001$) (Fig. 2). Specifically, the winter and late winter communities did not differ from each other but both differed from the late thaw ($A = 0.11$, $P = 0.01$ and $A = 0.095$, $P = 0.02$, respectively), the spring ($A = 0.13$, $P = 0.0014$ and $A = 0.11$, $P = 0.006$, respectively), the summer ($A = 0.18$, $P = 0.003$ and $A = 0.17$, $P = 0.009$, respectively) and the fall sampling dates ($A = 0.16$, $P = 0.003$ and $A = 0.14$, $P = 0.006$, respectively). Community profiles on these last four dates were similar and only differed between spring and fall ($A = 0.061$, $P = 0.03$). The best-fitting NMS solution contained 3-dimensions (stress = 8.08, final instability = 0.00001, iterations = 83; Fig. 2, only 2 axes shown). The first axis explained 57.7% of the variation in the ordination. The winter and late-winter PLFA communities (i.e. the pre-thaw soils) on this first axis were associated with greater snow depth ($r^2 = 0.53$), high fungal and protozoal relative abundances ($r^2 = 0.67$ and 0.32, respectively), and high DON, DIN, DOC and PO_4 ($r^2 = 0.41$, 0.35, 0.34 and 0.23, respectively). By contrast, the PLFA communities of the post-thaw soils sampled in late thaw, spring, summer and fall on the first axis (positively correlated) were associated with a distinctly different set of environmental variables including increasing soil liquid water content ($r^2 = 0.45$), higher total soil N ($r^2 = 0.51$) and warmer soil temperatures ($r^2 = 0.51$). Furthermore, the post-thaw soil communities were characterized by high bacterial numbers (microscopy; $r^2 = 0.44$) and relative abundances (PLFA; $r^2 = 0.59$), in particular, Gram positive bacteria ($r^2 = 0.41$). The second NMS axis explained 22.7% of the variance in the annual PLFA profiles and was positively correlated with Gram-positive bacteria, 16S and ITS copy numbers ($r^2 = 0.28$, 0.28 and 0.20, respectively), and was negatively correlated with MBC, MBN and DON ($r^2 = 0.23$, 0.20 and 0.21, respectively). The third NMS axis (not shown) explained 14.9% of the variation in the ordination and was positively correlated with actinomycetes and Gram-negative bacteria ($r^2 = 0.39$ and 0.25, respectively) and negatively correlated with a high saturated/monosaturated ratio ($r^2 = 0.32$).

The Indicator Species Analysis (ISA) identified several fatty acids (FA) that had a non-random distribution across the five sample dates. The winter and late winter communities contained relatively high proportions of a common monounsaturated FA (15:1 ω6c) and a branched FA (15:1*i*) that are both associated with Gram-negative (*Flavobacterium*) low-temperature membrane adaptations to enhance fluidity, the fungal biomarker (18:2 ω6,9c), a commonly associated fungal FA (18:1 ω9c), and the protozoan biomarker (20:4 ω6,9,12,15c). The late thaw sample contained relatively high proportions of a branched FA (15:0 *a*) that is associated with Gram-positive cold-stress, and a monounsaturated FA (16:1 ω5c) that is commonly associated with arbuscular mycorrhizae or Gram-negative bacteria. There were no statistically significant indicator species in the spring community, and the summer and fall

communities were distinguished by a Gram-negative biomarker (10:0 2OH), a common monounsaturated FA (19:1 ω6c), and two saturated FAs (12:0, 17:0).

3.4. Fungal, bacterial and fungal:bacterial ratio response to season

Bacterial cell counts measured with epifluorescent microscopy differed significantly among sample dates, increasing from late winter lows to peak values in late thaw (day 157) ($F_{8,32} = 3.8$, $P = 0.0032$) (Fig. 3a), simultaneously with the significant overall PLFA-based community shift (Fig. 2). Bacterial cell counts declined significantly from the late thaw peak to spring low then increased in mid-summer to levels similar to those in the fall. Bacterial carbon followed a similar seasonal pattern as cell counts ($F_{8,31} = 4.3$, $P = 0.001$). Fungal total hyphal length also exhibited significant seasonal change ($F_{8,32} = 4.5$, $P = 0.001$), following a very similar pattern as bacterial numbers except that they declined substantially between the winter and late winter sampling dates before rising to reach a peak in late thaw that was, once again simultaneous with the significant overall PLFA-based community shift (Fig. 2). Furthermore, after the post-thaw decline, fungal total hyphal length then increased significantly on the summer and fall sample dates to match the spring peak values (Fig. 3b). Fungal C followed a similar seasonal pattern as hyphal length ($F_{8,30} = 2.2$, $P = 0.06$). Despite increasing bacterial cell numbers, bacterial C/cell declined after winter ($F_{8,32} = 3.3$, $P = 0.0079$; data not shown directly, but note in Fig. 3a how the bacterial cell number trajectory increases more rapidly than the bacterial C) suggesting more, smaller cells as a result of increased replication at this time. There was a delayed, similar (but not significant) trend in hyphal length relative to volume (Fig. 3b). This winter to late winter bacterial growth of many small cells (increased bacterial cell count relative to bacterial C) was coincident with an increase in soil DOC, a decrease in MBC and MBP (Table 1) and a decrease in fungal length relative to fungal C. Fungal length relative to fungal C then increased, indicating new fungal hyphal growth, from late winter to thaw as soil DOC declined (Table 1).

Bacterial gene copy numbers varied seasonally ($F_{8,31} = 3.5$, $P = 0.003$), tending to decline from winter through to the thaw and then climbing to seasonal highs over the post-thaw period and through to summer, before gradually declining towards the fall (Fig. 4). By contrast, corresponding fungal gene copy numbers had larger within-date variation and there was no significant seasonal variation.

As expected from the ordination results (Fig. 2), several of the PLFA biomarkers varied significantly with sampling date (Table 2). In particular, the fungal biomarker was more abundant in winter and late winter than all other sample dates ($F_{5,20} = 13.4$, $P < 0.0001$). The sum of all the bacterial biomarkers illustrated the opposite, highest during late thaw and low in winter, late winter and fall ($F_{5,19} = 6.2$, $P = 0.0014$). Gram-positive bacteria were also highest in late thaw, but lowest during late winter ($F_{5,20} = 5.0$, $P = 0.0039$). Gram-negative bacteria also peaked in late thaw, yet were less abundant in winter, summer and fall ($F_{5,20} = 4.4$, $P = 0.0074$). Actinomycete bacteria were most abundant in late thaw and spring (i.e. immediately after the overall PLFA-based community shift), but became relatively scarce in the fall ($F_{5,20} = 3.2$, $P = 0.03$). Saturated/monounsaturated ratios (a potential indicator of nutritional limitation) were significantly higher in summer relative to winter and late thaw ($F_{5,20} = 4.4$, $P = 0.008$).

Our methods for determining microbial community structure over the six dates revealed some consistent patterns. Fungal to bacterial ratios measured with PLFA, qPCR, epifluorescent microscopy and as CFE (MBC/N ratio) were positively correlated between the PLFA and CFE ($r = 0.43$, $P = 0.02$) and qPCR and CFE ($r = 0.31$, $P = 0.04$) data. There were significant differences among sampling dates for these PLFA and CFE data, indicating in both cases that high F/B ratios in winter declined sharply to annual lows at varying points in the spring (Fig. 5). Specifically, CFE F/B in winter was higher than summer, and winter, spring and fall were higher than spring ($F_{8,31} = 5.4$, $P = 0.0003$); and PLFA F/B in winter was higher than all three post-thaw spring dates ($F_{5,18} = 5.7$, $P = 0.003$). The magnitude of the estimated F/B ratio declined by method (CFE > PLFA > qPCR ≈ microscopy), indicating that fungi were

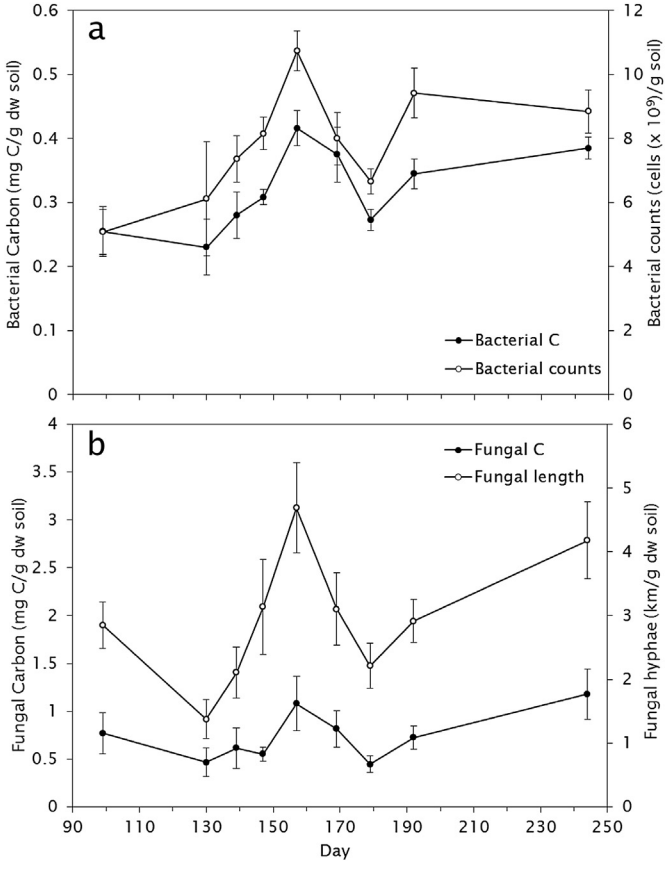

Fig. 3. Quantitative microbial community analyses using epifluorescent microscopy to estimate seasonal bacterial carbon and cell numbers (a) and fungal carbon and hyphal length (b).

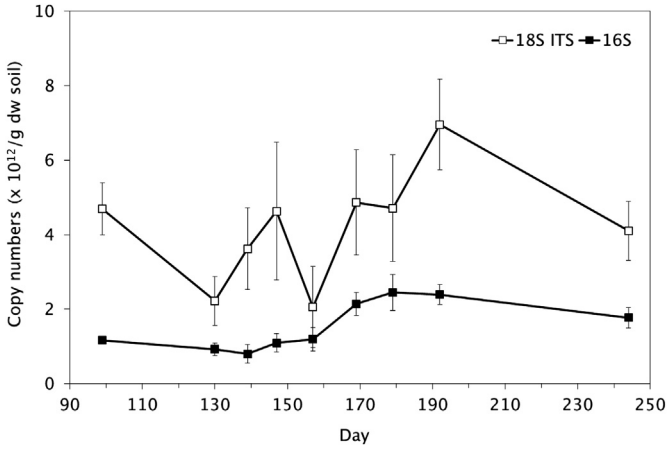

Fig. 4. Quantitative microbial community analysis using qPCR to estimate the copy numbers of the 18S ITS rRNA in fungi and the 16S rRNA in bacteria.

Table 2
The seasonal pattern of soil microbial groups categorized according to PLFA biomarkers.[a]

Date	Fungi (mol%)	Bacteria (mol%)	Gram-negative bacteria (mol%)	Gram-positive bacteria (mol%)	Actinomycetes (mol%)	Protozoa (mol%)	cy17:0 + cy19:0/ precursors[b]	Total saturated/total monosaturated[b]
Winter (DOY 99)	23 (1.7)	32 (1.7)	24 (1.2)	9.5 (0.82)	5.7 (0.21)	0.54 (0.17)	0.25 (0.0096)	0.90 (0.069)
Late winter (DOY 130)	22 (2.5)	29 (3.5)	25 (1.3)	8.3 (0.68)	5.5 (0.84)	0.56 (0.19)	0.33 (0.068)	1.1 (0.12)
Late thaw (DOY 157)	12 (1.5)	43 (2.9)	29 (1.3)	13 (1.2)	8.6 (1.1)	0.088 (0.088)	0.33 (0.027)	0.85 (0.17)
Spring (DOY 169)	12 (2.6)	40 (2.2)	25 (1.1)	11 (0.32)	8.4 (1.1)	0.24 (0.24)	0.37 (0.069)	1.1 (0.11)
Summer (DOY 192)	9.8 (2.1)	38 (1.3)	24 (1.0)	12 (0.68)	7.3 (0.68)	0	0.33 (0.22)	1.6 (0.25)
Fall (DOY 244)	11 (2.4)	31 (2.9)	23 (0.90)	10 (0.61)	4.1 (1.4)	0.30 (0.30)	0.33 (0.27)	1.4 (0.090)

[a] See methods for identification of the PLFAs used to calculate microbial group and the ratio abundances. Parentheses indicate one standard error ($n = 5$).
[b] Ratios that may be indicative of the physiological status of microbial communities.

consistently more abundant than bacteria in this mesic tundra soil throughout the study period ($F/B > 1$) (Fig. 5).

4. Discussion

Our goal was to investigate the seasonal pattern of the soil microbial community in mesic low Arctic tundra, with emphasis on the environmentally- and biogeochemically-variable thaw period. Our CFE and PLFA data indicate a strong statistically significant shift in the soil microbial community of mesic arctic tundra between the pre-thaw (winter and late winter) and the post-thaw (early and late spring, summer and fall) seasons, supporting Hypothesis 1. Bacterial community shifts between frozen and non-frozen soils has been noted previously in Antarctic tundra soils (Schafer et al., 2009). In our study, the shift to non-frozen soil was characterized by increasing relative abundances of certain bacteria (especially Gram +ves) as well as a decline in fungal biomass, and corresponded with a decrease in organic C and nutrient availability in the soil solution.

The significant declines ($\sim 1/2$) in F/B ratios observed with these two methods strongly suggest that fungal biomass was more dominant in winter than summer in these soils, supporting Hypothesis 2. This latter result is consistent with previous research at this site (Buckeridge and Grogan, 2008), with greater fungal biomass during winter in alpine tundra (Schadt et al., 2003), and with a similar magnitude of decline in the F/B ratio between winter and summer in alpine tundra (Björk et al., 2008). Winter fungal biomass peaks have not been recorded in non-tundra ecosystems, that we are aware of (Bardgett et al. 1999). Fungi may be favoured over bacteria in cold, dry soil, since their mycelial growth habit may allow exploitation beyond individual microsites of liquid water, or across thin films of liquid water in frozen soils. However, there is little research on the relative activity of fungi and bacteria in permafrost soils. Although our other two methods (microscopy and qPCR) indicated no significant winter peak in the F/B ratio, there were thaw peaks in bacterial and fungal biomass (microscopy). This result is consistent with relatively high fungal activity at −2 °C as compared to summer soils in Alaskan tussock tundra (McMahon et al., 2009). High fluorescein-active F/B ratios have been detected in alpine winter soils (Lipson et al., 2002). However, our relatively small increase in 18S ITS/16S ratio in the winter suggests that the majority of this fungal increase in arctic winter permafrost soils may be due to enhanced biomass (possibly preserved from the previous fall), instead of enhanced fungal growth during the very cold (i.e. −20 °C minima) winter months.

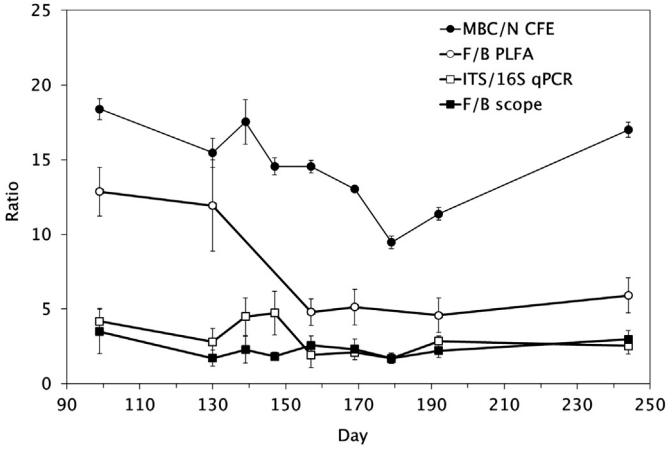

Fig. 5. Estimates of the seasonal soil fungal/bacterial ratios as calculated by CFE (MBC/N), PLFA lipid biomarkers, epifluorescent microscopy of the fungal and bacterial carbon, and Q-PCR, based on gene copies of the ITS rRNA in fungi and the 16S small-subunit rRNA in bacteria. Error bars indicate ± one standard error ($n = 5$). Note the 3 fewer sampling dates for PLFA data.

Our microbial community analysis (PLFA) indicated that the predominant bacteria within the winter microbial community had membranes that were specifically adapted to cold temperatures, such as anteiso-branching and unsaturated fatty acids (Kraft, 1992). Snowmelt defines the transition from of the cold season to warm season microbial community composition as indicated by fatty acid profiles. However, our microscopy data indicate new growth of small-celled bacteria and thin fungal hyphae from late winter up until the same time as high CFE biomass on day 157, which is 10 days after snowmelt. Perhaps related to this hyphal growth, our ISA results suggested that the FA 16:1 w5c, commonly associated with arbuscular mycorrhizal fungi (AMF), was characteristic of the thaw period. Although low arctic ecosystems are dominated by dark-septate fungi, and ecto- and ericoid-mycorrhizae, AMF are also common symbionts in tundra (Newsham et al., 2009), and thaw in particular may be associated with the growth of AMF-associated herbaceous plants. These patterns of nutrient immobilization and new growth of fungal hyphae and bacterial cells during thaw are associated with only a slight increase in 16S and variable ITS gene copy numbers. Although we expected an increase in genetic material with new cell growth, absolute numbers generated with qPCR are less useful than relative abundances, since the efficiency of PCR amplification can vary across DNA samples. The new cell growth detected with microscopy occurred while soil temperatures were rising from −5 °C to near 0 °C. Microbial growth below zero has also been reported as respired C from frozen soils in laboratory incubations (Schimel and Mikan, 2005; Panikov et al., 2006) or ^{13}C incorporation into phospholipids in frozen soil microcosms (McMahon et al., 2009); our results are the first to indicate microbial growth in sub-zero soils *in situ*.

The post-thaw microbial community composition was associated with biomarkers indicative of nutritional stress, such as small cell sizes and significant increases in the saturated/mono-unsaturated ratio during summer and fall (Kieft et al., 1994). Other studies have also found an increase in this ratio in nutrient-limiting conditions, such as with increasing soil depth (Fierer et al., 2003).

Despite large changes in aboveground productivity from spring to fall, the microbial community only varied slightly over this period, at least between our sampling dates. A stable fungal and bacterial community has been recorded with 16S and 18S rRNA clone libraries at the phyla level in Alaskan tundra at the start and the end of the winter (late fall and early spring) (Wallenstein et al., 2007); our results also indicate that tundra organic soil horizon communities do not change strongly across a single growing season. Spatial and temporal variation in the PLFA-based microbial community across the growing season was positively associated with the abundance of bacteria (microscopy and PLFA), especially Gram-positives, suggesting that growth of this group was particularly responsive to environmental conditions during the summer, or else that predation was correspondingly low. Bacterial 16S gene copies also peaked over this late thaw to summer period. Certain Gram-positive bacteria have been associated with a K-selected growth strategy indicative of low resource availability (Fierer et al., 2007; Cruz-Martinez et al., 2009), which may explain their prevalence during the post-thaw period if substrate competition between plants and microbes was occurring at that time. Together, in summary, many of the changes in microbial PLFA signatures that occurred as winter soils thawed and entered summer can be functionally linked to the changes in environmental conditions and soil nutrient status that occurred over the same period, supporting Hypothesis 3.

The pattern of microbial community change over the course of a year may be considered a dynamic stable state, responding to environmental drivers (i.e. soil water thaw and soil temperature), community interactions (i.e. predation and competition) and the impacts of both environment and community on resource availability as seasons change. The decline in fungal biomass in our study from winter to late winter, and then again in spring, was associated with a relatively high abundance of protozoan biomarkers, some of which may be fungal feeders (Ekelund and Rønn, 1994; Rodríguez–Zaragoza, 1994). Furthermore, the microbial community change just after thaw in this study was very similar to the successional pattern characterized in laboratory studies with recently rewetted and glucose-amended humic soils (Polyanskaya et al., 2008). Although fatty acid biomarkers are not definitive, the β-hydroxy (3OH) fatty acids are generally associated with the Gram-negative lipopolysaccharide layer (Zelles, 1999), especially Bacteroidetes (Moat et al., 2002), and fatty acids with a methyl group on the 10th C are commonly associated with actinomycetes (Zelles, 1999). Both groups had a significantly higher relative abundance on day 157 (late thaw) of our field study. In the Polyanskaya et al. (2008) successional pathway, fast-growing Gram-negative bacteria colonized, typical of an r-selected functional group (Fierer et al., 2007), followed by increases in actinomycetes and an increase in chitinase production, believed to contribute to the decline in fungal biomass in these laboratory studies. Our results, albeit at a low sampling frequency relative to the successional changes observed in the lab, are nevertheless clearly consistent with the described successional pathway, are at a high-frequency as compared to other field studies, and suggest that community interactions may be an important driver of seasonal succession.

Our estimates of fungal/bacterial ratios differed in both magnitude and pattern between methods across arctic seasons (Fig. 5). These results are a reflection of the effectiveness of the measured variable as a proxy for abundance. For instance, microbial biomass C/N ratios are used by ecosystem modellers as indicators of community change because cytoplasmic stoichiometries tend to shift towards greater C content with more fungal biomass (Strickland and Rousk, 2010). However, the CFE-flush is a rough approximation of F/B ratios as there is a large amount of overlap in the stoichiometry of microbial functional groups (Strickland and Rousk, 2010). PLFA F/B ratios may vary with lipids that are included in the unstandardized 'fungal' or 'bacterial' classification, as biomarkers often have greater degeneracy than implied (Zelles, 1999). Enumeration of fungi and bacteria with microscopy is well-known to underrepresent biomass, although the reasons for this vary widely between sample locations and investigators; our microscopy technique does not distinguish between live and dead tissues (especially for fungi) and may underestimate certain fungal strains (Joergensen and Wichern, 2008). Relatively low fungal gene copies of ITS rRNA relative to bacterial gene copies of 16S rRNA (as compared to the other F/B ratios in this study) are perhaps not surprising, given the high biomass/genomic material ratio for fungi (i.e. empty mycelia) and the high variability in 18S ITS gene copy numbers (i.e. 375 ± 294 gene copies g^{-1} dry fungal biomass; Baldrian et al., 2012) and 16S gene copy numbers (1–14 gene copies $cell^{-1}$; Farrelly et al., 1995). As a result, gene copy numbers may vary more according to growth rate or species differences than from biomass. Overall, gene copies were high in this study, as has been detected in other Arctic soils (Banerjee et al., 2011). The method to 'trust' may therefore reflect the investigator's interest in either biomass C content or genomic material. For instance, fungal biomass growth is not necessarily tied to genetic biomass, and genetically-empty fungal hyphae may provide an important ecosystem source of soil microbial biomass C for predation, decomposition and soil organic matter formation.

The persistence (i.e. turnover) of the measured variable in its environment also impacts our results. Microbial biomass C/N ratio can vary by a factor of 2 over a period of days (Buckeridge and Grogan, 2010), reflecting the wide range of stoichiometric responses of cells or hyphae to environmentally- or biotically-stimulated nutrient storage or growth (Elser et al., 2003; Pokarzhevskii et al. 2003), suggesting rapid potential turnover of microbial cytoplasmic nutrients. Despite this high-frequency variability, we found a clear seasonal pattern in this index. Likewise, fatty acids are also believed to be short-lived in the environment, at least relative to total soil DNA or live and dead cell membranes (Joergensen and Wichern, 2008), perhaps explaining the good correlation between microbial lipid responses and the CFE-flush in our data. We anticipate that turnover rates of these variables in the environment follow a hierarchy of CFE > PLFA > qPCR > microscopy, however it is unlikely that any of these proxy materials decompose slowly enough in ideal conditions to cause a lag in results that spans the 10-day gaps between our sample dates. More importantly for interpreting these results may be the combined effect of this lag with the non-ideal environmental conditions for decomposition at varying points in the season. For example, fungal biomass may be high in winter in this study and in others because fungal biomass accumulates in late fall and because chitin may be slow to decompose in frozen soil. Therefore, just as in biogeochemical studies where turnover rates compliment pool sizes when discussing nutrient availability, estimates of microbial seasonality could be strengthened by investigating the seasonal persistence of the microbial material being measured.

5. Conclusions

Using an extensive field investigation, we have shown that there are strong seasonal patterns in the soil microbial community in mesic tundra, in particular during the transition from frozen to thawed soil. Analysis of the microbial community with PLFA indicated that seasonal community shifts during thaw were strongly associated with differences in soil nutrient status between winter and the growing season, and that declines in fungal biomass at

thaw may be driven by predation. PLFA and CFE estimates of F/B ratios were positively-correlated and indicated larger fungal biomass in winter, consistent with studies from alpine tundra. Our DNA-based method (qPCR) of bacteria and fungi revealed much less seasonal variation in genomic material or ratios, but may be confounded by species shifts in gene copy numbers. However, large winter fungal biomass without increases in fungal gene copy numbers may also indicate that new fungal growth is not occurring in the deep arctic winter but that the winter fungal biomass is preserved from the preceding fall. Fungal and bacterial biomass estimated with epifluorescent microscopy indicated growth of new cells and hyphae in still-frozen, but thawing soils. This was followed by a different but consistent microbial community in the spring, summer and fall; one that was dominated by Gram-positive bacteria and adapted to low nutrient conditions. The results from this study lay an important foundation for understanding the possible consequences of a rapidly changing Arctic on soil microbial community and ecosystem function.

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