

Molecular investigations into a globally important carbon pool: permafrost-protected carbon in Alaskan soils

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Abstract

The fate of carbon (C) contained within permafrost in boreal forest environments is an important consideration for the current and future carbon cycle as soils warm in northern latitudes. Currently, little is known about the microbiology or chemistry of permafrost soils that may affect its decomposition once soils thaw. We tested the hypothesis that low microbial abundances and activities in permafrost soils limit decomposition rates compared with active layer soils. We examined active layer and permafrost soils near Fairbanks, AK, the Yukon River, and the Arctic Circle. Soils were incubated in the lab under aerobic and anaerobic conditions. Gas fluxes at -5 and 5°C were measured to calculate temperature response quotients (Q_{10}). The Q_{10} was lower in permafrost soils (average 2.7) compared with active layer soils (average 7.5). Soil nutrients, leachable dissolved organic C (DOC) quality and quantity, and nuclear magnetic resonance spectroscopy of the soils revealed that the organic matter within permafrost soils is as labile, or even more so, than surface soils. Microbial abundances (fungi, bacteria, and subgroups: methanogens and Basidiomycetes) and exoenzyme activities involved in decomposition were lower in permafrost soils compared with active layer soils, which, together with the chemical data, supports the reduced Q_{10} values. CH_4 fluxes were correlated with methanogen abundance and the highest CH_4 production came from active layer soils. These results suggest that permafrost soils have high inherent decomposability, but low microbial abundances and activities reduce the temperature sensitivity of C fluxes. Despite these inherent limitations, however, respiration per unit soil C was higher in permafrost soils compared with active layer soils, suggesting that decomposition and heterotrophic respiration may contribute to a positive feedback to warming of this eco region.

Keywords: carbon cycling, enzymes, methanogenesis, microbial communities, permafrost, respiration

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Introduction

Boreal forest ecosystems cover nearly 350 million hectares of North America, and significant portions of the boreal forest are underlain by discontinuous and continuous permafrost (Jorgenson & Osterkamp, 2005; CCSP, 2007), which has acted as a carbon (C) sink for thousands of years. Permafrost regions contain approximately 1600Pg of C, nearly equal to the amount of C stored in all nonpermafrost soils globally (Tarnocai *et al.*, 2009). These large C pools make northern forest ecosystems particularly important to the global C cycle and global climatic change (Zimov *et al.*, 2006). Since microbial activity is dramatically reduced in frozen soils, the C contained within permafrost is not appreciably decomposed. However, much of the permafrost in boreal regions, such as interior Alaska, is at a tempera-

ture near 0°C, and has been warming at a rate of 1°C per decade, rendering permafrost C increasingly vulnerable to decomposition (Osterkamp & Romanovsky, 1999; Lawrence & Slater, 2005). If the C that is locked away in permafrost is metabolized by microorganisms, this opens a latch into a globally important C reservoir that could be released to the atmosphere (as CO_2 and CH_4) and rivers (as dissolved organic C, DOC and dissolved inorganic C, DIC), affecting greenhouse warming and aquatic chemistry (Striegl *et al.*, 2005; Wickland *et al.*, 2006; Prater *et al.*, 2007; Schuur *et al.*, 2008).

A gap in our current knowledge is the extent to which permafrost-protected C is available for microbial metabolism once soils thaw. Current indications are that organic matter contained within permafrost is relatively labile since it is not protected from decomposition by physical protection or humification mechanisms (Fan *et al.*, 2008). However, we have little understanding of the microbiology of permafrost soils, which could

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significantly affect the rate of decomposition of permafrost C after thaw, if decomposer abundance is low. Several studies suggest that the number of bacteria in permafrost soils are equal to or lower than the active layers above them (Vorobyova *et al.*, 1997; Robinson, 2001; Steven *et al.*, 2008). Although data are sparse, soil fungi are often very reduced in number in permafrost soils (Gounot, 1999; Robinson, 2001; Gilichinsky *et al.*, 2005). If fungal biomass is lower in permafrost soils compared with active layer soils, this could alter the way in which these soils function after they thaw. We particularly focused on Basidiomycete fungi because they play important roles as strong decomposers and mycorrhizal fungi. Reductions in the size of such a major group of soil organisms could reduce the quantity of C-degrading enzymes released into the environment and subsequently reduce the rate at which C compounds in soils are decomposed.

Methane released from thawing permafrost is of great concern because fluxes can be very large and methane has a high global warming potential (Zimov *et al.*, 1997; Wickland *et al.*, 2006). Studies have shown high rates of methane production from the thermal erosion of permafrost at the borders of arctic lakes (Walter *et al.*, 2008), and thermokarst bogs (Wickland *et al.*, 2006). However, methanogenesis in intact deep active layer soils and permafrost is not necessarily higher than surface soils (Ganzert *et al.*, 2006). Little is known about methanogen abundance in permafrost, how their abundance compares to active layer soils, and if low methanogen population abundances might limit methanogenesis in permafrost soils.

The chemical composition of permafrost soils is another major factor affecting the rate at which permafrost C decomposes upon thaw. Available evidence suggests that in many instances permafrost C has not undergone significant decomposition and, therefore, is very labile once thermal restrictions are released (Dutta *et al.*, 2006; Schuur *et al.*, 2008). The C chemistry of permafrost soils is likely very diverse, because C trapped in permafrost can come from a variety of sources, including undecomposed plant roots, infiltrating DOC, or mineral-associated organic matter in loess. Proper understanding of the inherent chemical composition of organic matter in permafrost and how it responds to changes in climatic conditions is critical to determine the amount of C that can likely be released to the atmosphere (as CO₂ or CH₄) and aquatic ecosystems (as DOC) under warmer environmental conditions.

The goal of this study was to examine the chemical and biological characteristics of permafrost soils pre- and postthaw, and to determine whether the chemistry or microbiology of permafrost soils may limit C fluxes from those soils. We examined microbial control on C

processing by examining the abundance of microbial decomposer functional groups in permafrost soils, the enzymes present in soils, and the rates of microbial respiration and methanogenesis under aerobic or anaerobic conditions following thaw. We compared microbial and chemical characteristics of active layer and permafrost soils from black spruce stands in three distinct geographic regions of interior Alaska: Coldfoot, Hess Creek, and Smith Lake. We chose these regions because they span a range of permafrost conditions from high C storage permafrost (Hess Creek), to intermediate C storage (Smith Lake), and low C storage (Coldfoot).

Materials and methods

Our strategy was to test the chemical and biological constraints on decomposition of permafrost C in boreal black spruce forests from intact cores collected in interior Alaska. We sampled three sites that represent broadly different permafrost environments (Table 1). One site was a lowland soil near Hess Creek (65°40'12.84"N, 149°04'36.24"W), just south of the Yukon River, that contains both organic active layer horizons and organic permafrost. Depth to permafrost at Hess Creek was 60.5 cm in Fall 2006. Organic lowlands near Hess Creek have low mean annual soil temperatures (Table 1). A second site was located north of the arctic circle near Coldfoot, AK (67°12'04.6"N, 150°16'20.1"W), a site with continuous mineral permafrost and an organic-mineral active layer that was 63 cm in 2006 (Table 1). Coldfoot soils at depth are generally warmer than Hess Creek or Smith Lake soils, and soil temperatures are more stable than other locations (Table 1). The third set of cores were collected from the Smith Lake area adjacent to the University of Alaska, Fairbanks (64°52'07.47"N, 147°51'37.61"W). Smith Lake soils contain discontinuous organic-mineral permafrost, and the depth to permafrost at Smith Lake has increased from 52 cm in 1999 to 75 cm in 2006 (V.E. Romanovsky, personal communication). Surface soils at Coldfoot are warmer than Smith Lake or Hess Creek, and active layer and permafrost soils at some locations have been warming over the past 10 years (Table 1). All sites have an overstory of black spruce (*Picea mariana*).

Soil sampling

Sites were visited in September 2006 to measure maximum seasonal active layer thickness. Soil cores (1 m depth) were collected in March 2007 while soils were still frozen to the surface. We collected three replicate cores per site within a ~100 m² area. In the field, cores were scraped of surface organic matter contaminants that froze to the core during the drilling process, wrapped in aluminum foil, placed into 4 in diameter PVC tubes and capped. Cores were taken to the Cold Regions Research and Engineering Laboratory at Fort Wainwright Army Base in Fairbanks, AK. In a large -20°C cold room, cores were again scraped of surface organic matter and cut into segments using a band saw. Active layers and

Table 1 Characteristics of active layer and permafrost samples within the soil profile at three site locations

	Hess Creek		Smith Lake		Coldfoot	
	Active layer	Permafrost	Active layer	Permafrost	Active layer	Permafrost
Active layer depth (cm)	60.5		55		63	
Depth of sample (cm)	35–50	85–100	35–50	65–80	35–50	65–80
Horizon designation	Fibric/mesic	Fibric/mesic	Humic/mineral	Mineral	Fibric/mineral	Mineral
Mean annual temperature (°C)*	–2.7 (2002) –1.76 (2006)	Not available	–2.15 (1999) –0.75 (2008)	–2.2 (1999) –0.9 (2008)	–0.21 (2003) –0.16 (2008)	–0.26 (2003) –0.27 (2008)
Volumetric moisture (%)	60 ± 9	70 ± 9	34 ± 12	57 ± 13	35 ± 18	44 ± 7
Carbon (mg kg ^{–1})	403 ± 38	389 ± 43	66 ± 68	39 ± 52	32 ± 14	19 ± 2
Nitrogen (mg kg ^{–1})	18 ± 4	14 ± 4	3.7 ± 3.8	2.6 ± 3.4	2.0 ± 1.0	1.2 ± 0.1
Bulk density (g cm ^{–3})	0.16 ± 0.02	0.12 ± 0.05	0.66 ± 0.44	0.48 ± 0.23	0.82 ± 0.38	0.45 ± 0.05
¹⁴ C age of SOM	585 ± 212	1277 ± 95	2653 ± 967	4952 ± 2041	3363 ± 750	7252 ± 2155
¹⁴ C age of respiration	Modern ± 400	1458 ± 131	725 ± 1131	1363 ± 1545	923 ± 471	2853 ± 697
*Q ₁₀ †	6.6 ± 1.8	2.7 ± 1.6	9.0 ± 5.5	2.3 ± 0.7	6.8 ± 3.8	3.1 ± 1.1

*Mean annual temperature followed by parentheses to indicate earliest year of measure and latest year of measure. Washington Creek, an organic lowland with similar thermal properties to Hess Creek (V. E. Romanovsky, personal communication) was used in lieu of Hess Creek thermal data. Active layer temperature was measured at 50 cm (except at Washington Creek, 35 cm) and permafrost temperature was measured at 80 cm.

†Temperature response was measured from –5 to +5 °C, over the freezing point of water and thus cannot assume a purely exponential relationship between temperature and activity. Thus, the term *Q₁₀ is used.

Error bars are ± 1 SD (*n* = 3). Significant differences were depth or site main effects. See text for details.

SOM, soil organic matter.

permafrost were represented by both mineral and organic horizons, depending upon site location (Table 1). We separated the soils into two 15 cm segments: a surface active layer (35–50 cm depth) and permafrost horizon (~ 70–100 cm depth). The resulting core sections were cut lengthwise into four wedges. Each wedge was used for one of four measurements: (1) DOC and solution chemistry, (2) microbial community composition and lab incubation, (3) bulk density, moisture content, and C and N content, (4) ¹³C nuclear magnetic resonance (NMR) spectroscopy. Cores were then shipped on dry ice to the USGS Menlo Park, CA and USGS Boulder, CO labs.

Soil chemical techniques

Moisture content was determined by drying soils at 65 °C for 48 h and measuring the weight before and after drying. Bulk density was determined by measuring the volume (length, width, height) of a section of frozen core, and then drying the segment and determining its mass. C and nitrogen (N) concentrations and ¹³C and ¹⁵N of soil organic matter (SOM) were measured on a C/N analyzer (CE Elantech, Lakewood, NJ, USA) coupled to an isotope ratio mass spectrometer IRMS (Micromass Optima, Waters Corporation, Milford, MA, USA).

Dissolved organic matter was leached from active and permafrost soils by placing preweighed, frozen soils (20–200 g wet weight) in glass beakers and adding 500 mL of deionized (DI) water. The soils were allowed to thaw for 24 h at 5 °C while on a shaker table. The solution was then decanted and filtered using prebaked 0.7 µm glass fiber filters (GF/F).

We measured DOC concentrations using an OI700 analyzer (O.I. Analytical, College Station, TX, USA), and we characterized DOC chemistry using specific ultraviolet absorbance (SUVA), a measure of aromatic DOC (Weishaar *et al.*, 2003), and fluorescence spectroscopy. Fluorescence has been used to characterize DOC chemistry and to infer DOC precursors, and it has potential to be used to identify the presence of labile compounds (Wickland *et al.*, 2007). Total dissolved nitrogen (TDN) concentrations were measured using a Shimadzu TOC-V_{CPH} and Total Nitrogen analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a chemiluminescence detector. We calculated DOC and TDN yield by normalizing to dry soil weight (and dry soil C weight in the case of DOC) after accounting for the volume of water in the frozen soil and the added DI water for leaching.

We determined the inherent chemical composition of SOM and changes that occurred during the experimental treatments by analyzing spectra obtained from solid state ¹³C NMR. The ¹³C NMR spectra were obtained from a Varian Infinity CMX 300 MHz spectrometer at the William R. Wiley Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory. Each sample was packed in 5 mm zirconia rotor, fitted with boron-nitride spacers and KEL-F (low C) caps. A ramped (¹³C pulse) cross-polarization magic angle spinning (CP MAS) pulse sequence was used. We used a contact time of 1 ms, spinning rate of 10 kHz, and a decoupling field of 63–55 kHz. The ¹³C chemical shifts were referenced to tetramethylsilane (0 ppm) using an external reference, hexa-meta-benzene (HMB, 16.81 ppm). Once the spectra were obtained, we performed Fourier transformation of the data and applied line

broadening of 200 Hz for all samples. Chemical composition was inferred by dividing the ^{13}C NMR spectra into seven chemical shift regions that correspond to different functional groups: 0–45 ppm for alkyl-C, 45–60 ppm for -alkyl and methoxyl groups, 60–95 ppm for O-alkyl C, 95–110 ppm for di-O-alkyl C, 110–145 ppm for aromatic C, 145–165 ppm for phenolic C, and 165–210 ppm for amide and carboxyl groups (Nelson & Baldock, 2005).

The proportion of C in each functional group was computed by integrating the area under the individual peaks of the functional group region (Swift, 1996). We used the relative difference of the peaks [alkyl to O-alkyl ratio (A:O-A)] as a measure of the degree of decomposition of organic compounds in soils (Baldock *et al.*, 1997) and the aromaticity index was calculated by dividing the area under aromatic groups by the total area under 0–165 ppm. In this computation, the area under the carboxyl peak (165–220 ppm) is not included because it can be assigned to both straight-chain and aromatic-C structures (Rovira & Vallejo, 2002). Spectra processing was performed using MESTERC software (version 4.59, Universidad de Santiago de Compostela, Spain).

Incubation experiment

We measured CO_2 and CH_4 flux from soils under aerobic and anaerobic conditions. During the 98-day aerobic treatment, we measured CO_2 fluxes over the first 40 days from soils incubating in 1 pint jars. Fluxes were measured when soils were at -5°C (on day 1), and again after soils had been warmed to 0°C (on day 3). The soils were then incubated at 5°C for the rest of the period (days 6–98). We measured CO_2 concentrations in the headspace of the aerobic samples on a Licor 6252 infrared gas analyzer (Li-Cor, Lincoln, NE, USA) having a nitrogen carrier gas. Headspace gases were sampled through a rubber septa on the container lids immediately after closing the containers, and again 2 h later. Soil respiration rates were calculated as the change in headspace CO_2 concentration over time (adjusted for headspace volume and ambient temperature). The change in dissolved CO_2 concentration was calculated from headspace concentrations, soil water contents, and known CO_2 equilibrium constants (Plummer & Busenberg, 1982) adjusted for ambient temperature and pressure (Striegl *et al.*, 2001). Q_{10} s were calculated as the flux rate at -5°C (day 1) divided by the flux rate at 5°C (day 6). Because the incubation crosses the freezing point of water, the classic Q_{10} relationship should not be used. Therefore, we have written the temperature sensitivity as $^*Q_{10}$ throughout this manuscript. The jars were opened between each measurement to allow for the headspace CO_2 to equilibrate with the atmosphere. The jars were weighed weekly after respiration measurements to determine evaporative water loss and, when necessary, water was added to bring the soil samples to their initial weight. Normally, jars were kept closed for 1–5 days, but from days 16 to 37 the jars were continuously closed for $^{14}\text{CO}_2$ collection. For $^{14}\text{CO}_2$, 50 mLs of headspace gas were injected into a pre-evacuated glass serum bottle with septa. The serum bottles, along with subsamples of the incubated soils, were submitted to

the W.M. Keck Accelerator Mass Spectrometry Laboratory at UC Irvine for CO_2 purification, graphitization, and radiocarbon measurement.

Anaerobic incubations were conducted by placing frozen soil subsamples into 50 mL serum bottles which were flushed with N_2 for 1 min and sealed with butyl rubber stoppers. The sealed bottles were alternately put on a vacuum pump and flushed twice with N_2 for 20 min at a time to ensure that O_2 was removed. The samples were incubated at 5°C , and bottles were kept sealed for the 117 days. Methane concentrations were measured using a HP5890 gas chromatograph (Hewlett Packard Corporation, Palo Alto, CA, USA) having a flame ionization detector. Cumulative CH_4 production rates were calculated as the change in headspace CH_4 concentrations (adjusted for headspace volume and ambient temperature and pressure) and dissolved CH_4 concentrations with time. Dissolved CH_4 concentrations were calculated from headspace concentrations, soil water contents, and with the Bunsen adsorption coefficient for CH_4 equilibrium (Yamamoto *et al.*, 1976) adjusted for ambient temperature. Production rates became nonlinear over time, so we calculated the CH_4 flux based upon the linear portion of the data set (generally >30 days).

Microbial communities

We extracted DNA from all soils using the Powersoil DNA extraction kit (Mobio, Carlsbad, CA, USA), using 0.15 g of homogenized frozen soil. DNA was quantified using PicoGreen[®], according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). We used quantitative PCR (qPCR) to quantify the abundance of bacterial, fungal, and basidiomycete genes in soils. We used the abundance of genes specific for microbial groups as an indicator of changes in the biomass of these groups (Zak *et al.*, 2006). All qPCR reactions were performed in a Stratagene MX3000P quantitative PCR machine (Agilent, Santa Clara, CA, USA) using SybrGreen master mix. All reactions consisted of 3 μL of template DNA (diluted to $1\text{ ng } \mu\text{L}^{-1}$), 1 μL of the forward and reverse primers (10 μM), 12.5 μL of master mix, 0.5 μL ROX (diluted 10000 $\times$) as an internal standard, and 9 μL of sterile water. Quantification of the threshold concentration (C_t) was computed using the MX3000P software algorithm. Standards were created by amplifying the gene fragment of interest, quantifying it using PicoGreen[®] (Molecular Probes, Invitrogen, Carlsbad, CA, USA), and diluting the standard to concentrations within the expected range of the samples. All standards and samples were run in triplicate.

Bacterial gene abundance was quantified using primers eub338 (forward) and eub518 (reverse) (Fierer *et al.*, 2005). The thermocycling parameters were 95°C for 15 min, followed by 40 cycles of $95^\circ\text{C}/0.5\text{ min}$, $53^\circ\text{C}/0.5\text{ min}$, $72^\circ\text{C}/1\text{ min}$; and extension $72^\circ\text{C}/10\text{ min}$. Fungal gene abundance was estimated by quantifying the fungal internal transcribed spacer (ITS) region of soil DNA using the primers ITS1 and ITS4 (Zak *et al.*, 2006) and the following temperature program: $95^\circ\text{C}/10\text{ min}$, 40 cycles of $95^\circ\text{C}/1\text{ min}$, $57^\circ\text{C}/1\text{ min}$, and $72^\circ\text{C}/2\text{ min}$. Basidiomycete gene abundance was quantified using the

primers ITS1F and ITS4B (Zak *et al.*, 2006) and the following temperature program: 94°C/5 min, 40 cycles of 94°C/1 min, 54°C/2 min, and 72°C/5 min. We estimated methanogen population abundance using the primers mcrAf and mcrAr (Luton *et al.*, 2002). The thermal profile was 94°C/0.5 min, 55°C/1 min, 72°C/2 min, and 72°C/10 min. Efficiencies for all reactions were near 100%.

Soil enzymes

Enzyme activity potentials involved in the extracellular decomposition of organic molecules were assayed on permafrost soils, both before the incubation, and after the aerobic and anaerobic incubations. The enzymes under study were *B*-glucosidase, involved in cellulose decomposition; *N*-acetyl glucosaminidase (NAGase) involved in the degradation of chitin; phenol oxidase and peroxidase, involved in the degradation of lignin; and phosphatase, an enzyme that releases ester-bound phosphate from organic matter. Assays were conducted on 1 g soil samples using a 96-well plate and *p*-nitrophenol (pnp) linked substrates. The substrates were pnp-glucosidase, pnp-*N*-acetyl glucosaminidase, and pnp-phosphate. Substrate concentrations were 10 mM. Phenol oxidase and peroxidase were assayed using *L*-dihydroxyphenylalanine (*L*-DOPA) as a substrate. Peroxidase also received 0.1% H₂O₂ as a co-substrate. Substrates were made in pH 7.0 10 mM acetate buffer. Soil slurries were made by adding 1 g soil to 100 mL pH 7 acetate buffer and rapidly mixing on a magnetic stir plate while pipetting. Assays were conducted by adding 100 μ L substrate solution with 100 μ L of soil slurry. Substrate controls and buffer controls were conducted at the same time. The 96-well plates were covered and incubated at room temperature for 1–24 h with gentle mixing on a benchtop horizontal shaker. Standards were made using pnp or oxidized *L*-DOPA products at concentrations that spanned the range of observed activities.

Statistics

Comparisons were made using analysis of variance (ANOVA) with site (Coldfoot, Smith Lake, Hess Creek), and depth (active layer vs. permafrost) as the main factors for all physical and chemical measurements. For microbial community and enzyme measurements, a 3-way ANOVA including pre- and post-incubation was added. Data were normalized using log transformation when necessary to have normal distributions before ANOVAs were conducted. Comparisons of means were performed using Tukey's HSD *post hoc* test. Statistical package used was Statistica (Statsoft Inc., Tulsa, OK, USA).

Results

Soil C and nitrogen storage and depth patterns varied widely among our three sites. Soil C concentrations were higher at the lowland Hess Creek site compared with the two upland sites at Coldfoot and Smith Lake (site effect, $F = 421$; $P < 0.0001$; Table 1). Soil C concen-

trations were also higher in active layer soils than permafrost soils (depth effect, $F = 13.4$, $P = 0.0006$), and there was no site $\times$ depth interaction (Table 1). Soil C storage (C concentration multiplied by bulk density) was lower in the permafrost horizons compared with the active layer at Smith Lake and Coldfoot, but there was no difference in C storage between permafrost and active layer at the Hess Creek site (ANOVA, site $\times$ depth interaction, $P = 0.002$, data not shown). Soil N concentration was also higher in Hess Creek soils compared with Smith Lake or Coldfoot soils (site effect, $F = 126$, $P < 0.0001$, Table 1). Also, N concentrations were higher in permafrost soils compared with active layer soils (depth effect; $F = 11.7$, $P = 0.0013$). Soil C/N ratios were highest at Hess Creek site ($C/N = 26A \pm 1A$) compared with Smith Lake (11.8 ± 1.6) or Coldfoot sites (15.7 ± 0.4), but there was no difference in soil C/N ratios between active layer and permafrost horizons.

The chemistry of the aqueous phase differed in certain respects between active layer and permafrost soils. Permafrost soils had higher volumetric moisture content than active layer soils ($57 \pm 14\%$ compared with $43 \pm 17\%$; ANOVA depth effect $P = 0.005$) and Hess Creek had a higher volumetric moisture content ($65 \pm 4\%$) than either Smith Lake ($45 \pm 7\%$) or Coldfoot ($40 \pm 5\%$; ANOVA site effect; $P = 0.01$). There was no site $\times$ depth interaction on soil moisture content. DOC and TDN concentrations did not differ between active layer and permafrost soils ($P > 0.05$, $n = 3$), but differed significantly among sites ($P < 0.05$). Hess Creek had the highest DOC and TDN concentrations, followed by Smith Lake and Coldfoot (Table 2). Across the sites, DOC yield (mg DOC/g soil) was greater from permafrost soils than from active layer soils. However, DOC yield did not differ by site (Table 2). TDN yield exhibited a site by depth interaction (ANOVA; $P = 0.009$), where the permafrost soil at Hess Creek had higher TDN yield than all other sites and horizons. DOM 'quality' showed a similar trend; UV absorbance measures differed among the sites, but did not differ by depth. SUVA did not differ significantly by either site or by depth (Table 2).

NMR was only conducted on samples with organic C concentrations $> 5\%$, to avoid the effect of paramagnetic interference from Fe and Mn on the ¹³C NMR analysis in soils with low organic matter. Thus we were only able to compare our Hess Creek samples and two Smith Lake samples (one active layer and one permafrost). Although we could not statistically compare Hess Creek and Smith Lake, the patterns support the notion that there are differences in the distribution of organic molecules between these two sites. The organic matter at Hess Creek was dominated by O/N substituted alkyl groups (easily metabolizable alkanes e.g.

carbohydrates), whereas at Smith Lake there was a more even distribution of alkyl, O-alkyl, aromatic, and amide groups (Fig. 1a). At Hess Creek, there was no statistically significant difference between active layer and permafrost horizons in the relative abundance of organic matter functional groups (Fig. 1a). Over the course of the aerobic incubation, the relative abundance of the different functional groups did not show significant changes in the Hess Creek soil. During the anaerobic incubation of Hess Creek samples, there was accumulation of alkyl groups (relatively recalcitrant fraction) and higher loss of O-alkyls, aromatics, and phenolics compared with the aerobic incubation (Fig. 1a). The alkyl/O-alkyl ratio indicates that all samples underwent more significant transformation (more intense humification) under anaerobic than aerobic incubation (Fig. 1b). For Hess Creek Samples, active layer soils were more humified than deeper horizons at the same location, indicating that permafrost C is even more chemically labile than active layer soils.

In the one Smith Lake sample that could be analyzed, there tended to be more change in the anaerobic incubation than the aerobic incubation (Fig. 1a). In the active layer, there tended to be increases in the alkyl and O-alkyl fractions and declines in the aromatic, phenolic, and carbonyl fractions over time. In the permafrost, the changes over time tended to differ from the active layer soil in that there were declines in the O-alkyl fraction and increases in the carbonyl fraction over time (Fig. 1a). The ratio of alkyl to O-alkyl moieties tended to increase over the course of the incubation (particularly the anaerobic incubation), similar to the Hess Creek soils (Fig. 1b). Although we cannot make statistical inferences about Smith Lake samples, if we assume that the standard deviation is similar to those at Hess Creek (which is true for other chemical data), then

these differences in organic matter composition are likely real.

Incubation experiment

While frozen at -5°C , soil respiration was higher in permafrost soils compared with active layer soils (Fig. 2, $F = 16.14$, $P = 0.002$). After soils were thawed, respiration rates between active layer and permafrost soils did not differ from each other (Fig. 2), but normalized CO_2 fluxes ($\mu\text{gC-CO}_2/\text{h/gC}$) were higher in permafrost soils compared with active layer soils (for Smith Lake and Coldfoot sites, Fig. 3; $P < 0.05$). $^*\text{Q}_{10}$ values were calculated while comparing flux rates at -5°C and 5°C (see 'Materials and methods' for description of $^*\text{Q}_{10}$). Temperature sensitivity of decomposition ($^*\text{Q}_{10}$) values were lower in the permafrost soils (2.7 ± 0.98) compared with the active layer soils (7.5 ± 0.95 ; $P = 0.005$). These values (7-8) are within the range of values observed for Q_{10} values above and below freezing (Mikan *et al.*, 2002). However, this is the first time a lower Q_{10} value has been reported in permafrost soils.

In the anaerobic treatment, CH_4 production displayed a site $\times$ depth effect where CH_4 production was higher in the Hess Creek active layer soil compared with all other soils two sites (ANOVA site $\times$ depth effect, $F = 6.99$, $P = 0.01$, Fig. 4). Although it appears that CH_4 fluxes were higher in permafrost soils compared with active layer soils at Coldfoot, this was not Significant. Regression analysis between methane flux and methanogen abundance indicates that methanogen abundance (fg ML gene / g soil) was a strong predictor of methane flux [methane flux = $4.4 \log(\text{abundance}) - 134$; $r^2 = 0.881$, although this was very nearly a two-point

Table 2 Chemical characteristics of dissolved organic matter among the three sites

	Hess Creek		Smith Lake		Coldfoot	
	Active layer	Permafrost	Active layer	Permafrost	Active layer	Permafrost
DOC (mg/L)	17.57 ± 2.12	17.30 ± 3.87	17.20 ± 8.86	10.33 ± 6.91	9.05 ± 5.52	3.18 ± 0.79
DOC yield (mg DOC/g dry soil)	0.46 ± 0.031	0.72 ± 0.28	0.11 ± 0.07	0.35 ± 0.18	0.063 ± 0.041	0.087 ± 0.07
DOC yield (mg DOC/g soil C)	1.14 ± 0.19	1.84 ± 0.57	2.4 ± 1.17	26.8 ± 27.6	1.89 ± 0.81	4.40 ± 3.32
TDN (mg/L)	0.50 ± 0.09	1.52 ± 0.81	0.71 ± 0.41	0.73 ± 0.80	0.29 ± 0.81	0.18 ± 0.01
TDN yield (μg TDN/g dry soil)	13 ± 3	59 ± 18	5.2 ± 5	22 ± 17	3.1 ± 3	4.3 ± 2.7
UV absorbance	0.36 ± 0.15	0.39 ± 0.07	0.41 ± 0.11	0.35 ± 0.21	0.17 ± 0.03	0.074 ± 0.021
SUVA	2.09 ± 1.02	2.28 ± 0.16	2.6 ± 0.90	3.45 ± 0.29	2.18 ± 0.86	2.34 ± 0.54
Fluorescence index	1.22 ± 0.12	1.38 ± 0.04	1.34 ± 0.18	1.34 ± 0.02	1.41 ± 0.11	1.49 ± 0.04

There was no effect of depth (permafrost vs. active layer) on any of these variables. Errors are ± 1 SD.

DOC, dissolved organic carbon; TDN, total dissolved nitrogen; SUVA, specific ultraviolet absorbance.

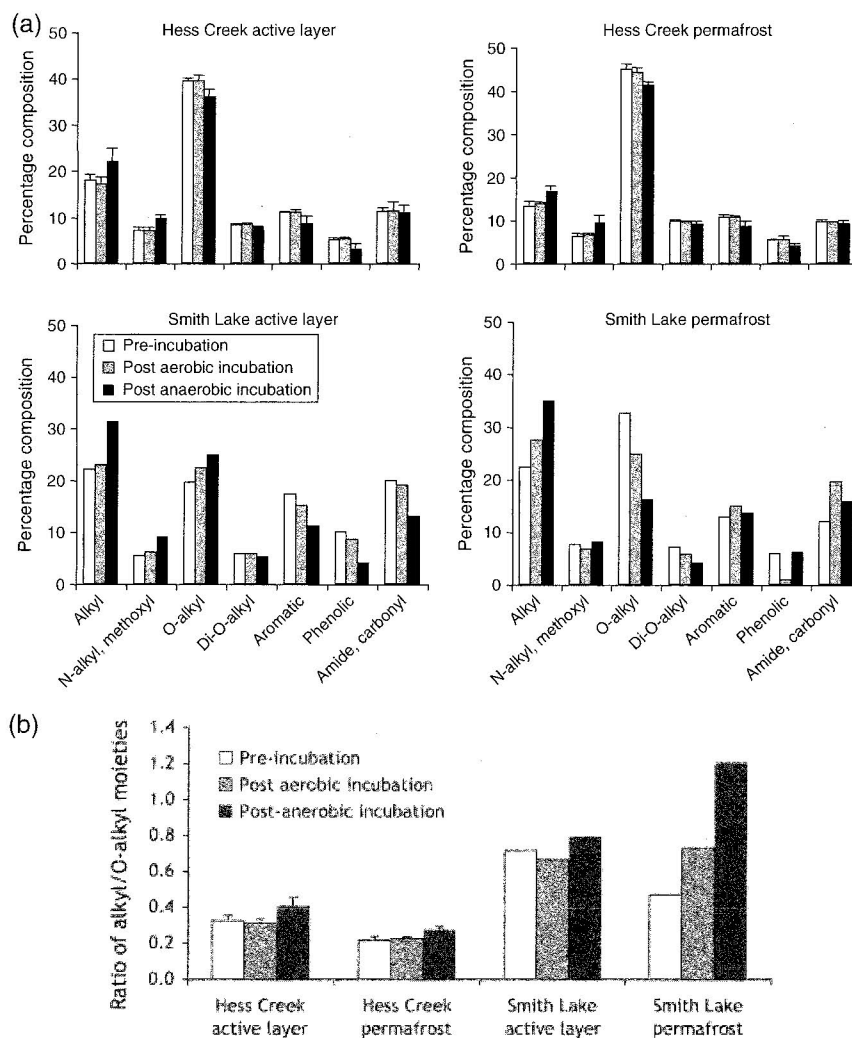


Fig. 1 Relative abundance of organic moieties contained within active layer and permafrost soils at Smith Lake and Hess Creek sites. The Coldfoot site is not shown because low organic matter content prevented nuclear magnetic resonance (NMR) data from being collected. (a) Preincubation and postincubation data are shown, from both the aerobic and anaerobic incubations. (b) Alkyl/O-alkyl ratios in pre and post incubation soils. Higher ratios indicate higher levels of humification.

regression due to the high methane flux and methanogen abundance of Hess Creek active layer soils.

Radiocarbon

The radiocarbon age of SOM showed both a site effect and a depth effect by ANOVA, but no interaction, in which Hess Creek SOM averaged 931 ± 165 years bp which was significantly younger than either Smith Lake (3803 ± 777 years bp) or Coldfoot (5307 ± 1050 years bp; ANOVA site effect; $P < 0.001$). Radiocarbon age of the permafrost SOM was significantly greater at 4493 ± 1000 years bp than the radiocarbon age of the active layer SOM [2200 ± 465 years bp (ANOVA depth effect; $P = 0.003$)]. The radiocarbon age of respired C

showed a depth effect by ANOVA ($P = 0.006$) but no site effect ($P = 0.08$), in which active layer soils ranged from modern to 923 years old, and C respired from permafrost soils ranged from 1360 to 2850 years bp (Table 1). Radiocarbon age can be used for comparison purposes, but does not represent calendar age. Also, SOM and respired C radiocarbon ages do not represent C that was fixed all at one time and then isolated. Rather it is the average radiocarbon age of many C molecules with differing ages.

Microbial populations

We observed lower abundances of the microbial groups in permafrost soils compared with active layer soils at

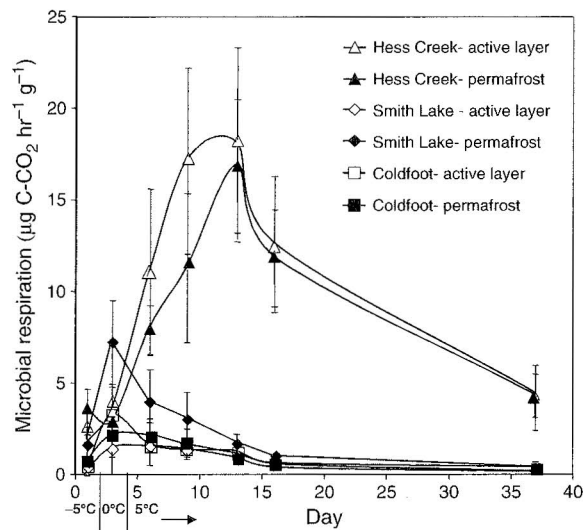


Fig. 2 Aerobic microbial respiration from active layer and permafrost soils in laboratory incubations. Soils were incubated at -5 and 0°C early in the incubation and then at 5°C from day 5 onward. Values are means ± 1 standard error ($n = 3$).

Smith Lake and Hess Creek, but not at Coldfoot (Fig. 5). Bacterial abundance was 70%–90% lower in permafrost soils compared with active layer soils, except at the Coldfoot site (site $\times$ depth interaction; $P = 0.001$; Fig. 5). Similarly, total fungal abundance was 66% lower in permafrost compared with active layer soils, but this difference was not present after soils were thawed and incubated (depth $\times$ time interaction, $P = 0.03$; data not shown). Basidiomycete abundance was 95% lower in permafrost soils compared with active layer soils at Hess Creek and Smith Lake, but not Coldfoot (site $\times$ depth = 0.043). There was a marginally significant result noting a doubling or tripling of Basidiomycete abundance postthaw (depth $\times$ time interaction, $P = 0.06$; data not shown). Methanogen abundance showed a significant site effect ($P < 0.001$) and a significant depth effect ($P = 0.016$), but there was no site $\times$ depth interaction. Methanogens were more abundant in the organic rich Hess Creek soil compared with either Smith Lake or Coldfoot soils (Fig. 5). In addition, methanogens were more abundant in the active layer than in the permafrost soil (Fig. 5). Although it appears that methanogen abundance is higher in the permafrost soil at Coldfoot, ANOVA did not show a significant site $\times$ depth interaction.

Soil enzyme activity potentials were lower in permafrost soils compared with active layers soils at Smith Lake and Coldfoot, but not at Hess Creek (Fig. 6), a result that does not correspond to differences in microbial population sizes between active layer and permafrost soils.

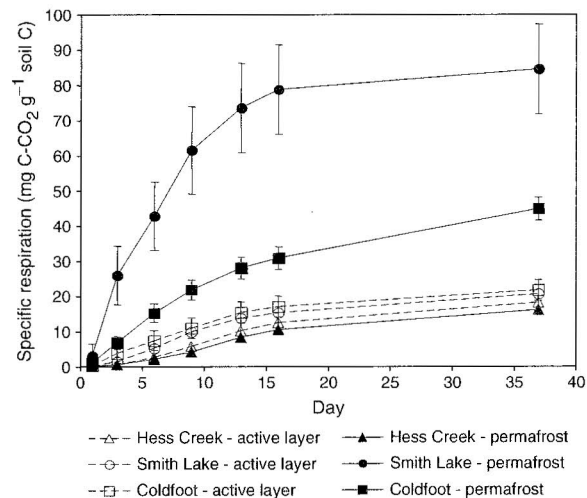


Fig. 3 Cumulative microbial respiration from active layer and permafrost soils in laboratory incubations. Respiration values were normalized to soil C to account for large differences in soil C among sites. High respiration rates per unit soil C indicates high amounts of labile carbon. Values are means ± 1 standard error ($n = 3$).

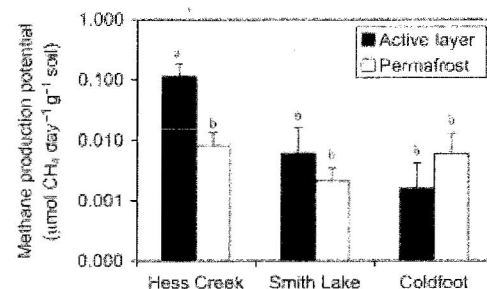


Fig. 4 Methane flux rates from active layer and permafrost soils during anaerobic laboratory incubations. Methane production potential was the rate of methane production in incubation jars. Values represent means ± 1 standard error ($n = 3$).

frost soils. *B*-Glucosidase, *N*-acetyl-glucosaminidase, phosphatase, and peroxidase potential enzyme activities were lower in permafrost soils from Coldfoot and Smith Lake, but lower activities for these enzymes were not observed in Hess Creek permafrost (site $\times$ depth interaction; $P < 0.05$). Over time, *B*-glucosidase, *N*-acetyl-glucosaminidase, and phosphatase, activities in the active layer soils declined to the same range as permafrost soils (depth $\times$ time interaction; $P < 0.05$), likely due to the depletion of substrate. Interestingly, there was a 2.5-fold increase in peroxidase activity after thaw ($P < 0.0001$; data not shown). Phenol oxidase activity was also lower in the permafrost at all sites compared

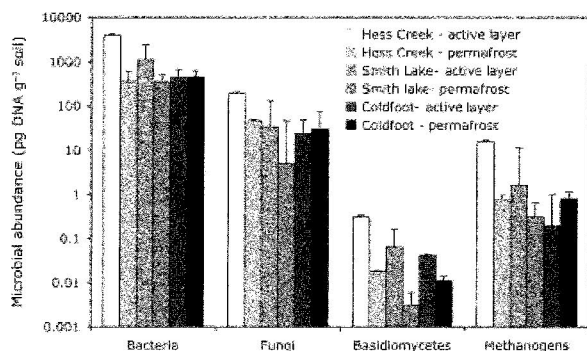


Fig. 5 Microbial functional group abundance quantified using quantitative PCR (qPCR).

with the active layer (depth effect, $P = 0.007$), but there was no statistically significant change over time.

Discussion

The three study sites differed markedly in organic matter storage, age, lithologies, and organic matter quality. Despite these differences, there were similar patterns that resulted when comparing permafrost and active layer soils. First and most surprising was that the apparent temperature sensitivities of the microbial community differed between active layer and permafrost soils. This may indicate that microbial communities in permafrost soils were adapted to life in frozen conditions as seen by their higher respiration rates (per g C) at -5°C compared with the active layer. Because edaphic factors also differ between active layer and permafrost soils, we cannot conclude that the difference in temperature sensitivity is solely due to differences in microbial physiology. However, several lines of evidence show that permafrost communities are adapted to lower temperatures than surface soils (Robinson, 2001; Mangelsdorf *et al.*, 2009). Cold adapted 'psychrophilic' microorganisms can be as active or even more active than mesophilic or psychrophilic organisms that can survive at low temperatures, but prefer warmer conditions (Robinson, 2001; Mangelsdorf *et al.*, 2009). Although respiration rates (per g soil) did not differ between active layer and permafrost soils over the course of the entire 5°C incubation (Fig. 2), the temperature sensitivity of decomposition ($^*Q_{10}$) was lower in permafrost soils ($^*Q_{10} \sim 2$) compared with active layer soils ($^*Q_{10} \sim 7.5$ for the range of -5 to $+5^{\circ}\text{C}$). $^*Q_{10}$ s from our active layer samples were at the high range of other studies (Mikan *et al.*, 2002; Dutta *et al.*, 2006; Rodionow *et al.*, 2006), but given that our study was at a lower temperature than several of these other studies, and crossed the freezing point of water,

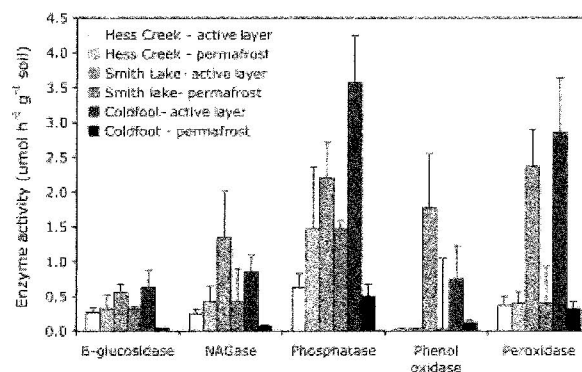


Fig. 6 Soil potential enzyme activities in active layer and permafrost soils. β -glucosidase degrades cellulose; *N*-acetyl glucosaminidase (NAGase) degrades chitin; phenol oxidase and peroxidase degrade lignin; phosphatase releases organically bound P.

higher $^*Q_{10}$ values would have been expected (Schimel & Mikan, 2005).

Several environmental constraints can affect the temperature sensitivity of organic matter decomposition in northern soils, the most significant of which is freezing, which severely limits microbial activity and the diffusion of substrates and products. After thaw, however, both chemical and biological factors can limit the temperature sensitivity of decomposition. The chemical recalcitrance of organic matter is the primary 'intrinsic' property of soils that affects its decomposition. Low enzyme abundance, microbial population size, and oxygen availability also reduce the 'apparent' decomposition rate of soils by limiting rates of substrate diffusion, consumption, and electron donation (Davidson & Janssens, 2006; Conant *et al.*, 2008). Thus, reduced temperature sensitivity in permafrost soils could be explained by higher chemical lability of organic matter, and/or by reduced pool sizes of microbial populations and enzymes (Mikan *et al.*, 2002; Michaelson & Ping, 2003).

The chemical and isotopic data suggest that permafrost C is more intrinsically labile than surface soils. For example, microbial respiration, when normalized to the quantity of bulk soil C ($\mu\text{gCO}_2/\text{h/gC}$, Fig. 3) is higher in Smith Lake and Coldfoot permafrost soils compared with active layer soils. This indicates that a large portion of the total C pool is easily metabolized by soil organisms. This is further supported by DOC data which indicate that DOC yields (mgDOC/g soil) and TDN yields were higher in permafrost than active layer across sites (Table 2). Dissolved organic matter concentrations have been shown to be higher in permafrost soils, likely because DOC becomes entrapped at the bottom of the active layer (Michaelson & Ping, 2003). Solid phase NMR data also indicate that permafrost C,

at least for the samples with organic contents >5%, is less humified than surface soils, and therefore easier to decompose. Michaelson & Ping (2003) and Dutta *et al.* (2006) also have shown that the quality of permafrost C can be high because the organic substrates have not been actively processed by microbial activity, despite their older age.

Soil enzyme concentrations and the size of microbial populations were lower in permafrost soils compared with the active layer and likely contribute to the reduced temperature sensitivity of decomposition of permafrost samples. Reduced enzyme and microbial populations are consistent with substrate limitation for microbial activity (Davidson & Janssens, 2006). In permafrost, low potential enzyme activity also may be due to low production rates by a smaller population size of microbes. Although it is well understood that permafrost environments can contain active microorganisms in high abundance, comparisons of the abundance of organisms between active layer and permafrost soils did not previously exist (Wagner, 2008). In our study, Basidiomycete fungi were the least abundant in permafrost soils in absolute terms, possibly due to these organisms' cold avoidance, low spore germination rates, or large cell size (Robinson, 2001). Alternatively, many Basidiomycete fungi are ectomycorrhizal, and since permafrost soils do not contain plant roots, Basidiomycete abundance could be limited. Interestingly, it was only the Basidiomycete fungi that increased in abundance after permafrost thaw. There are two possible explanations for this increase in abundance. First, Basidiomycete fungi are adapted to decomposing recalcitrant C, and thus as decomposition of SOM proceeds during a lab incubation, Basidiomycete fungi will increase in abundance as labile substrates are depleted. Basidiomycete fungi may also be increasing in abundance post-thaw because they are the most negatively impacted by long-term frozen conditions. Peroxi-dase activity, which is often specific to Basidiomycete fungi (Hammel, 1997), increased at the end of the incubation. It remains to be determined whether this increase in Basidiomycete abundance and potential enzyme activity has the potential to alter the types of substrates decomposed by the soil community.

Methanogens were also found to be in low abundance in two of the three permafrost soils examined, coincident with low rates of methane production in this study. Interestingly, deeper, colder soils at the base of the active layer have been found to have lower temperature sensitivities for methanogenesis compared with surface soils (Ganzert *et al.*, 2006), similar to our Q_{10} results for CO_2 flux which showed lower temperature sensitivities at depth. The low abundance of methanogens in per-

mafrost might seem odd given that methanogens in permafrost have been found to be highly adapted to stressful conditions (Morozova & Wagner, 2007). However, low methanogen abundances or methane production rates have been found in permafrost environments before (Steven *et al.*, 2007; Wagner *et al.*, 2007; Onstott *et al.*, 2009). There seem to be zones of both high and low methane production with depth (Elizaveta *et al.*, 2007) yet the cause for this variability is not well understood but could be due to available electron acceptors, pH; or C substrate. Low rates of methanogenesis observed in lab studies are in apparent contrast to high rates of methane emissions from thermokarst in and around northern lakes and bogs (Wickland *et al.*, 2006; Walter *et al.*, 2008). This apparent contradiction may be explained if methanogen abundance increases postthaw as methanogens increase their growth rate under ice-free anaerobic conditions. Also, our lab studies preclude other biological and physical changes that can occur on the landscape such as land slumping and bog formation. The timescale of methanogen and methane oxidizer response to thaw is thus an important consideration for understanding the dynamics of methane fluxes within changing permafrost environments.

Multiple lines of physical, chemical, and biological evidence suggest that permafrost underlying Alaskan boreal forests contains organic C that can be readily processed aerobically by microbial communities over the short term to contribute trace gas emissions in a positive feedback to regional warming. Temperature sensitivity of decomposition is lower in permafrost soils than in active layer soils, a finding that is consistent with higher substrate availability, lower potential enzyme activities, and lower microbial abundances in permafrost soils as compared with active layers. Despite higher lability of soil C in permafrost, the lower microbial population sizes and lower potential enzyme activities constrain decomposition such that soil respiration rates are similar between active layer and permafrost soils after thaw. Under anaerobic conditions in the lab, methane emissions from permafrost can be lower than active layer soils and likely constrained by low methanogen abundances and growth rates. However, lab studies preclude natural physical and biological processes such as thermokarst formation, changes in hydrology and plant communities, which will have a profound effect on microbial community development and greenhouse gas production. Studies of microbial community change over natural thermokarst chronosequences would be an ideal place to further test the hypotheses presented in this paper. The large quantity of C contained within permafrost makes our understanding of its decomposition an important global concern, and the study of permafrost

microbiology and the microbial response to thaw and adaptation to freezing temperature will be necessary to clearly understand future C fluxes in permafrost environments.

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