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Temperature sensitivity of organic matter decomposition of permafrost-region soils during laboratory incubations

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ABSTRACT

Permafrost soils contain more than 1300 Pg of carbon (C), twice the amount of C in the atmosphere. Temperatures in higher latitudes are increasing, inducing permafrost thaw and subsequent microbial decomposition of previously frozen C, which will most likely feed back to climate warming through release of the greenhouse gases CO_2 and CH_4. Understanding the temperature sensitivity (Q_{10}) and dynamics of soil organic matter (SOM) decomposition under warming is essential to predict the future state of the climate system. Alaskan tundra soils from the discontinuous permafrost zone were exposed to *in situ* experimental warming for two consecutive winters, increasing soil temperature by 2.3 °C down to 40 cm in the soil profile. Soils obtained at three depths (0–15, 15–25 and 45–55 cm) from the experimental warming site were incubated under aerobic conditions at 15 °C and 25 °C over 365 days in the laboratory. Carbon fluxes were measured periodically and dynamics of SOM decomposition, C pool sizes, and decay rates were estimated. Q_{10} was estimated using both a short-term temperature manipulation ($Q_{10\text{-ST}}$) performed at 14, 100 and 280 days of incubation and via the equal C method ($Q_{10\text{-EC}}$, ratio of time taken for a soil to respire a given amount of C), calculated continuously. At the same time points, functional diversities of the soil microbial communities were monitored for all incubation samples using a microbial functional gene array, GeoChip 5.0. Each array contains over 80,000 probes targeting microbial functional genes involved in biogeochemical cycling of major nutrients, remediation strategies, pathogenicity and other important environmental functions. Of these, over 20,000 probes target genes involved in the degradation of varying C substrates and can be used to quantify the relative gene abundances and functional gene diversities related to soil organic matter turnover. The slow decomposing C pool (C_S), which represented close to 95% of total C in the top 25 cm soils, had a higher Q_{10} than the fast decomposing C pool (C_F) and also dominated the total amount of C released by the end of the incubation. Overall, C_S had temperature sensitivities of $Q_{10\text{-ST}} = 2.55 \pm 0.03$ and $Q_{10\text{-EC}} = 2.19 \pm 0.13$, while the C_F had a temperature sensitivity of $Q_{10\text{-EC}} = 1.16 \pm 0.30$. In contrast to the 15 °C incubations, the 25 °C microbial communities showed reduced diversities of C-degradation functional genes in the early stage of the incubations. However, as the incubations continued the 25 °C communities more closely paralleled the 15 °C communities with respect to the detection of microbial genes utilized in the degradation of labile to recalcitrant C substrates. Two winter seasons of experimental warming did not affect the dynamics and temperature sensitivity of SOM decomposition or the microbial C-degradation

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genes during incubation. However, under the projected sustained warming attributable to climate change, we might expect increased contribution of C_S to organic matter decomposition. Because of the higher Q_{10} and the large pool size of C_S, increased soil organic matter release under warmer temperatures will contribute towards accelerating climate change.

1. Introduction

Permafrost zone soils contain approximately 1330–1580 Pg of organic C, which is twice the amount of atmospheric C (Schuur et al., 2008; Tarnocai et al., 2009; Hugelius et al., 2014; Schuur et al., 2015). Though they cover less than 15% of global soil area, permafrost zone soils store about one-third of total global soil C to 3 m depth (Schuur et al., 2015). Temperatures in high latitude regions are increasing faster than in the rest of the world (Hassol, 2004; Fyfe et al., 2013) and future climate projections indicate a potential increase between 7 and 8 °C by the end of the 21st century (Trenberth et al., 2007; IPCC, 2013). Sustained warming thaws permafrost (Romanovsky et al., 2010; Smith et al., 2010; Koven et al., 2013), leading to a thicker seasonal active layer that exposes a large pool of previously frozen organic C to microbial decomposition (Harden et al., 2012). The release of CO_2 and CH_4 from this newly thawed C by increased microbial activity could add significant quantities of C to the atmosphere. Recent efforts to model permafrost C in response to warming project a shift from a C sink to a source in the arctic and sub-arctic regions by the end of the 21st century, leading to a positive feedback to a warming climate (Koven et al., 2011; Schaefer et al., 2011, 2014).

Soil organic matter is composed of a continuum of C compounds. For simplicity, it is often conceptualized as fast, slow, or passively decomposing C pools (Trumbore, 1997; Amundson, 2001; Schädel et al., 2014). In permafrost zone soils, the fast C pool, with turnover times of a few days to weeks at laboratory temperatures (Schädel et al., 2014), represents less than 10% of total soil C, while the majority belongs to the slow C pool, with turnover times from years to decades (Knoblauch et al., 2013; Schädel et al., 2014) Since slow C is a large proportion of total soil C and has a long residence time, it will dominate the long term response of permafrost soil C decomposition to warming (Schuur et al., 2007; Sistla et al., 2013, 2014).

Soil organic matter decomposition in arctic and subarctic ecosystems undergoing permafrost thaw is controlled by a complex of biophysical interactions including soil temperature, soil moisture, physical and chemical protection, C quality, changes in microbial biomass and microbial communities, and the dominant plant community composition (Hirsch et al., 2002; Wickland and Neff, 2008; Karhu et al., 2010; Waldrop et al., 2010; O'Donnell et al., 2011; Schmidt et al., 2011; Hugelius et al., 2012; Sistla et al., 2013, 2014). Changes in the biophysical factors that control SOM decomposition will likely be reflected most rapidly in the abundance and structure of the microbial communities as they adapt to their new physical and chemical environment (Deslippe et al., 2011; Rinnan et al., 2011; Sistla et al., 2013, 2014). Functional adaptations of microbial communities to seasonal environmental changes have been documented in alpine and arctic tundra soils; winter microbial biomass is fungus dominated, while growing season microbial biomass is bacteria dominated (Schadt et al., 2003; Wallenstein et al., 2007; Buckeridge et al., 2013). New dominant microbial communities and the C substrates they decompose may have different temperature sensitivities, and small changes could have a significant effect on the C balance of permafrost soils (Davidson and Janssens, 2006; Fan et al., 2008).

Field warming experiments often show an initial burst of respiration after the application of the warming manipulation associated with consumption of the fast C pool, followed by a decline in C release rates as slow C increasingly dominates respiration (Kirschbaum, 1995, 2004; Melillo et al., 2002; Eliasson et al., 2005; Knorr et al., 2005; Hartley et al., 2007, 2009; Streit et al., 2014). In these field warming experiments, microbes can acclimate to warming by adjusting their metabolism to the new temperature regime, thus reducing their respiration rate at a given temperature and improving their carbon use efficiency (CUE) (Luo et al., 2001; Barcenas-Moreno et al., 2009). Microbial communities may also shift in composition as a new C balance is established perhaps reflecting the changes in the environment as well as in C availability. Experimental field warming in arctic and low arctic ecosystems have been shown to shift microbial communities toward dominance of fungi over bacteria leading towards increased use of more recalcitrant slow decomposing C and change in the plant–microbial associations that accompany shifts in plant community composition and productivity (Deslippe and Simard, 2011; Deslippe et al., 2011, 2012; Natali et al., 2011; Natali et al., 2012; Sistla et al., 2013, 2014).

Accurate measurements of the turnover rates and temperature sensitivity of fast and slow C decomposition are difficult in field conditions. Field studies measure only the apparent temperature sensitivity because of environmental constraints and the confounding effects of different C pools' contribution to total respiration (Davidson and Janssens, 2006; Kirschbaum, 2013). Laboratory soil incubations are a valuable means for estimating the long-term potential for C release from thawing permafrost soils because environmental constraints over SOM decomposition can be carefully controlled (Holland et al., 2000; Reichstein et al., 2000; Dutta et al., 2006). Continuous C loss measurements from long-term incubation experiments provide information on potential C release, kinetics of soil organic matter decomposition, separation of different C pools comprising the SOM, their decay rates and temperature sensitivities, and the composition and abundance of associated microbial populations (Dutta et al., 2006; Karhu et al., 2010; Lavoie et al., 2011).

While many connections between microbial community compositions and ecosystem functions have been made, it has been recently suggested that ecosystem processes may be more dependent on the abundance and diversity of related functional genes rather than the phylogenetic structures of communities (Philippot et al., 2013; Paula et al., 2014). The utilization of the GeoChip functional gene array provides a platform in which a variety of important functional genes, including critical genes for C-turnover, can be detected even when present at low abundances. The sensitivity of the GeoChip arrays was reported previously over half of probes producing positive signal with 10 or fewer pg of DNA template (He, 2007). It is also specific as probe design accounts for minimal-to-no cross-hybridization of sequences with less than 90% similarity and was designated a quantitative tool in which R^2 values averaged 0.93 for signal intensity and DNA concentration correlations (Liebich et al., 2006; Wu et al., 2006; Zhou et al., 2010, 2012). These arrays allowed for comparison of samples throughout the

incubation process with respect to microbial functional diversity, which is the variety of functional genes detected in each sample. We used all functional genes covered on the arrays to broadly compare communities. For analyses centered on traits involved in tundra C degradation we focused on 20,000 probes targeting hundreds of bacterial and fungal genes involved in the degradation of various C substrates.

The main objectives of this incubation study of permafrost zone soils were: (1) to assess the effects of *in situ* experimental soil warming on initial soil C pools, and to estimate C pool sizes and their relative turnover rates, (2) to characterize the relative abundance and structure of microbial functional genes associated with fast and slow C pool decomposition, as well as the response to field warming, (3) to determine the temperature sensitivity of SOM microbial decomposition (Q_{10}) for the fast and the slow C pools, and to characterize how Q_{10} responds to field warming. We expected warming-induced changes in microbial community functional gene abundances and diversities to accompany changes in the kinetics of SOM decomposition. On this matter, we hypothesized that both the abundances and diversities of genes involved in the degradation of fast decomposing C would decrease as these C pools were depleted over the course of the incubation study, whereas genes involved in slow decomposing C would show a relative increase. We also expected that there could be differential temperature sensitivities, with a higher Q_{10} for the slow C pool compared to the fast C pool.

2. Materials and methods

2.1. Site description and experimental design

The Carbon in Permafrost Experimental Heating Research project (CiPEHR) was established in September 2008 (Natali et al., 2012, 2014) on moist acidic subarctic tundra within the Eight Mile Lake research watershed (63°52′59″N, 149°13′32″W) (Schuur et al., 2007, 2009). Vascular plant cover is dominated by the tussock-forming sedge *Eriophorum vaginatum* and the deciduous shrubs *Betula nana* and *Vaccinium uliginosum*, non-vascular biomass is dominated by mosses and lichens (Natali et al., 2012). Soils are Gelisols (Soil Survey Staff, 1999) with a thick organic horizon (0.45–0.65 m) on top of cryoturbated mineral soil and a C content between 50 and 70 kg m^{-2} down to 1 m depth (Pries et al., 2012). Long-term mean annual temperature is −1.0 °C; mean growing season (May–September) air temperature is 11.2 °C and monthly averages range from +15 °C in July to −16 °C in December (Schuur et al., 2009). Average annual precipitation is 378 mm with a growing season mean of 245 mm (National Climatic data center, NOAA). The soil warming treatment was applied by increasing snow cover behind snow fences during winter time, coupled with early spring snow removal to keep water input and snow melt timing similar to control plots (Natali et al., 2011). As a result of this treatment, soil warming plots reached an average of −3.9 °C in the soil profile (5–40 cm depth) during the winter time, 2.3 °C warmer than control plots, this warming effect continued into the growing season when temperature at 40 cm reached 2.9 °C in warmed soils and 1.6 °C in control soils.

2.2. Soil sampling and preparation

Soil cores were collected frozen from control (n = 6) and field warmed (n = 6) plots in May 2010 after exposure to two consecutive winter warming seasons (winters of 2008–2009 and 2009–2010) and one full summer growing season (2009), and kept frozen until further processing. Soil cores were separated in the lab into three depth increments for incubation: 0–15 cm, a fibric Oi-Oe horizon, with a lot of litter and coarse roots, 15–25 cm, Oe-Oa horizon with less coarse roots and more decomposed soil, and 45–55 cm, soils with a mineral-organic mix located just above the surface permafrost because the surface soil thaws each summer to about 55–65 cm and refreezes in the winter. Each soil layer increment was split longitudinally into four sections: one for each lab incubation temperature (15 °C and 25 °C), one for microbial analysis, and one for soil physical characterization. Soil moisture content, bulk density, and mass-based nitrogen (N) and C content (ECS 4010, Costech Analytical, Valencia, CA) were measured for each soil increment (Table 1).

2.3. Incubation design and soil carbon fluxes

Each incubation sample was further divided into 8 subsamples of the same wet weight (~10 g). Each subsample was kept largely intact and placed into a perforated foil cup over a bed of 3 mm glass beads to allow drainage and maintain the soils at field capacity soil moisture. Each incubation sample thus consisted of eight 30 cm^3 vials inside a 1 L mason jar. Each jar was covered with a perforated lid to allow air exchange while minimizing contamination. Control and field warmed soils were aerobically incubated at each of two laboratory incubation temperatures (15 °C and 25 °C) for 365 days. Optimal temperature for field microbial growth is well above the mean annual air temperature and can be considered as the average highest temperature during the year (Rousk and Baath, 2011), except for when Q_{10} was being assessed. Soil temperature at the research site can reach up to 25 °C in the surface few centimeters of soils exposed to direct sunlight during the growing season; laboratory temperatures were chosen to span the soil temperatures found in the soil profile while keeping them warm enough to observe changing dynamics through time.

Jars were placed in a water bath set to the incubation temperature and connected to an automatic soil incubation system (ASIS). Air from each sequential jar was circulated by a pump through an infrared gas analyzer (IRGA, Li-820 Licor, Lincoln, Nebraska) at 0.9 L min^{-1}. A mass flow controller (Mass Flow meter GFM, Aalborg Instruments & Control) maintained a constant flow, while CO_2 concentration and pressure inside each jar were measured by the IRGA and recorded every three seconds by a datalogger (CR1000, Campbell Scientific, Logan UT) over 8 min. Jars were flushed with CO_2-free air at any point when CO_2 concentration in the jar head space reached 10,000 ppm in order to avoid suppression of microbial activity due to oxygen deprivation. Carbon fluxes (F_c) were calculated as the rate of CO_2 increase in the headspace of the jars over time after at least four cycles of eight and an half hours each, and expressed in micrograms of C per gram of initial soil C per day ($\mu gC\ gC_{initial}^{-1}\ d^{-1}$). Fluxes were measured every 48 h during the first two weeks of incubation in order to capture the initial phase of SOM decomposition, then twice a week up to 45 days of incubation, biweekly up to 180 days, and at least once per month up to one year of total incubation time. Total amount of $C_{respired}$ (C_R) at any measurement point was estimated by linear interpolation between measurement points.

Total $C_{respired}$ (C_R, $mgC\ gC_{initial}^{-1}$) through time (t) up to 365 days was fitted with a two-pool C model (Andren and Paustian, 1987) (Proc NLIN, SAS 9.3. SAS Institute Inc. North Carolina):

$$C_R(t) = C_F\left(1 - e^{-k_F t}\right) + C_S\left(1 - e^{-k_S t}\right) \quad (1)$$

where C_F and C_S are the sizes of the fast and slow decomposing C pools relative to the initial C content ($mgC\ gC_{initial}^{-1}$), and k_F, and k_S are the first order kinetic decomposition rate constants for the fast and slow decomposing C pools (day^{-1}). This model assumes that C_F and C_S sum to 1000 mg C per gram of initial C and $k_F > 0$ and $> k_S$.

Table 1
Initial properties of soils from a tundra warming experiment (mean ± SE; N = 6).

Treatment	Depth (cm)	%C	%N	C:N	Bulk density	pH
Control	0–15	41.80 ± 0.35	1.16 ± 0.09	37.21 ± 3.03	0.09 ± 0.01	4.67 ± 0.03
	15–25	34.83 ± 4.14	1.69 ± 0.21	20.89 ± 0.55	0.30 ± 0.11	4.73 ± 0.09
	45–55	17.70 ± 2.97	0.74 ± 0.17	25.50 ± 1.56	0.42 ± 0.05	5.15 ± 0.14
Field warming	0–15	39.88 ± 0.69	1.27 ± 0.04	31.66 ± 1.34	0.12 ± 0.03	4.71 ± 006
	15–25	33.67 ± 2.96	1.66 ± 0.17	20.54 ± 0.58	0.30 ± 0.07	4.97 ± 0.07
	45–55	12.53 ± 2.51	0.48 ± 0.09	26.42 ± 1.44	0.86 ± 0.18	5.05 ± 0.10

2.4. Temperature sensitivity of soil organic matter decomposition (Q_{10})

In order to determine if and how temperature sensitivity of SOM decomposition was affected by field warming, we calculated the change in decomposition rate in response to an increase in temperature of 10 °C (Q_{10}) using two different methods. Short-term Q_{10} ($Q_{10\text{-}ST}$) was calculated by measuring respiration while simultaneously exposing soils to the range of 5–30 °C via 5 °C steps over the total period one week, ~1 day at each temperature level (Hamdi et al., 2013). Short-term Q_{10} was calculated by exposing soils to this temperature range at 14, 100 and 280 days following initiation of the incubation (DOI). We expected the contribution of C_F to dominate the total C flux at the beginning of incubation and because of this, the $Q_{10\text{-}ST}$ at 14 DOI would reflect primarily the temperature sensitivity of C_F. Similarly, more than 95% of C_F was expected to be consumed by 280 DOI (Schädel et al., 2014) thus the $Q_{10\text{-}ST}$ at 280 DOI in contrast was expected to reflect primarily the temperature sensitivity of C_S.

Measured C fluxes were fitted to an exponential function to describe the short-term temperature sensitivity of microbial decomposition ($Q_{10\text{-}ST}$) (Proc NLIN, SAS 9.3. SAS Institute Inc. North Carolina):

$$F_C = ae^{bT} \quad (2)$$

Where a and b are estimated parameters; a is the basal C flux at 0 °C, T is the incubation temperature and b is the parameter related to the sensitivity of microbial respiration, where:

$$Q_{10-ST} = e^{(10b)} \quad (3)$$

Temperature sensitivity of SOM decomposition was also calculated using a second methodology: the equal C method ($Q_{10\text{-}EC}$) (Rey and Jarvis, 2006; Conant et al., 2008a). $Q_{10\text{-}EC}$ was calculated as the ratio of time taken for a soil to respire a given amount of C. For example, comparing rates at steps of 1 mg (or 0.1% of total C) at each of the two constant incubation temperatures (15 °C and 25 °C) throughout the incubation experiment. The $Q_{10\text{-}EC}$ method allowed us to track the trend in Q_{10} continuously as organic matter was decomposed. This method assumes that a given amount of respired C comes from the same C pool across different temperatures. At the early stages of incubation, when only a small amount of C has been respired (i.e. the first 0.5–1% of initial C), whole soil Q_{10} is often used as a proxy for the temperature sensitivity of the fast C pool (Rey and Jarvis, 2006; Conant et al., 2008b; Haddix et al., 2011; Zhu and Cheng, 2011). However, contributions of the slow C pool to total C flux can also be significant at these early stages of the incubation (Schädel et al., 2014; Liang et al., 2015), potentially confounding the estimated Q_{10} for the fast pool alone. To avoid this bias, we used equation (1) to separate the slow and fast C pools, then tracked total $C_{respired}$ from each C pool over time, and estimated $Q_{10\text{-}EC}$ for each C pool separately (Conant et al., 2010). Since soils below 15 cm had very low C_F, no $Q_{10\text{-}EC}$ was calculated.

2.5. Microbial analysis

One vial containing a soil subsample from each depth was removed from each jar after 14, 90, and 280 days of incubation for microbial analysis. A PowerSoil® DNA isolation kit was used to extract microbial community DNA from soil subsamples following standard procedures (MoBio Laboratories, Inc, Carlsbad, California). In some samples, DNA of high purity (Nanodrop 260/280 and 260/230 absorbance ratios above 1.70) could not be obtained via the kit alone so a freeze-grind method was used to obtain raw DNA (Zhou et al., 1996), which was subsequently purified with the PowerSoil® kit. We assessed the microbial functional gene structure using GeoChip 5.0, which contains over 80,000 probes targeting microbial environmental functional genes (Wu et al., 2006; Zhou et al., 2012; Xue et al., 2013; Tu et al., 2014; Zhou et al., 2014). For this, 500 ng of soil community DNA was labeled with the fluorescent dye Cy-3, hybridized to GeoChip 5.0 60K microarrays, and scanned with a NimbleGen MS200 Microarray Scanner using techniques described previously (Cong et al., 2015). The images data were processed using the Agilent Feature Extraction program that designates values for probe signal intensities and background (noise) signal intensities based on the scanned images. Extracted data was then loaded onto an in-house GeoChip data analysis pipeline (ieg.ou.edu/microarray/). Data normalization and quality filtering were performed with multiple standard steps (Liang et al., 2010; Deng and He, 2014). Briefly, poor quality spots were removed, the signals of all spots were transferred into relative abundances, and spots with signal-to-noise (SNR) ratios less than 2 were set to 0 or removed, depending on the analysis. Probes with positive signal in only 2 or fewer samples were removed. Probes with high signal intensities reflect a greater amount of hybridization and targeted genes more abundant in the sample. We analyzed the microarray data in three ways. First, we used microarray data from all probes, which included genes involved in C fixation and degradation, nitrogen and sulfur cycling, antibiotic resistance, and contaminant remediation or degradation. These data represent a broad picture of the microbial functional communities in each sample. Next, we analyzed only probes targeting genes involved in C-degradation. For this, we focused on the abundances of C degradation genes present in each sample by quantifying the signal intensities of only probes with positive signal. We also reflected the diversity of C-degradation genes in a sample by incorporating the probes that did not have an SNR >2 into the data as having a signal intensity of 0. Thus, samples with gene categories containing more 0's represented conditions wherein there were lower functional diversities of the given gene.

2.6. Statistical analysis

We investigated the effects of field warming and sampling depth on soil properties (%C, %N, C:N, bulk density and pH) using linear mixed effects models in R. The same analysis was applied for parameters (C_F, C_S, k_F, k_S), total $C_{respired}$ and $Q_{10\text{-}ST}$, using field warming, sampling depth, incubation temperature, C:N, bulk

density and pH as fixed factors. We used graphical inspection and variance inflation factors (VIF) with the full model to avoid variable collinearity. The cutoff VIF value to drop a variable was 3 (Zuur et al., 2010). After selecting the fixed factors we used the full model with all selected fixed factors to optimize the random structure using the 'lmer' function in the lme4 package (Bates et al., 2014), which was field replicate (fence) and soil core nested within field replicate (fence). Once the random structure was optimized, we performed stepwise multiple regression analysis using the 'lme' command with restricted maximum likelihood from the 'nlme' package in R (Pinheiro et al., 2012). For each model the optimal model was selected by dropping the least significant individual explanatory variable one by one and refitting the model every time with the model selection criterion being the smallest Akaike Information Criterion (AIC) as described in Zuur et al. (2010). For short-term Q_{10} ($Q_{10\text{-ST}}$), we additionally added two-way interactions after graphical exploration of the data in order to test if the effect of a main factor on the dependent variable was affected by another independent factor. Data were log or arcsine transformed as needed and residuals were checked for normality and heterogeneity of variance. For $Q_{10\text{-EC}}$ analysis, a linear mixed effects model was used with field warming and C_R as fixed effects and soil core as random effect. $Q_{10\text{-EC}}$ was analyzed for each depth. For 0–15 cm soils $Q_{10\text{-EC}}$ was compared at different amounts of C_R (1, 25, 45, 65 and 90 mgC $gC^{-1}_{initial}$) and at 1, 5 and 10 mgC $gC^{-1}_{initial}$ for 15–25 cm and 45–55 cm soils. $Q_{10\text{-EC}}$ for all depths was compared after C_S contributed to 75% or more to C_R.

The GeoChip data was analyzed for the entire array (containing probes targeting genes involved in C, nitrogen, phosphorus, and sulfur cycling as well as bioremediation processes) and for C gene targets alone. Samples collected at 2 weeks, 3 months, and 9 months from both incubation temperatures and all 3 depths were used for all analyses. Dissimilarity tests were based on Bray–Curtis dissimilarity index using non-parametric multivariate analysis of variance (ADONIS) (Anderson, 2001) under the package Vegan (v.2.0–3) (Oksanen et al., 2012). Detrended correspondence analysis (DCA) was used to determine the overall functional changes in the microbial communities with respect to environmental and temporal variables (Hill and Gauch, 1980). All DCA plots were prepared in R package vegan and contained data from all GeoChip probes include those with SNR<2 filled as 0's. The signal intensities for GeoChip probes in subcategories "carbon degradation" and "organic remediation" were isolated for all analyses focused on traits involved in tundra C degradation. We removed all probes with SNR <2 for all analyses focused on gene abundances and included them as 0's for all analyses focused on C-degradation functional gene diversity. Mann-Whitney-Wilcoxon tests, performed in R, were used to determine statically significant differences between categorized probes in different treatments and diversity and evenness indices of C-degradation functional genes.

3. Results

3.1. Initial soil properties

In general, initial mass based soil C (%C) and nitrogen (%N) concentrations varied systematically by depth (Table 1), but were not affected by field warming over this initial phase of the tundra warming experiment (Table 2). Carbon concentration decreased from about 40% in the 0–15 cm layer, to 35% in 15–25 cm, and to less than 20% in the 45–55 cm layer (Table 1). Nitrogen concentration also significantly changed with depth, with the highest value in the intermediate layer (15–25 cm). Carbon to nitrogen ratio (C:N) slightly decreased with depth although in general, the lowest values were found in the 15–25 cm depth interval. Bulk density and pH both increased with depth with no difference between control and field warmed soils, although there was a trend towards higher bulk density with warming at the deepest soil depth (Tables 1 and 2).

Table 2
Statistics for initial properties of soils from a tundra warming experiment.

Model	Coefficient	Estimate	SE	Df	t-value	P-value
Soil properties						
C (%)	Intercept	0.809	0.028	29	29.318	<0.0001
	Depth	−0.008	0.001	29	−9.886	<0.0001
N (%)	Intercept	0.139	0.007	29	18.472	<0.0001
	Depth	−0.001	0.000	29	−5.127	<0.0001
Bulk density	Intercept	−1.231	0.094	28	−13.130	<0.0001
	Depth	0.017	0.002	28	7.472	<0.0001
	Field warming	0.131	0.078	28	1.676	0.1050
pH	Intercept	0.659	0.009	28	73.171	<0.0001
	Depth	0.001	0.000	28	5.555	<0.0001

3.2. Carbon pools, decay rates and total carbon respired

The fast decomposing C pool (C_F) accounted for less than 5% of the total initial C pool and decreased from 30–50 mgC $gC^{-1}_{initial}$ in 0–15 cm soils to 5–10 mgC $gC^{-1}_{initial}$ in 45–55 cm (Fig. 1a, Supplemental Table 1) whereas the slow decomposing C pool size (C_S) accounted for 950–990 mgC $gC^{-1}_{initial}$ (Fig. 1b). Both C pools significantly changed with soil depth and incubation

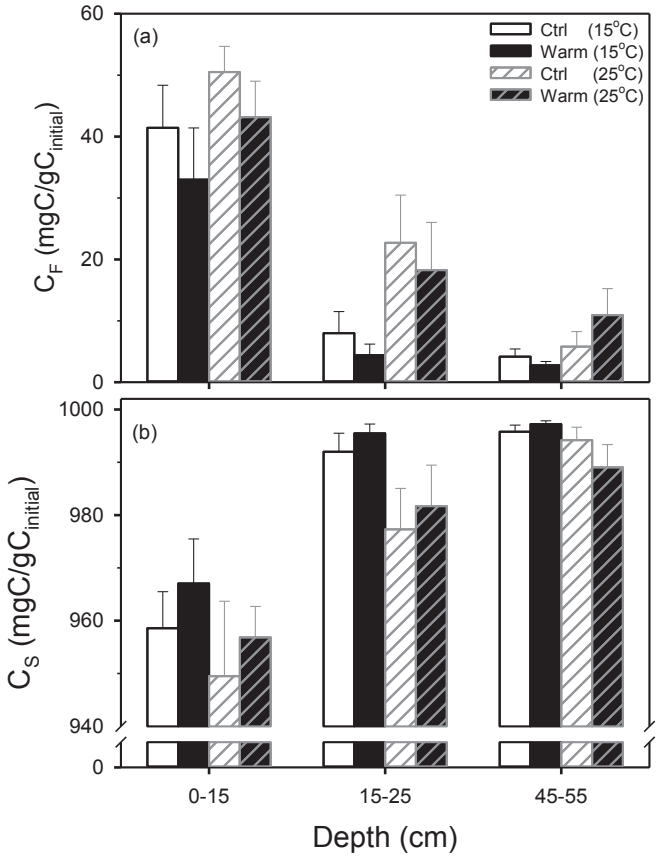

Fig. 1. Size of the fast (C_F) (a) and slow (C_S) (b) decomposing carbon pools (mgC/$gC_{initial}$) for field control (Ctrl) and field warmed (Warm) Alaskan tundra soils incubated at 15 °C and 25 °C (hatched bars). (Mean ± SE, n = 6).

temperature (Table 3). Because the sum of both pools is defined as1000 mgC $gC^{-1}_{initial}$, they showed opposite patterns versus depth and incubation temperature: C_F decreased with depth but increased with incubation temperature, while C_S increased with depth and decreased with incubation temperature (Fig. 1, Table 3). Carbon pool sizes were not affected by field warming in this initial phase of the experiment. Of all the variables that went into the mixed effects regression model, only C:N showed a significant relationship with both C pool sizes ($p < 0.0001$, Table 3), with higher C:N related to larger C_F and the reverse for C_S.

The decomposition rate of the fast decomposing C pool (k_F) ranged from 0.004 to 0.1 day^{-1} (i.e. turnover times of 10–250 days, Fig. 2a, Supplemental Table 1) and was negatively influenced by incubation temperature and bulk density (Table 3). On average, 40% and 60% of C_F from control and field warmed soils was consumed by 14 DOI at 15 °C incubation temperature. By day 100, 85% and 95% of C_F was consumed from soils incubated at 15 °C and 25 °C, respectively. By day 280 of incubation virtually all of C_F was consumed from all soils. The decomposition rate of the slow C pool (k_S, Fig. 2b, Supplemental Table 1) ranged from 0.00003 to 0.0012 d^{-1} (i.e. turnover times of 2.3–97.8 years). The slow pool decomposition rate decreased three-fold from 0-15 cm to 15–25 cm soils, and doubled when incubation temperature was increased from 15 to 25 °C (Table 3, Supplemental Table 1); k_S also increased with C:N while pH had a marginal negative effect on k_S.

Total cumulative $C_{respired}$ (C_R) in 0–15 cm soils after 365 days of incubation reached an overall average of 137.27 ± 15 and 232.60 ± 24 mgC $gC^{-1}_{initial}$ for 15 °C and 25 °C incubations (Fig. 3, Supplemental Table 1). The total amount of $C_{respired}$ significantly decreased with depth and increased with incubation temperature, but no significant field warming effect was detected (Fig. 3, Table 3, Supplemental Figure 1). Linear mixed effects regression results identified C:N as a significant predictor for total C loss over the one year incubation period (Table 3), with higher initial C:N associated with increased total C loss.

Table 3
Statistics for parameters derived from laboratory incubations of soils from an Alaskan tundra warming experiment incubated at 15 °C and 25 °C for 365 days.

Model	Coefficient	Estimate	SE	Df	t-value	P-value
Parameters						
C_F	Intercept	−0.039	0.142	60	−0.277	0.7830
	Depth	−0.008	0.001	60	−5.953	<0.0001
	$Temp_{incubation}$	0.015	0.004	60	3.474	0.0010
	C:N	0.015	0.003	60	4.739	<0.0001
C_S	Intercept	1.566	0.037	60	42.107	<0.0001
	Depth	0.002	0.000	60	6.323	<0.0001
	$Temp_{incubation}$	−0.004	0.001	60	−3.622	<0.0001
	C:N	−0.004	0.001	60	−4.713	<0.0001
k_F	Intercept	0.282	0.031	61	9.115	<0.0001
	$Temp_{incubation}$	−0.004	0.001	61	−2.959	0.0040
	Bulk density	−0.069	0.019	61	−3.580	0.0010
k_S	Intercept	−3.517	0.592	59	−5.945	<0.0001
	Depth	−0.012	0.002	59	−6.676	<0.0001
	$Temp_{incubation}$	0.032	0.004	59	7.408	<0.0001
	C:N	0.020	0.003	59	6.257	<0.0001
	pH	−0.224	0.123	59	−1.824	0.0730
$C_{respired}$	Intercept	1.913	0.531	59	3.606	0.0010
	Depth	−0.012	0.002	59	−7.846	<0.0001
	$Temp_{incubation}$	0.031	0.004	59	8.042	<0.0001
	C:N	0.019	0.003	59	6.470	<0.0001
	pH	−0.170	0.110	59	−1.539	0.1290
$Q_{10\text{-}ST}$	Intercept	0.392	0.018	194	22.277	<0.0001
	Field warming	0.066	0.023	194	2.861	0.0050
	Depth	0.001	0.000	194	1.637	0.1030
	Day of incubation	0.000	0.000	194	−1.597	0.1120
	Field warming:Depth	−0.002	0.001	194	−3.827	<0.0001

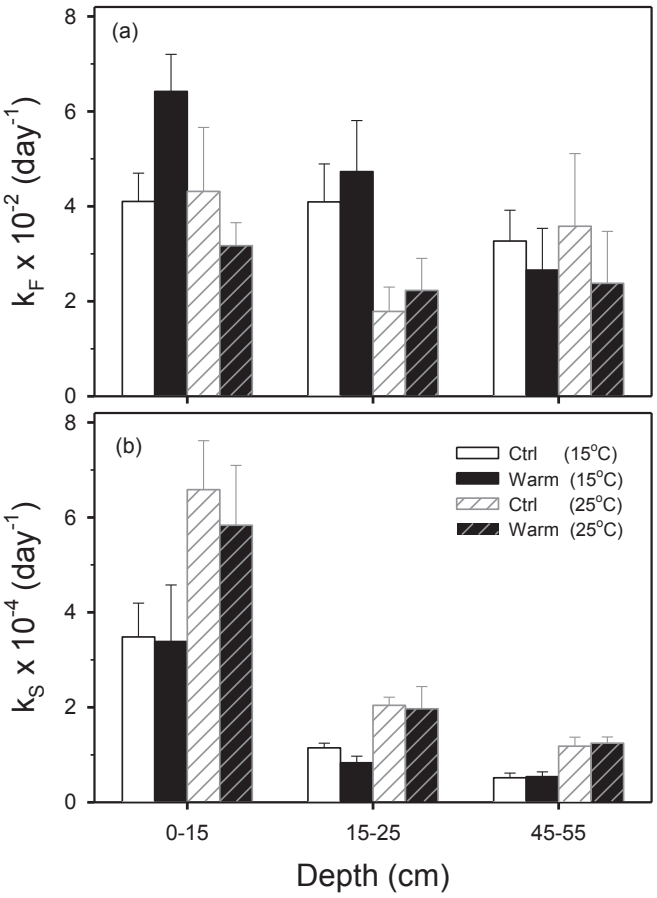

Fig. 2. Decay rates for the fast (k_F) (a) and slow (k_S) (b) decomposing carbon pools for field control (Ctrl) and field warmed (Warm) Alaskan tundra soils incubated at 15 °C and 25 °C (hatched bars). (Mean ± SE, n = 6).

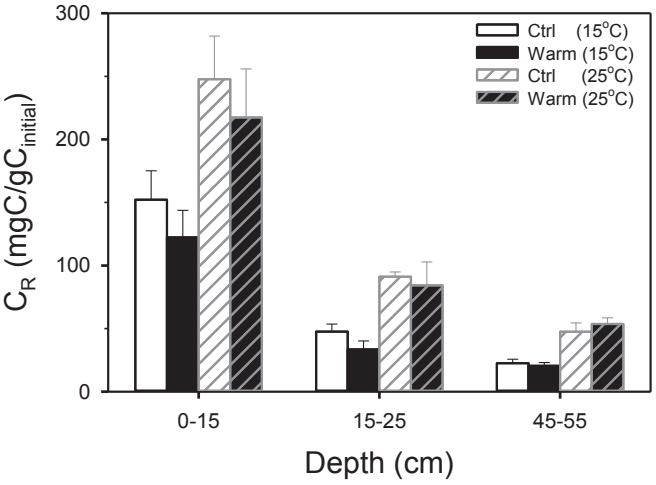

Fig. 3. Total carbon respired, C_R (mgC/$gC_{initial}$) for field control (Ctrl) and field warmed (Warm) Alaskan tundra soils from different depths incubated at 15 °C and 25 °C (hatched bars) after one year of incubation. (Mean ± SE, n = 6).

3.3. *Temperature sensitivity (Q_{10})*

Short-term calculated temperature sensitivity of SOM decomposition ($Q_{10\text{-}ST}$) ranged from 1.6 to 4.8, with an average value of 2.55 ± 0.03 across all soils (Supplemental Table 2). Field warming seems to have an effect on $Q_{10\text{-}ST}$ at 14 DOI in 0–15 cm soils

(Supplemental Table 2, Fig. 4), however, a significant interaction was seen between field warming and depth on $Q_{10\text{-}ST}$ (Table 3, Fig. 4). The strongest effect of field warming was observed for the 45–55 cm depth interval, where a 20% reduction in $Q_{10\text{-}ST}$ was observed in field warmed soils. We expected to observe $Q_{10\text{-}ST}$ dominated by C_F at 14 DOI however, time of incubation had only a marginal to no effect on $Q_{10\text{-}ST}$. On average, more than 50% of C_F was already consumed in 0–15 cm soils at both temperatures of incubation by 14 DOI, and C_S already dominated total C flux by that time. This effect was stronger in deeper soils, where C_S dominated respiration even at the very beginning of the incubation.

Temperature sensitivity of SOM decomposition estimated by the equal C method ($Q_{10\text{-}EC}$) in 0–15 cm soils showed a significant increase ($p = 0.0097$) from 1.18 ± 0.37 to 2.40 ± 0.37 as the total amount of $C_{respired}$ (C_R) changed from 1 to 90 mgC $gC^{-1}_{initial}$ (Fig. 5a,d), no significant field warming effect was detected. Contribution of C_S to the first mg of C_R in 0–15 cm soils was in average 24.6% at both temperatures of incubation, and 87% and 97% at 60 and 90 mg of C_R, respectively. In soils below 15 cm (Fig. 5b, c; e, f), $Q_{10\text{-}EC}$ was marginally affected by C_R ($p < 0.1 > 0.05$), contribution of C_S to the first mg of $C_{respired}$ was in average 50% and 70% for 15–25 and 45–55 cm soils, respectively. However, $Q_{10\text{-}EC}$ was higher ($p < 0.01$) for field warmed than control at both 15–25 cm (3.09 ± 0.58 vs. 2.07 ± 0.54) and 45–55 cm (2.93 ± 0.18 vs. 1.98 ± 0.19 vs.) soils, respectively (Fig. 5b, e; c, f). $Q_{10\text{-}EC}$ after C_S contribution to C_R was greater than 75% was not affected by depth or field warming ($p > 0.05$) showing an overall average of 2.66 ± 0.40.

Temperature sensitivity for each C pool independently was estimated using equation (1) (Supplemental Table 2). $Q_{10\text{-}EC}$ for C_F was estimated for the 0–15 cm soils as the ratio of time to respire the first 10 mg of C_F. Surface soils' $Q_{10\text{-}EC}$ for C_F (1.16 ± 0.30) was not significantly affected by field warming ($p = 0.28$), but was significantly less than $Q_{10\text{-}EC}$ for C_S at the same depth (2.05 ± 0.23). In general, estimated $Q_{10\text{-}EC}$ for C_S was not significantly affected by field warming ($p = 0.21$) or by depth ($p = 0.13$), with an overall average of 2.19 ± 0.13.

3.4. Total microbial functional traits analysis

Dissimilarity analysis for overall microbial functional traits, as identified by GeoChip 5.0, revealed that functional diversity of microbial communities in incubated soils was significantly altered (Table 4, $p = 0.001$) with soil depth, incubation time and temperature of incubation. While there was no overall field warming effect on functional diversity (Table 4, $p > 0.1$), there was a marginal significant interaction between field warming and incubation time ($p = 0.08$). This showed that dissimilarities in functional diversity with respect to field warming tended to increase as the incubation progressed and happened in combination with the depletion of C_F from the soil.

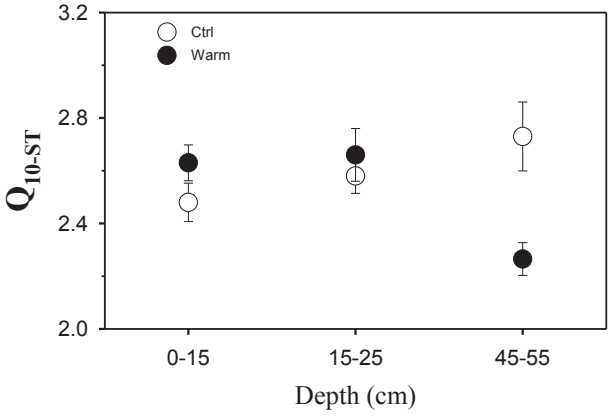

Fig. 4. Overall average $Q_{10\text{-}ST}$ (15 °C & 25 °C) for field control (Ctrl) and field warmed (Warm) Alaskan tundra soils from different depths after one year of incubation. (Mean ± SE, n = 12).

Detrended correspondence analysis (DCA) of GeoChip data indicated that the structure of the microbial functional communities in specific soil samples were separated with respect to the variables depth, time and temperature of incubation. For example, throughout the incubation, distinct microbial communities dominated at 14, 100 and 280 days in 15–25 cm soils incubated at 25 °C, with a more clear separation in field warmed than control soils (Fig. 6). Functional communities in the top (0–15 cm) differed from those in the bottom (45–55 cm) in field warmed soils (Supplemental Figure 2). Also, temperature of incubation selected different microbial communities in 15–25 cm in both control and field warmed soils (Supplemental Figure 3). To some extent, field warming tended to select different microbial communities in the soil profile in samples incubated at 25 °C for 280 days although individual outliers samples are present at both depths (Supplemental Figure 4).

3.5. Microbial traits involved in tundra carbon degradation

Focusing on genes involved in C-degradation, we did not see significant differences in the gene abundances or diversities in field warmed or control soils. However, after two weeks of incubation soils incubated at 25 °C had lower mean signal intensity values for the overall set of genes involved in C-degradation that were explored here as compared to the 15 °C incubations (Fig. 7). This was largely caused by higher numbers of absent (zero) signals for genes involved in C-degradation in the 25 °C incubations (Fig. 8). This trend was consistent for probes targeting genes involved in the degradation of a wide range of C substrates and was strongest in the surface soils and less so at depth. However, by 3 and 9 months the microbial communities from soils incubated at 15 °C and 25 °C showed similar mean and median signal intensities for C-degradation genes. When only probes with positive signal were considered (0's excluded), the difference in gene abundances between the 15 °C and 25 °C 2-week samples was no longer apparent (Fig. 9). Furthermore, microbial communities assayed from the 2 weeks, 25 °C incubations, which corresponded to the highest measured daily C fluxes, also displayed the lowest alpha diversity estimations for functional genes as determined by Shannon and Simpson indices and Pielou and Simpson evenness (Supplemental Table 3). These direct and quantitative measures show that the assayed genes at the two weeks of incubation point were not equivalently abundant in the samples at the different incubation temperatures. This indicates that the 10 °C temperature difference used in the incubation had a short-term, but highly significant effect on the functional diversity of microbial C degradation genes.

4. Discussion

4.1. Dynamics of soil organic matter decomposition

Decomposition of SOM in tundra from permafrost zone soils incubated under controlled conditions was affected by differences in substrate quality (%C, %N) and C availability (Total C) in the soil profile, as well as incubation temperature and time of incubation. These differences were reflected in microbial community diversity, decay rates, and in total $C_{respired}$ at the end of the incubation experiment. Slow decomposing C contributed between 75% and 90% to total $C_{respired}$ by the end of the incubation term, even in 0–15 cm soils where fast decomposing C was relatively higher

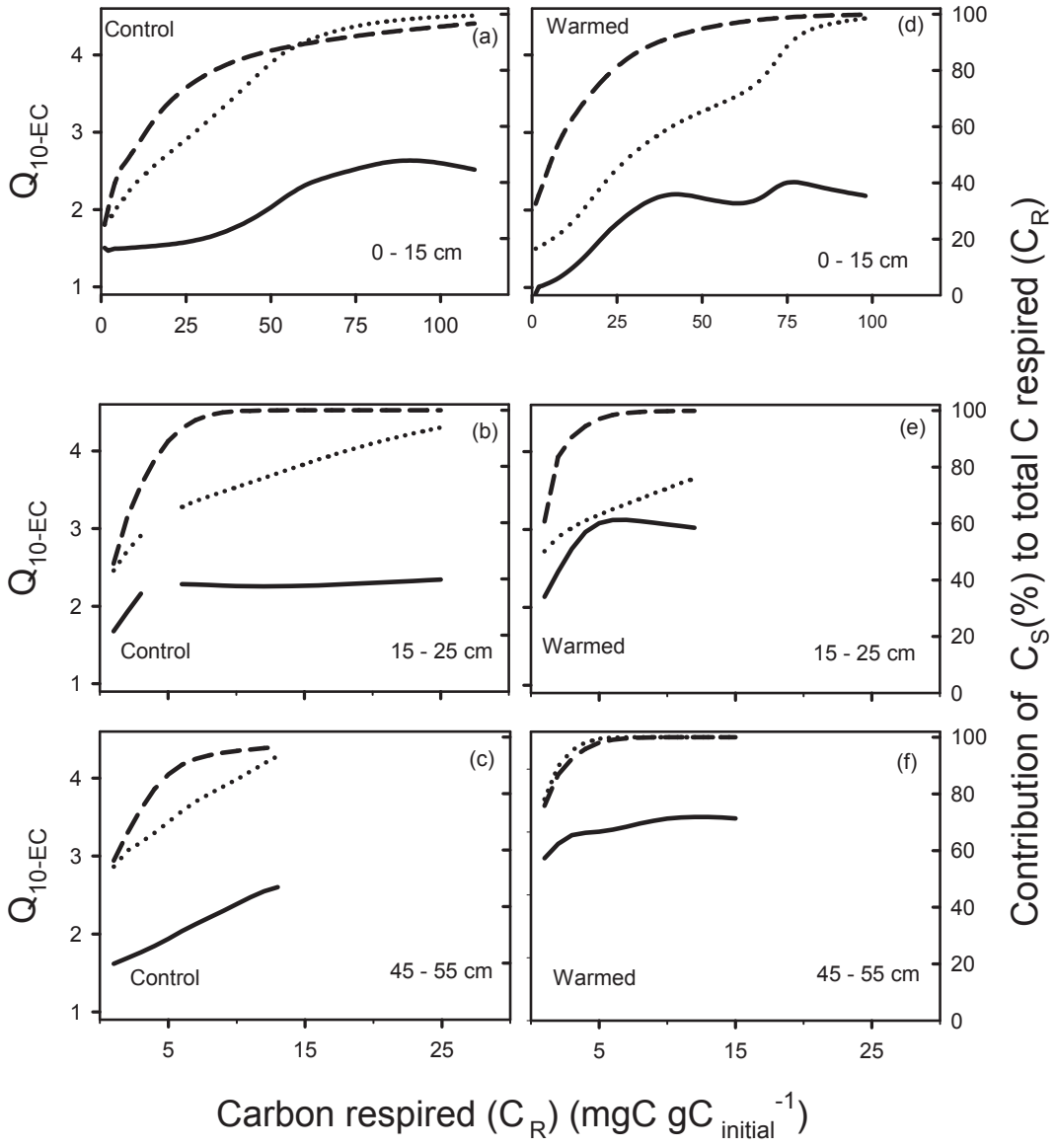

Fig. 5. Relationship between $Q_{10\text{-}EC}$ (continuous line) and total amount of carbon respired (C_R, mgC/$g_{initial\_C}$), and contribution of the slow decomposing C pool (%C_S) (right Y axis) to C_R at 15 °C incubation (dotted line) and 25 °C incubation (dashed line). Control soils (Panels a–c, left), field warmed soils (Panels d–f, right). Top, intermediate, and bottom panels correspond to 0–15, 15–25 and 45–55 cm depths. Data are the average of six replicates, error bars omitted for simplicity of visualization.

Table 4
Dissimilarity analysis of the microbial communities based on Geochip data by Adonis in Alaskan tundra soils incubated at 15 °C and 25 °C for 365 days.

	Geochip		
	F.Model	R^2	P-value
Plot	0.803	0.01225	0.611
Depth	13.289	0.04053	0.001
Field warming	1.459	0.00445	0.198
DOI	34.552	0.10538	0.001
$Temp_{incubation}$	31.161	0.09504	0.001
Field warming:depth	1.662	0.00507	0.169
Depth:DOI	1.036	0.00316	0.297
Field warming:DOI	2.327	0.0071	0.084
Depth:$Temp_{incubation}$	1.048	0.0032	0.325
Field warming:$Temp_{incubation}$	1.808	0.00551	0.142
DOI:$Temp_{incubation}$	33.635	0.10258	0.001

DOI = Day of incubation.
$Temp_{incubation}$ = Temperature of incubation.

compared to deeper in the soil profile, stressing the importance of C_S in long-term C release from these soils. The field warming manipulation did not have much effect either on the soil properties or the decomposition dynamics in the laboratory but this was not surprising because the field warming had been recently implemented and so there was not a long period of time for substantial change to the bulk soil pool. Soils were collected before the beginning of the second growing season after the field warming experiment was implemented and changes to the bulk soil might be expected to take multiple years to manifest (Sistla et al., 2013, 2014). The one exception to this was a change in bulk density at the deepest layer that was likely a result of decreasing ice/water content as the thaw depth increased in the warming manipulation.

Size and distribution of C_F and C_S in the soil profile of this research site is typical for permafrost zone soils (Knoblauch et al., 2013; Schädel et al., 2014). The largest proportion of C_F was found in 0–15 cm soils is due to fresh litter inputs, microbial biomass, and the presence of roots and root exudates. In tundra soils, more than

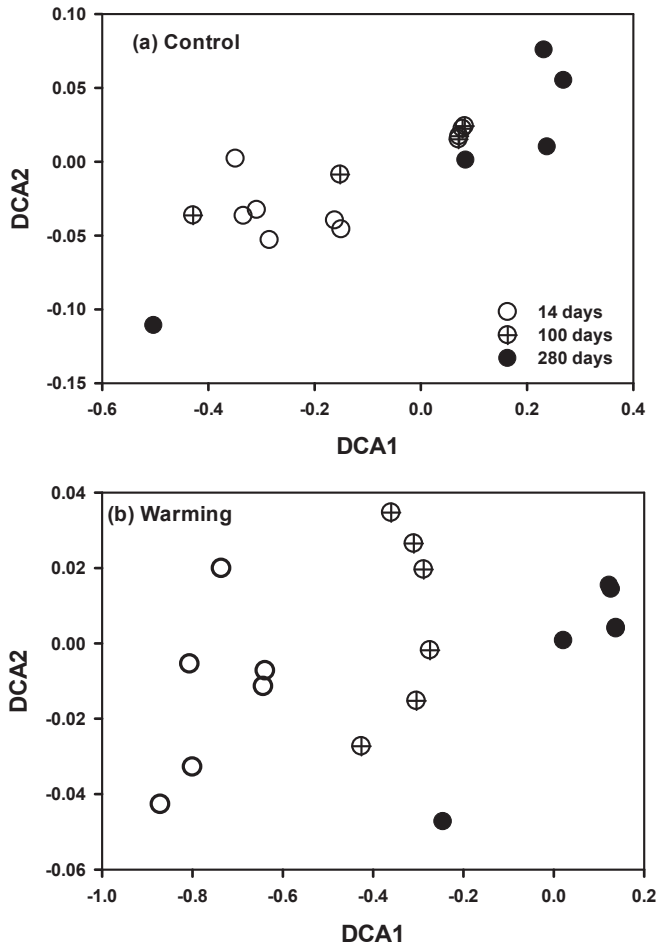

Fig. 6. DCA plots reflecting effects of time of incubation on microbial functional communities for 15–25 cm Alaskan tundra soils incubated at 25 °C. The placement of the samples along the axes are output of the DCA ordination algorithm, which functions to find maximum correlations between all GeoChip probe scores and sample scores.

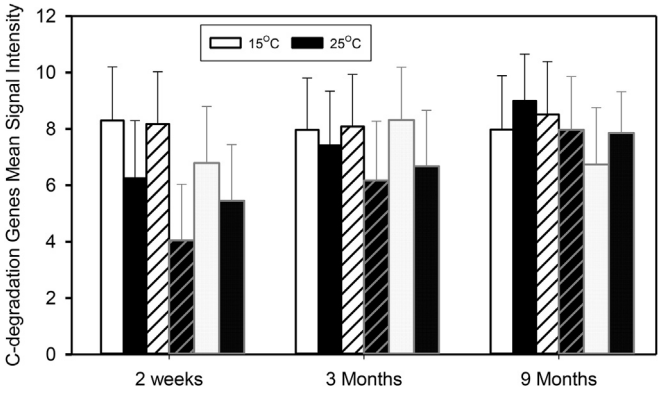

Fig. 7. Mean signal intensities of GeoChip 5.0 probes targeting genes involved in C-degradation. Solid fill (white and black): 0–15 cm, stripped fill: 15–25 cm, doted fill: 45–55 cm.

80% of root biomass is found in the top 20 cm (Dennis and Johnson, 1970; Dennis, 1977; Jackson et al., 1996; van Wijk et al., 2003). Root exudates comprise low molecular weight C compounds that can be easily assimilated by microbes. As well, microbial biomass turnover due to winter soil freezing likely contributes additional substrate to C_F (Schimel and Mikan, 2005). However the contribution of C_S was still higher than contribution of C_F to total $C_{respired}$, even in 0–15 cm soils at the end of the incubation, as C_F was depleted rapidly by new microbial activity in the incubation.

Although C_F and C_S have complementary patterns with depth relative to initial C content, both decreased on dry soil basis as total soil C decreased with depth. These depth patterns in C were accompanied by changes in functional microbial diversity (Conant et al., 2011; Sistla et al., 2013), affecting mostly decomposition rate for C_S. A higher k_S at the surface than at depth is likely a function of the fact that these soils accrete vertically over time such that C_S in the deeper layers has already been through significant previous decomposition and remaining SOM has slower turnover, reflected in the significant relationship with C:N. At all depths, k_S was higher in 25 °C relative to 15 °C incubation reflecting standard temperature effects on microbial metabolism.

The two fold increase in k_S with an increase of 10 °C of incubation was driven by increased activation energy and enzymatic activity, eliminating physical protection and breaking down complex organic C into more simple molecules (Marschner and Bredow, 2002; Davidson and Janssens, 2006; Allison et al., 2010). As result, more C was transferred to C_F and C_R doubled at 25 °C incubation. In contrast to k_S, k_F was not sensitive to depth nor C:N and was seemingly less sensitive to incubation temperature. This suggests that $C_{respired}$ from this fast decomposing pool have a similar molecular composition through the soil profile (Mueller et al., 2015), and perhaps that temperature sensitivity of this material was less. It is important to recognize though, that estimates for pool sizes and turnover rates are not completely independent using these modeling procedure (Katterer et al., 1998).

There did not appear to be variation in specific functional genes along with the phases of C degradation measured during these incubations, which was contrary to our original hypothesis. In fact, functional groups representing all gene subcategories present on the functional gene array were abundant throughout the incubation, rather than genes associated with labile C degradation declining over time. This likely reflects concurrent C_S and C_F decomposition and the ability of the microbial communities to perform both functions simultaneously at all points during the incubation. It has been shown that microbial biomass and their products, as well as plant detritus, are simultaneously contributing to total microbial respiration, albeit at different rates, as wet arctic tundra surface soils thaw (Schimel and Mikan, 2005). These substrates contrast greatly in C quality and their decomposition should be reflected in varied microbial functional genes involved in their degradation. Additional support of these findings was indicated in a previous study wherein microbial communities from different depths in moist acidic tundra were able to decompose a wide range of C substrates with increasing recalcitrance and this capacity increased with temperature (Ernakovich and Wallenstein, 2015).

4.2. Microbial function during OM decomposition

As a whole, the DCA clustering plots reflect that microbial functional community structure varied with the divergent environmental parameters, but that the incubation conditions resulted in greater dissimilarities than did field treatment or depth. Soils also differed in the state of C quality as a result of these parameters. While there was some separation in microbial functional communities from field warmed and control plot soils they were often not complete, with individual outlier replicates consistently observed through the dataset. In contrast, separation between communities based on the variables time and incubation temperature were notably clearer, in particular when field warmed soils were considered. This greater separation may indicate some initial

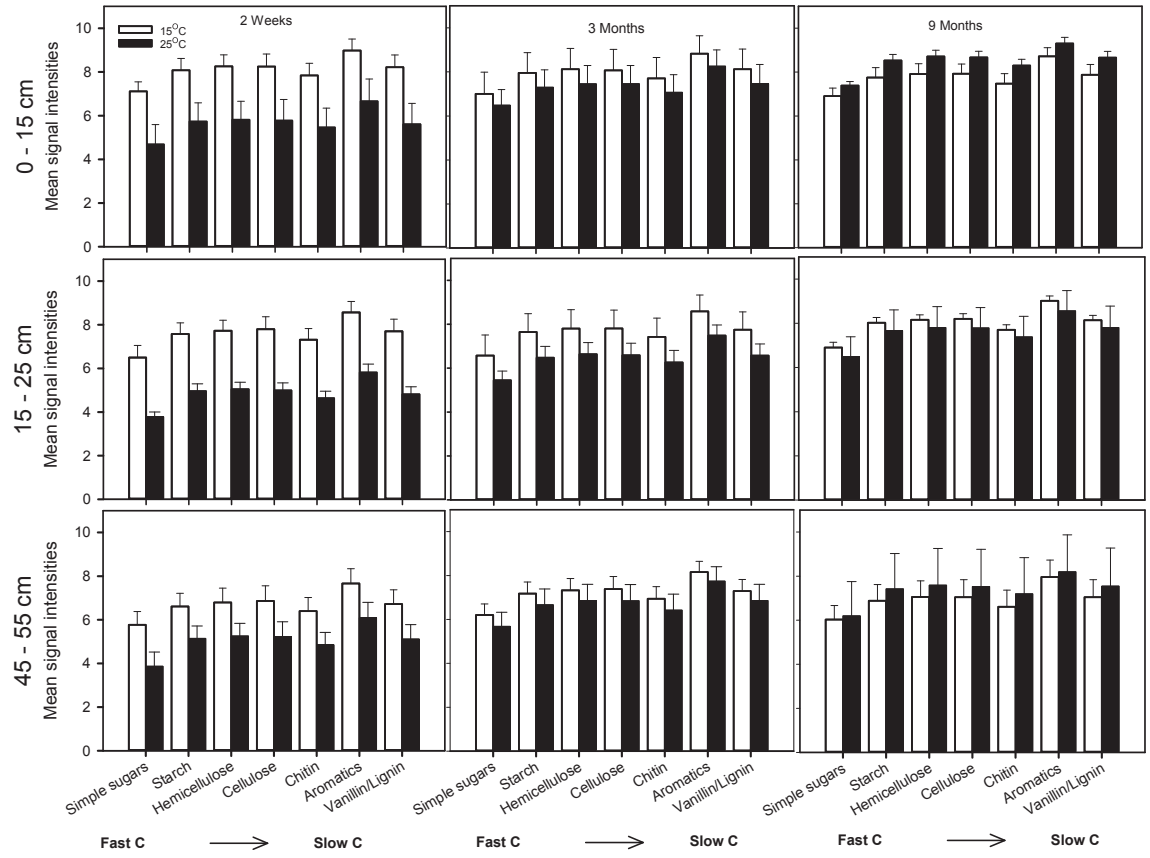

Fig. 8. Mean signal intensities form GeoChip 5.0. Values represent means and standard deviations from 6 replicate soils and all probes categorized as targeting genes involved in degradation of denoted carbon sources. Carbon substrates range from fast to slow decomposing C (left to right).

functional community shifts that were present in the field warming samples, which then became more apparent as C quality declined, although this was not directly tested here.

In contrast to some findings in literature, we did not find a significant difference in C-degradation genes as a product of field warming as determined by GeoChip analyses. Previous studies showed microbial functional diversity to decrease in response to warmer soils (Yergeau et al., 2012; Yang et al., 2014) and other reports quantified increased abundances of functional genes in response to warming (Wang et al., 2012), particularly those involved in labile C-degradation (Zhou et al., 2012). A common trend was a correlation between plant biomass (Yergeau et al., 2012; Yang et al., 2014) and C_4 plants (Zhou et al., 2012) to microbial functional gene abundances and diversities in warming experiments. However, changes in plant biomass or aboveground net primary productivity had not yet been detected in this study at the time when soils were sampled (Natali et al., 2012; Salmon et al., 2016). Consequently, changes in soil microbial communities would more likely be tied to environmental changes imposed by the experiment rather than in response to changing plant processes.

The monitoring of the SOM decomposition kinetics provided us a unique opportunity to analyze the corresponding microbial communities. One interesting finding was that after two weeks of incubation, communities with higher respiration rates had lower functional diversity than the communities present in cases of both 1) greater abundance of C_F and lower incubation temperatures and 2) lower abundance of C_F and high incubation temperatures. Thus the temperature-stimulated communities may have had some key degraders that initially dominated the C degradation activity as opposed to an equivalent increases in biomass across a diverse flora. As C_F became less available these opportunistic organisms may have declined in abundance. There were no C inputs to the soils during these incubations therefore the temporal increase in functional diversity of the 25 °C samples reflected the presence of diverse functional genes and the degradation capacity of these communities that were potentially overshadowed by genetic material from fast-growing organisms earlier in the incubation. Prior studies support this idea, reporting that bacterial diversity was positively correlated with time of litter decomposition and the reduced substrate quality at later stages of decomposition (Dilly et al., 2004). Another study showed soil bacterial diversity to decrease following exposure to freeze-thaw cycles, even though total microbial abundance was unaffected (Sharma et al., 2006). That study also noted that fungal communities were more stable under freeze-thaw conditions and suggested that stronger shifts in bacterial diversity could be associated with the ability of bacteria to respond to C substrates mobilized following thaw.

An alternative explanation for the lower gene signal intensity may be that many groups were hindered at this higher 25 °C temperature, rather than some individual groups of organisms flourishing. The success of microorganisms in tundra can be attributed to freeze-tolerance (Schimel and Mikan, 2005) and the organisms best acclimated to this environment may not thrive at 25 °C. This explanation corresponds to the observation that representative functional diversity was low after two weeks at 25 °C. Over time, organisms may have acclimated to the 25 °C temperatures and thus, their DNA reached detectable quantities, which was observed at levels similar to the 15 °C incubations later in the experiment. Previously, Steinweg et al. (2008) found that

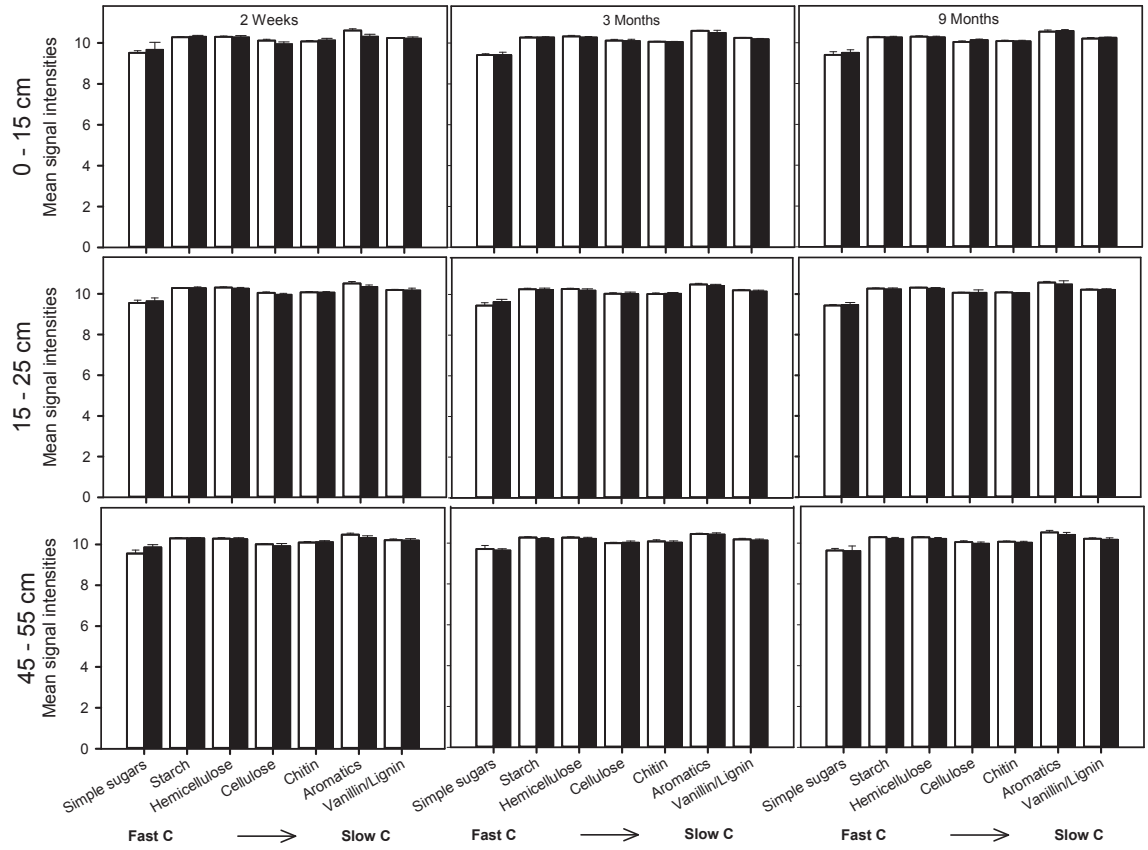

Fig. 9. Mean signal intensities from GeoChip 5.0 for only probes showing positive signal intensity. Values represent means and standard deviations from 6 replicate soils and all probes categorized as targeting genes involved in degradation of denoted carbon sources. Carbon substrates range from fast to slow decomposing C (left to right).

microbial carbon use efficiency (CUE) declined with warmer temperatures, resulting in less C allocated to cellular growth and more lost as CO_2, but this reduced efficiency was not maintained over time. Thus the high respiration rates of the 0–15 cm, 2 week, 25 °C incubation soils could have actually reflected soils with lower biomass, wherein many groups of microbes were inactive under the unusual temperature and those active had low CUE's leading to higher respiration rates.

We expected to see variation in the abundances of fungal and bacterial genes throughout the incubation process. We also anticipated an initial rise in labile C-degradation genes, followed by increased recalcitrant-degradation genes after 280 days of incubation. However, our data actually reflect consistency among fungi and bacteria as well as across the suite of C-degradation genes in response to the effects of time and C pools. This was reflected by lower functional diversities of genes degrading all subcategories of C substrates observed after two weeks 25 °C incubation. Additionally, when we focused on C-degradation gene abundances there are not significant differences among the soil depths, incubation times or temperatures.

4.3. Temperature sensitivity of SOM decomposition

Temperature sensitivity of microbial SOM decomposition reported here is the intrinsic Q_{10} (Davidson and Janssens, 2006; Karhu et al., 2010; Deslippe et al., 2012; Sistla et al., 2014). The temperature sensitivity for C_F estimated using the equal C method was $Q_{10\text{-}EC} = 1.16 \pm 0.30$ (Supplemental Table 2), indicating that decomposition of the fast C pool is not temperature sensitive or it may even decrease as shown by the negative effect of increasing temperature on the decay rate (Table 3). It was previously shown that microbes from the organic active layer are able to use the same amount of substrates when temperature of incubation changed from 1 °C to 10C° and even 20 °C (Ernakovich and Wallenstein, 2015). On the other hand, decomposition of the slow decomposing C pool was affected by changes in temperature with Q_{10} overall average values greater than 2, as calculated by two different approaches, the short term ($Q_{10\text{-}ST} = 2.55 \pm 0.03$) and the equal C ($Q_{10\text{-}EC} = 2.19 \pm 0.13$). Temperature sensitivity obtained using the short term method primarily corresponded to C_S since this C pool dominated C fluxes the first time Q_{10} was estimated at DOI 14 in all depths and throughout the incubation period. A similar conclusion can be made when a $Q_{10\text{-}EC} = 2.66 \pm 0.40$ was estimated across all samples after normalizing contribution of C_S pool to total $C_{respired}$ exceeded 75%. This value was not affected by depth or field treatment and may explain the overall lack of the effect of DOI/time and depth on $Q_{10\text{-}ST}$. The one shift in $Q_{10\text{-}ST}$ was the interaction of field warming and depth, where field warmed soils in the deepest depth had a slightly lower temperature response (Fig. 4). This corresponds to a mineral soil layer, which also had higher bulk density at this same sample. It is not clear if bulk density was a direct or indirect driving factor, or what the mechanism would be if these were actually linked. Our results indicate that significant amount of C can be released from this slow C pool with an increase of soil temperature even if flux rates are small, considering that substrate availability will not be a limiting factor. The higher temperature sensitivity for the slow decomposing C pool from tundra soils is consistent with the enzyme kinetics, where high activation energy is required to break down more complex C molecules (Davidson and Janssens, 2006; Wetterstedt et al., 2010; Craine et al.,

2010a,b; Conant et al., 2011).

Increases in Q_{10} ($Q_{10\text{-}EC}$), as C_R increased through time, were accompanied by changes in substrate use toward slower decomposable C (Streit et al., 2014), and the consequent shift in microbial communities (Conant et al., 2011; Deslippe et al., 2012; Sistla et al., 2014). This was reflected here by increased functional diversity of communities degrading C_S, primarily, which corresponded to changes in functional community structures with time of incubation and depth. When contributions of C_S to C_R reaches 50%, slow C decomposers probably dominate C decomposition showing a Q_{10} typical for the slow C pool. These microbes are able to use C compounds in the whole range of recalcitrance in the temperature range they were incubated (Ernakovich and Wallenstein, 2015), probably competing and dominating over the fast C decomposers. This is even more significant when contributions of C_S to C_R exceeds 60% and $Q_{10\text{-}EC}$ is the same independently of the depth the soil is coming. In general, shifts in microbial communities associated with the slow decomposing C mediate increases in temperature sensitivity of soil organic matter decomposition (Karhu et al., 2014), although we didn't see the shifts in C degradation genes that were expected. This increased temperature sensitivity of SOM decomposition as slow decomposing C becomes dominant (lower organic matter quality) has also been observed in soils from ecosystems with lower soil C content than permafrost soils (Hartley and Ineson, 2008; Conant et al., 2008a,b; Karhu et al., 2010).

Incubation results need to be extrapolated to the field with some caution, because for example, factors such as fresh soil C inputs are completely eliminated in laboratory incubations. C inputs in the field are not likely to remain the same and it is not completely clear how warming will affect soil C balance. Our results suggest that sustained warming appears to have more effect on the dominant slow C pool as compared to the fast C pool. Increased plant input due to sustained warming (Chapin et al., 2005; Tape et al., 2006; Schuur et al., 2007; Pearson et al., 2013; Sistla et al., 2013, 2014) could help to refill or maintain soil pools and counterbalance some soil C loss. Assuming both the quantity and quality of C inputs change or stay the same but temperature increases, microbial communities will shift towards slow C decomposers with a temperature sensitivity greater than 2 but, also with capacity to degrade a wider range of C compounds. This process will be amplified when additional slow C is exposed to decomposition by a deeper thaw as permafrost degrades (Natali et al., 2011). The microbial response to increased temperatures and the exposure of slow C will control regional C feedback to the atmosphere under the projected warming scenarios of 7–8 °C by the end of the 21st century (Trenberth et al., 2007; IPCC, 2013).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.02.008.

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