Title: Permafrost microbial community traits and functional diversity indicate low activity at *in situ* thaw temperatures

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Abstract:

Previously-frozen stores of organic carbon (C) are now subject to decomposition due to a warming Arctic climate and associated permafrost thaw; however, estimates of the amount of greenhouse gases (GHG) that may be released are not well constrained. Knowing more about the functions of the extant permafrost microbial community will inform this knowledge gap. The exploration of microbial functional traits may be useful to elucidate the relationship between microbial diversity and ecosystem function. We characterized the community traits and functional diversity of the bacterial and Archaeal component of the microbial community from three depths of permafrost, as well as the organic and mineral horizons of the seasonally-thawed active layer, by assessing ‘substrate-use richness,’ ‘substrate preference,’ ‘growth rate,’ ‘and substrate specific growth rate.’ We measured the microbial community response to 31 substrates with an EcoPlate (Biolog, Inc.) assay at three incubation temperatures (1, 10, and 20 °C) using a kinetic approach, and modeled the microbial response to each substrate with a modified logistic growth function. We hypothesized that the permafrost communities would be selected for high functional potential at cold temperatures. However, we found that the permafrost community did not have a higher functional diversity or activity at 1 °C than the organic active layer soils. In addition, permafrost communities increased their growth rates with increasing temperature, indicating that the highest incubation temperature (20 °C) was below their temperature optimum for growth. As predicted, the permafrost communities did exhibit temperature dependent substrate preferences. Thus, permafrost microbial communities did not appear to be selected for higher metabolism and the ability to use a broad suite of substrates at low temperatures, which suggests that they may have limited function immediately following thaw when temperatures are near 0 °C. However, changes in community composition or additional permafrost warming will

44 increase the functional capabilities of permafrost microbes to decompose the C stored in those
45 soils.

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1. Introduction

Previously-frozen stores of organic carbon (C) are subject to decomposition due to Arctic warming and associated permafrost thawing (Harden et al., 2012; Hinzman et al., 2005; Osterkamp, 2007; Schuur et al., 2008). Climate models forecast continued warming at the poles and increased rates of permafrost thaw (Koven et al., 2011; Schaefer et al., 2011). Decomposition of the organic matter in freshly thawed permafrost is dependent on interactions between C quality, abiotic conditions, geomorphology and decomposer activity (Schädel et al., 2014; Treat et al., 2014). But, decomposer activity is dependent on the functional traits of the microbial community in relation to these abiotic drivers, and these traits are likely to differ among microbial communities that differ in composition. The functional potential of the permafrost microbial community is likely to have a large effect on the decomposition of permafrost and C flux to the atmosphere, but this effect is difficult to predict with current knowledge (Graham et al., 2011).

Characterizing and employing microbial functional traits has been useful in explaining many ecosystem processes (Allison, 2012; Tang and Riley, 2013). However, the description of broad-scale ecological patterns based on traits of individual microbial species has been hampered by limited knowledge of the functions of specific taxa (Green et al., 2008). Rather, the aggregated traits of the microbial community may enhance our ability to predict the rates of ecosystem-level processes (Wallenstein and Hall, 2011). For example, Allison (2012) used the traits of enzymes and the physiology of microorganisms to model litter decomposition and found the level of enzyme production for the entire community was an important predictor of decomposition rates. Follows et al. (2007) developed a trait-based model using phytoplankton growth requirements to model ocean productivity, allowing phytoplankton community

composition and biogeography to emerge from the traits. Evans and Wallenstein (2014) discovered that ecological strategies employed by microbial communities were related to historical environmental conditions (such as the precipitation regime), and proposed that these strategies could be aggregated into community level traits that explained differences in functional responses to changing environmental conditions (Evans and Wallenstein, 2012). Other community-level functional traits, such as catabolic evenness (Degens et al., 2000), C mineralization rate (Santrucková et al., 2003), and microbial growth rate (Zak et al., 1994) have also been instrumental in linking community traits to ecosystem function. Thus, understanding the functional traits of the permafrost microbial community may improve our predictions of decomposer activity and potential greenhouse gas flux from permafrost after thaw.

Microbial community traits are often shaped by substrate availability, at least in temperate ecosystems. Degens et al. (2000) found that catabolic evenness, a component of functional diversity, was related to both total organic C and potentially mineralizable C. In addition, the native temperature regime of a microbial community appears to play a role in microbial functional potential. For example, Balser and Wixon (2009) found that the temperature optimum of microbial community growth for three soils from a broad range of native conditions was related to the mean annual temperature of the site. Because permafrost microbial communities are likely structured by environmental filtering based on their ability to withstand frozen conditions (Ernakovich, 2014; Rivkina et al., 2000; Santrucková et al., 2003) the native temperature regime might play an especially large role in determining their functional potential. Permafrost microorganisms are not all merely lying dormant, in wait of less metabolically restrictive conditions; they are actively performing cellular repair (Brinton et al., 2002; Johnson et al., 2007), metabolizing substrates (Bakermans et al., 2003; Rivkina et al., 2000), and growing

in situ (Drotz et al., 2010; McMahon et al., 2011; Tuorto et al., 2014). Thus, permafrost microbial communities may exhibit high functional potential at temperatures close to *in situ* temperatures.

We characterized the temperature dependence of community traits and functional diversity in permafrost and active layer soils. We hypothesized that environmental filtering for fitness at cold temperatures would structure the community traits and functional diversity of the permafrost community, which would be reflected in high community-level metabolism and substrate use. First, we predicted that both growth rate and the number of substrates degraded would be greater for the permafrost than the active layer microbial communities at the lowest incubation temperature (1 °C). Secondly, we predicted that permafrost microbes would have a greater response at 1 °C than 20 °C because the latter would be above the microbial community's optimum temperature for growth. Finally, we predicted that incubation temperature would affect the substrate preference of the microbial communities. We fit a logistic growth model to substrate utilization data from EcoPlate (Biolog, Inc.) assays to assess community traits, including 'growth rate' and 'substrate-specific growth rate,' and functional diversity, including 'substrate-use richness' and 'substrate preference.'

2. Methods

2.1. Description of the study site, soil sampling and processing

Organic active layer, mineral active layer, and permafrost soils were collected from Sagwon Hills, Alaska (N 69° 25' 32.190" W 148° 41' 38.731", 288 m above sea level), which is in the foothills north of the Brooks Range. The soils were collected from under moist acidic tundra vegetation, which are classified as Ruptic Histic Aquiturbels (Borden et al., 2010). The

permafrost at Sagwon Hills is of loess origin over gravel deposits (Borden et al., 2010). Cores were collected from 15 plots representative of the site on a grid covering 150 m². The depth of the seasonally thawed active layer was 26.8 ± 1.3 cm in August of 2009, and consisted of an organic and mineral horizon with evidence of cryoturbation. Further description of the site and soils can be found in Ernakovich et al., 2015.

At each plot, the active layer was removed and placed on a tarp as a monolith. Organic and mineral active layer soils were sampled from the monolith from the center of their respective horizons at a depth of 2 ± 0 cm for the organic horizon and 9.4 ± 1 cm for the mineral horizon. Permafrost soils were obtained as frozen cores using a Tanaka auger fitted with a SIPRE-style (Snow, Ice, and Permafrost Research Establishment, (Tarnocai, 1993) soil corer with carbide bits (Jon's Machine Shop, Fairbanks, Alaska). Permafrost cores were collected as deep as possible before encountering glacial till (30-47 cm below the frozen boundary).

The samples were kept frozen through sampling, transportation, and storage. Permafrost cores were processed frozen in a walk-in -10°C freezer. First, they were scraped under aseptic conditions to remove outer layers of soil. Then they were separated into 5 cm increments. If there was a natural fracture point at an ice lens within 2 cm of the 5cm fracture point, that point was chosen. Permafrost, organic and mineral active layer soils were homogenized as frozen, unthawed soil by crushing them with a hammer to an approximate 2-4 mm sieve size in sterile bags. After homogenization, the samples were processed and stored for 11 months at -10 °C until analysis. Although we acknowledge that this prolonged storage could have affected functional responses, -10 °C is below the field temperature (average = -1 °C at the time of sampling), and because the soils remained frozen from sampling to analysis, they should represent field microbial community composition. Cores from nine of the 15 plots were chosen at random for

this assay, but only eight organic and six mineral active layer samples were used for this assay due to errors in field collection. Thus for this analysis, we included organic active layer (n=8), mineral active layer (n=6), and permafrost samples from 0-5 cm (n=9), 10-15 cm (n=9), and 20-25 cm (n=9) below the maximum active layer thaw depth. Soil characteristics (Table 1) were described previously (Supplemental material; Ernakovich et al., 2015).

2.2. *EcoPlate experimental setup*

The Biolog EcoPlate™ (Biolog Inc., Hayward, CA) assay contains 31 substrates in triplicate and is similar to other widely used Biolog assays (such as Biolog GN and GN2). A description of the assay assumptions is included in the Supplemental materials. Field-moist active layer and permafrost soils (1 g dry weight equivalent) were weighed into sterile flasks and pre-incubated for three days. To reduce interference of soil organic matter and mineral particles in the colorimetric assay (Balser et al., 2002), 10^{-3} soil dilutions (with sterile 7% NaCl) were used for the two active layer soils and 2.8×10^{-2} for the permafrost layers. Permafrost soils were diluted to 2.8×10^{-2} because preliminary experiments showed no color development in the 10^{-3} dilution in 7 days. 10^{-1} dilutions were shaken for 30 minutes, and the dilutions were allowed to settle for 10 minutes before subsequent dilutions or inoculation into EcoPlate assay. 140uL of each soil dilution was dispensed into each well of 3 EcoPlate 96-well microplates. Plates were placed into 1, 10 or 20 °C incubators inside lidded boxes containing water to maintain humidity during the assay.

We used a kinetic approach by collecting absorbance readings every 12 hours at 590 nm (average from 10 measured reads) on a microplate reader after 15 seconds of shaking (Lindstrom et al., 1998) (Tecan Infinite M200, Tecan, Research Triangle Park, NC). Absorbance

measurements were collected until color saturation (from the exhaustion of the tetrazolium dye) was achieved. This occurred between 1 week and 3 months depending on temperature.

2.3. Obtaining kinetic parameter estimates

After subtracting the absorbance of the water well from that of each substrate, we fit a “modified logistic growth model” (Eq. 1, Lindstrom et al, 1998) to the data using PROC NLIN (SAS 9.3, Cary, NC).

$$y = A_{595\text{ nm}} = \frac{K}{(1 + e^{-\lambda(t-s)})} \quad (\text{Eq. 1})$$

$A_{595\text{ nm}}$ is the absorbance at 595 nm, K is the asymptote the absorbance approaches, λ is the exponential increase in the absorbance, t is the time since inoculation and s is the time at the halfway point of the exponential phase. We used this approach to minimize bias introduced by incubation duration and differing inoculum density (Supplemental material; Lindstrom et al., 1998; 1999).

We evaluated model fit with a pseudo- r^2 . Parameters were accepted for further statistical analysis if the model fit had a pseudo- r^2 between 0.8 and 1, and if the number of missing data points was below 50%. If the model did not fit the data, we determined whether this was because the substrate was not degraded or whether the model was simply a poor fit and the data should be classified as missing for that point. If the blank-corrected absorbance value for an analytical replicate of a substrate was less than zero for over 50% of the time points, we determined that analytical replicate did not degrade that substrate ($\lambda=0$). We designated the rest of the data as missing.

2.3.1. Determining functional traits: Growth rate

The parameter λ from Eq. 1 represents an exponential increase of absorbance over time and can be used to represent the exponential growth phase (Lindstrom et al., 1998). An estimate of the mean λ for each microplate was calculated from the substrates that had a good model fit and not more than 50% missing data (as described previously). Thus, the mean of λ represents the average growth rate only for substrates that were degraded. Boxplots were created after averaging across analytical replicates and all the substrates within a substrate class using BoxPlotR (<http://boxplot.tyerslab.com>; R Core Development Team, 2013; RStudio and Inc, 2013). We also made boxplots by averaging across replicates and substrates such that there were only field replicates included in the n for the boxplot. The latter boxplots displayed less variability, so we decided to be conservative and use the former for boxplots and statistical analysis to display a fuller range of variability in λ .

A Q_{10} function was applied to the parameter λ (after collapsing analytical replicates and averaging the λ for all substrates within a substrate class, as above) to understand the temperature sensitivity of microbial growth in the microplate assay using Eq. 2,

$$Q_{10} = \left(\frac{\lambda_2}{\lambda_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (\text{Eq. 2})$$

where λ_2 is the kinetic model parameter for the higher temperature, T_2 , and λ_1 for the lower temperature, T_1 . Boxplots of Q_{10} values were made as described above.

Analysis of variance (ANOVA) (SAS 9.3, Cary, NC) was used to evaluate the effect of incubation temperature and soil depth on λ and Q_{10} using a Welch test to satisfy the assumption

of equal variance. Tukey Honestly Significant Difference (HSD) was used to compare differences between λ and Q_{10} by depth ($p < 0.05$).

2.3.2. Determining functional traits: Substrate-specific growth rate

To determine the effect of temperature and soil depth on substrate-specific growth, we grouped the 31 substrates into substrate classes: amines, amino acids, carbohydrates, carboxylic acids, phosphorylated chemicals and polymers (Table 2), as in Zak et al., 1994. We calculated average λ for each of the substrate classes by averaging across analytical replicates and substrates within a class for each sample at each temperature (as described in the previous section). We reduced the dimensionality of λ using distance-based redundancy analysis (db-RDA) (using the vegan package in R; Oksanen et al., 2013; Ramette, 2007) to evaluate the effect of temperature and soil depth on the growth rates of the microbial community. For two samples at 1 °C and one at 10 °C, no substrates were used, and they were removed from the analysis. db-RDA was performed; firstly using the soil depth as the factor class and secondly using the incubation temperature. The component scores from the db-RDA were used in linear regressions (PROC REG, SAS 9.3, Cary, NC) for each substrate class to assess the substrate-specific growth rates of the different microbial communities (Balser and Wixon, 2009; Garland and Mills, 1991).

2.3.3. Determining functional diversity: Substrate-use richness

We determined substrate-use richness, or the number of substrates the community in each sample was able to degrade (Zak et al., 1994), from whether or not the use of a substrate was a good fit to the logistic growth model fit. Model fits (including whether data was determined to be missing or a zero) were performed as described above.

Substrate-use richness was calculated by summing the number of substrates degraded by the community within a plate (Zak et al., 1994) and dividing by 3 (the number of analytical replicates on the plate). ANOVA (SAS 9.3, Cary, NC) was used to evaluate the effect of incubation temperature and soil depth on the substrate-use richness. A Levene test confirmed that this data satisfied the assumption of equal variance. Tukey HSD was used to compare differences between the substrate-use richness by depth ($p < 0.05$).

2.3.4. Determining functional diversity: Temperature dependence of substrate preference

To determine the effect of temperature on substrate preference, we grouped the substrates into classes as described in 2.3.2 and analyzed the substrate-use richness (2.3.3) by temperature. db-RDA was performed for each soil depth with temperature as the factor class. In addition, we calculated the percentage of samples that used the substrates within a class for each temperature-depth combination by dividing the sum of the number of substrates used (averaged across the analytical replicates) by the product of the number of substrates in the substrate class and the number of samples at the soil depth.

3. Results

3.1. Functional traits of the communities

Average growth rate, λ , at 1 °C across all utilized substrates was equivalent for all soil depths, from the organic active layer to the deepest permafrost. At both 10 and 20 °C, the organic active layer soil had a significantly higher growth rate than the mineral active layer or any of the three permafrost depths (0-5, 10-15, and 20-25 cm below the maximum active layer thaw depth)

(Fig. 1a). The growth rates for all soil depths increased with temperature, indicating that the temperature optima for these microbes was not below 20 °C.

The Q_{10} of microbial growth was not different between the soil depths between 1 and 10 °C or 10 and 20 °C (Fig. 1b). The Q_{10} value decreased on average by 54% between the low (1 and 10 °C; mean Q_{10} = 4.5) and high (10 and 20 °C; mean Q_{10} = 2.4) incubation temperatures for the organic active layer ($p<0.001$), mineral active layer ($p=0.0476$), and the shallowest permafrost ($p=0.0374$). Greater variation in the Q_{10} values for the two deeper permafrost depths prevented a significant difference ($p>0.05$), but the means for the Q_{10} at 1 and 10 °C were also higher than those at 10 and 20 °C.

Differences in substrate-specific growth rates between the soil depths were determined with db-RDA models with the soil depth category as the factor class. The models were significant for all three incubation temperatures ($p<0.001$) (Fig. 2). At all three temperatures, the first axis, Partial Constrained Analysis of Principle Coordinates (CAP) 1, was significant but the second axis was not. At 1 °C, five substrate classes (amines, amino acids, carboxylic acids, polymers and phosphorylated chemicals) resulted in the separation along CAP 1 (Table 3), which separated the response of the microbial communities in the organic active layer from the mineral active layer and the permafrost (Fig. 2). Carbohydrate utilization at the lowest incubation temperature was the same for all soil depths (Table 3). CAP 2 was not significant, but amino acids and carboxylic acids appeared to influence the different responses of the organic and mineral active layers from one another (Table 3). For 10 and 20 °C, the substrate utilization of the organic active layer separated it from the other soils, but the mineral active layer and permafrost soils behaved the same.

The temperature dependence of substrate-specific growth rates was determined using db-RDA models of λ with the temperature as a factor class, which were significant for each soil depth category ($p < 0.001$) (Fig. 3). For all five soil depths, substrate utilization separated the 1 °C samples from 10 and 20 °C (Fig. 3) along CAP 1 (which was always significant). For the organic active layer soil, separation along the component 2 was also significant. For the organic active layer soils, all six substrate classes contributed to the separation of the 1 °C samples from 10 and 20 °C along CAP 1 (Table 4). Substrate utilization at 10 and 20 °C separated along CAP 2 due to differential growth rates on carbohydrates and phosphorylated chemicals. For the mineral active layer soil, the separation of 1 °C from 10 and 20 °C occurred due to differential growth rates on amines, amino acids, carbohydrates, carboxylic acids and phosphorylated chemicals. For the top of the permafrost (0-5 cm), component 1 separated not only the 1 °C samples from 10 and 20 °C, but also 10 °C from 20 °C. All of the substrate classes contributed to these differences (Table 4). The substrate utilization for the deepest (20-25 cm) and intermediate permafrost (10-15 cm) was similar, and was affected by all the substrate classes except phosphorylated chemicals.

3.2. Functional diversity

The number of substrates that the permafrost and mineral active layer microbial communities degraded at 1 °C was low compared to the organic active layer (Fig. 4); it increased to its maximum at 10 °C and remained the same at 20 °C (Fig. 4). The substrate-use richness of the organic active layer community was not affected by temperature ($p = 0.999$). In contrast, the difference in the number of substrates that the microbial communities from the mineral active layer and permafrost 0-5 cm, 10-15 cm, and 20-25 cm used at the different incubation temperatures indicates a dependence of substrate-use richness on temperature ($p < 0.05$, Fig. 4).

db-RDA models of the numbers of substrates used at different temperatures (factor class) were significant for each soil depth ($p < 0.001$) (Fig. 5). For the organic active layer and top two permafrost depths (0-5 cm and 10-15 cm below the maximum active layer thaw depth), degradation patterns within all six substrate classes explained the difference in the substrate preference between 1 °C and the higher incubation temperatures (Table 5). In the mineral active layer, five of the substrate classes (amines, amino acids, carbohydrates, carboxylic acids, and phosphorylated chemicals) explained the differences between the number of substrates degraded by the microbes between the 1 °C and at the higher incubation temperatures. The use of polymers did not explain the difference (Table 5), because polymers made up a greater proportion of the total substrate use for the mineral soil at 1 °C than they did at 10 or 20 °C (calculated from % in Table 6). For the deepest permafrost depth (20-25 cm), phosphorylated chemicals did not explain the differences in the number of substrates degraded by temperature because the community at this soil depth did not degrade these substrates effectively at any temperature (Table 6).

For all the soil depths, the number of samples that degraded the substrates increased with temperature, but this difference was less for the organic active layer, which already utilized a large proportion of the substrates even at 1 °C (Fig. 4; Table 6). The organic active layer soils utilized all of the substrate types fairly evenly regardless of temperature (Table 6). In contrast, the mineral active layer and permafrost soils degraded more N-containing compounds (amines and amino acids) at 1 °C than other substrates. The microbes in the mineral active layer soils also degraded less polymers and phosphorylated chemicals than in the organic active layer soils, except at 1 °C where the mineral active layer microbes successfully degraded polymers. The microbes from the permafrost did not degrade phosphorylated chemicals, but degraded polymers

321 nearly as well as other substrates. At 10 and 20 °C substrate degradation was fairly uniform
322 across the substrate classes for all soil depths.

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4. Discussion

The community of bacteria and Archaea inhabiting permafrost and mineral active layer soils from this Arctic tundra site are less functionally diverse and have lower activity than those from organic active layer soils. This suggests that the permafrost microorganisms are not selected for increased capability to metabolize substrates at low temperatures. There were, however, some differences in substrate preference of permafrost microorganisms between the lowest and higher incubation temperatures. Previous studies that have employed metagenomics found that the functional potential of soil microbial communities declined with depth towards the permafrost table in active layer soils from Svalbard, Norway and the Northwest Canadian Arctic (Frank-Fahle et al., 2014; Tveit et al., 2012). Microbial communities from the mineral active layer and permafrost soils thus appear capable of fewer C degrading functions than microorganisms from the organic horizon of the active layer, which could be a result of lower taxonomic diversity or less chemical complexity in the oligotrophic conditions found in permafrost. The limited ability of these permafrost microbes to degrade the diverse suite of compounds that become available upon permafrost thaw could limit gaseous C loss from thawing permafrost, however this delay is likely only transitory.

4.1. Permafrost microorganisms are not highly functional at low temperatures

Previous studies have reported that microbial communities in permafrost are selected for life at cold temperatures (Rivkina et al., 2000; Santrucková et al., 2003). Thus, we predicted that the growth rate of the permafrost microbes would be greater than the active layer at the lowest incubation temperature (1 °C). Rather, we found equal growth rates at 1 °C between the organic active layer, mineral active layer and permafrost communities (Fig. 1a). In a laboratory

incubation at 5 °C, Waldrop et al (2010) also found microbial respiration rates to be the same between the active layer and permafrost from three Alaskan sites. In this study, the mean growth rate, λ , was 0.366 day⁻¹ at 1 °C across the five soil depths which is similar to that reported by Lindstrom et al (1998) once corrected for temperature. This suggests that arctic microbial communities do not grow faster at low temperatures than soils from warmer climates. This seemingly universal similarity of growth rates at low temperatures regardless of the native temperature regime of the soils could be due to constraints on physiology at cold temperatures (Price and Sowers, 2004) or decreased affinity for substrates at low temperatures (Nedwell, 1999).

If permafrost microbes are selected for high metabolism at cold temperatures, the temperature sensitivity of growth would be less for the permafrost than the active layer at low temperatures, such as between the 1 and 10 °C incubation temperatures in this study. But, the temperature sensitivity of growth was the same between all the soils at both temperature ranges tested (1 vs. 10°C and 10 vs. 20 °C) ($p>0.05$) (Fig. 1b). In contrast, previous studies have found evidence for higher temperature sensitivity of microbial activity in organic versus mineral arctic soils. Waldrop et al. (2010) found that the temperature sensitivity of respiration for organic-rich, active layer soils was higher than for mineral permafrost soils, although this difference was exacerbated by the low activity of the organic soils and relatively higher activity of the permafrost soils at their lower incubation temperature (-5 °C). Lupascu et al. (2012) found the temperature sensitivity of methanogenesis decreased with depth in the active layer at Stordalen mire, Sweden. Likewise, Wallenstein et al. (2009) found that beta-glucosidase activity, an indicator of overall microbial activity, was more temperature sensitive for organic than mineral soils sampled under shrub tundra immediately following soil thaw in northern Alaska.

In the soil matrix, the temperature sensitivity of microbial processes can be confounded by other factors, such as organo-mineral interactions, aggregation, redox (Davidson et al., 2006) and substrate supply (Davidson et al., 2011; Weedon et al., 2013). Thus, it is possible that we did not observe a difference in the temperature sensitivity between the organic active layer and permafrost because, once removed from the limitations imposed by the micro-scale heterogeneity of chemical properties inherent in soils, these confounding factors were not present. Rather, it is possible that we observed the intrinsic temperature sensitivity of microbial substrate degradation under non-substrate limiting conditions. Alternately, degradation of labile compounds may not be temperature sensitive. For example, Boddy et al. (2008) found that microbial degradation of simple, dissolved substrates was not temperature sensitive, and temperature sensitivity was only observed later during the degradation of microbial necromass.

4.2. Permafrost activity and functional diversity increases with temperature

Selective pressures for fitness at cold temperatures would likely be balanced with trade-offs that restrict substrate degradation or growth at warmer temperatures. Life at cold temperatures requires specialized adaptations (Gilichinsky et al., 1993), such as modified cell walls (Bakermans et al., 2003) and altered enzyme kinetics (D'Amico et al., 2006). Thus, we expected permafrost microbes to have a greater growth rate at 1 °C than 20 °C. Contrary to our expectations, the permafrost microbial community was capable of growth at the highest incubation temperature (20 °C), suggesting that it was not beyond its temperature optimum for growth (Fig. 1). This indicates that permafrost communities are not dominated by specialists with a niche dependent on cold temperatures (psychrophiles), but rather are psychrotolerant microorganisms (Bakermans et al., 2003; Rivkina et al., 2004; Steven et al., 2006).

Evidence of trade-offs would also be evident if the number of substrates degraded by the permafrost microbial community at 1 °C exceeded that of 20 °C. Instead, the number of substrates the permafrost microbial community degraded increased with incubation temperature. This was also the case for the mineral soils in the active layer but not the organic soils (Fig. 4), and neither the permafrost nor the mineral active layer microbial communities reached the maximum potential substrate-use richness, even though the substrates were all relatively easy to utilize (Campbell et al., 1997) and many can be taken up without extracellular enzymatic breakdown. In contrast, the proportion of substrates degraded by microbes in the organic active layer was high at all temperatures (Fig. 4; Table 6). It has been proposed that substrate-use richness is correlated to soil C content (Chaer et al., 2009; Degens et al., 2000). In this study, substrate-use richness tracked soil C (Table 1; Ernakovich et al., 2015), suggesting that soil C content rather than whether or not a soil was from the permafrost shaped the functional diversity and traits of the community. Rather than being poised to take advantage of thaw with an adaptation of high metabolism at low temperatures, permafrost microbes seem to be composed of slow growing, conservative microbes adapted for persistence under C starvation conditions (Morita, 1997).

4.3. Permafrost microbial communities alter their substrate preference with temperature

The community of bacteria and Archaea from the permafrost and mineral active layer soils changed substrate preference with temperature (Fig 5). This could be due to temperature associated shifts in the substrate preference of a static community, or because members of the community, which have different substrate preferences, change their activity with temperature. In either case, the community-level reflection of the functional diversity changed with

temperature. At 1 °C, these soils used more N containing compounds (amino acids and amides) than other substrates as a proportion of total compounds used (Table 6). N mineralization has been observed in thawed permafrost at temperatures just above 0 °C (Keuper et al., 2012). At spring thaw in seasonally frozen soils, a large pulse of N is commonly observed and has been attributed to a turnover in the microbial biomass (Schmidt et al., 2007; Schmidt and Lipson, 2004). Thus, N mineralization in permafrost after thaw (such as in Keuper et al., 2012) could be due to a turnover in microbial biomass. But, our results suggest a preference for N-rich substrates just above thaw temperatures, which likely reflects an increased N demand at the onset of growth for cellular components (e.g. proteins and nucleic acids), as well as for the productions of enzymes necessary for organic matter degradation.

The microbes in the organic active layer had no temperature-dependent substrate preferences, and degraded all the substrate classes at all temperatures. Balser and Wixon (2009) found that microbial communities from various ecosystems had differential substrate utilization with temperature, which they supposed was dependent on the initial community composition. Different consortia of bacterial taxa have been shown to utilize different substrates (Goldfarb et al., 2011), and temperature niches also exist in soil microbial communities (Tuorto et al., 2014). The ability of the organic active layer microorganisms to utilize the substrates evenly across all temperatures is likely due to its greater microbial taxonomic diversity compared to the mineral and permafrost soils (Ernakovich, 2014).

Rare microorganisms impart important C-cycling functions on the traits of the microbial community in permafrost. None of the 27 permafrost samples incubated at 1°C degraded all three analytical replicates of the 31 substrates, resulting in notable variability among analytical replicates in the assay. In contrast, the organic active layer had a more consistent response

among replicates; 51% of samples had the same response in all three replicates at 1 °C. Only at 20 °C did the permafrost samples achieve the same level of variability (52-67%) as the organic active layer. This suggests that permafrost microorganisms able to function at low temperatures were more easily excluded from the assay due to the dilution. Soils have high spatial heterogeneity, and the resultant micro-habitats can foster different microbial communities (Vos et al., 2013). These results suggest that the permafrost is comprised of more rare species, which could be a result of greater spatial heterogeneity in the permafrost versus the organic soils from the active layer. Alternatively, the functional diversity of the permafrost may have been greater if we had assayed the soils under anoxic conditions, because the permafrost community is partly comprised of strict anaerobes (Rivkina et al., 1998).

4.4. EcoPlate (Biolog) assays mirror changes in community composition after thaw

Although Biolog plates can differentiate between microbial communities from closely related natural systems (Bell et al., 2009; Garland and Mills, 1991; Zak et al., 1994), it has been proposed that its inherent biases affect its applicability as a tool to assess the microbial ecology of a system. One of the criticisms was that it is a culture-based method (Konopka et al., 1998; Preston-Mafham et al., 2002) that induces shifts in the native community. Changes in community composition will occur naturally in permafrost as it thaws (Mackelprang et al., 2011) due to the availability of previously frozen substrates and changes of the metabolic state of microbes, many of which persist in frozen permafrost through maintenance and survival metabolism (Price and Sowers, 2004). Shifts in community structure during growth in liquid media are due to variation in initial cell densities and specific growth rates for the members of the community (Konopka et

al., 1998), as well as competition (Allison, 2005) and mutualism, all of which would be mirrored during *in situ* permafrost thaw.

The physiological state of microbes in an environmental sample can affect their cultivability (Konopka et al., 1998). For example, if microbes are sporulated in a dormant state, they will not be able to respond quickly in the assay and will be left out of the assessment of functional diversity and activity using Biolog plates. We argue that this is relevant to field conditions, where the activity and abundance of members of the microbial community is a reflection of their physiological state in response to the abiotic conditions at any given moment (Lennon and Jones, 2011). After permafrost thaw, drastic shifts in microbial abundance, activity, and diversity can be expected in the transition from slow growth, maintenance, and survival (Brinton et al., 2002; Price and Sowers, 2004) to more rapid metabolism and growth.

A drastic turnover in the active microbial community composition of arctic active layer soils and alpine soils occurs between frozen and thawed conditions during seasonal transitions (Buckeridge et al., 2013; McMahon et al., 2011; Schmidt et al., 2007). Many possible mechanisms are likely at play in this turnover of microbial biomass, including temperature niche selection (Jefferies et al., 2010). Similar shifts in permafrost community composition after thaw should also be expected. In this study, we have captured the effect of temperature on the traits and functional diversity of the microbial communities that emerge along with the shift in community composition, thus justifying the use of a tool that allows the community to shift. We assessed the bacterial and Archaeal component of the microbial community because they are a larger part of the community than fungi in permafrost (Gilichinsky et al., 1993; Yergeau et al., 2010). However, many fungi in permafrost are cultivable, indicating that they could be a viable part of the microbial community (Ozerskaya et al., 2009). Little is known about the ability of

fungi in permafrost to degrade organic matter under *in situ* conditions, which are often sporulated in permafrost. Because this assay excludes the activity of fungi (Dobranic and Zak, 1999), the total function of the microbial community is not represented in its entirety, and should be considered in future work due to the ability of fungi to degrade chemically recalcitrant organic material.

5. Conclusions

When permafrost temperatures increase, previously frozen organic C is subject to decomposition. However, the limited capacity of the permafrost bacteria and Archaea community to degrade even simple substrates at low temperatures suggests that it is comprised of slow growing, conservative microbes adapted for persistence under C starvation conditions (Ekschmitt et al., 2005; Morita, 1997) and not for high activity at low temperatures. This could result in slower decomposition rates than might otherwise be expected from extrapolating Q_{10} 's of organic active layer microbial community growth rates to predictions of permafrost activity. There may be a delay of at least a few growing seasons after permafrost thaw to allow for the microbial community composition to shift and increase its ability to degrade organic matter at a higher rate. As temperatures rise, the permafrost community will become more active and able to degrade a larger diversity of substrates. Because the functional diversity and growth rates of organic active layer are much greater than in permafrost soil, decomposition rates in warming active layer soils will likely rapidly increase with climate change induced increases in soil temperature.

6. Figure Captions

6.1. **Figure 1.** Boxplots of (a) the estimated growth rate, λ , of microbial communities from different soil depths at different incubation temperatures, and (b) the temperature sensitivity, Q_{10} , of the growth rate, λ , for microbial communities from the different depths. The number of estimated values in each boxplot is shown. The numbers vary due to differences in the number of substrates used at the different temperatures. For (a) and (b), center lines show the medians, and box limits indicate the 25th and 75th percentiles as determined by R software. Whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by dots. Crosses represent sample means and grey bars indicate 95% confidence intervals of the means. Different letters indicate differences between the means ($p < 0.05$).

6.2. **Figure 2.** The db-RDA of λ on substrate classes at each temperature reveals differences in substrate-specific growth rates for the different soil depths at each temperature. Ellipses are the 95% confidence intervals of the microbial substrate-specific growth rates for each soil depth (factor class). The axes indicate the percent variance explained.

6.3. **Figure 3.** The db-RDA of λ on substrate classes reveals the differences in substrate-specific growth rates by temperature for each of the different soil depths. Ellipses are the 95% confidence interval of the growth rates at each temperature. The axes indicate the percent variance explained. The location of the name of the substrate classes indicates the direction of axis loading (which corresponds to the r^2 and p-value in Table 4).

6.4. **Figure 4.** Substrate-use richness, as defined by the number of substrates a microbial community is able to use (Zak et al., 1994). The maximum number of substrates possible is 31.

6.5. **Figure 5.** The db-RDA of the temperature dependence of substrate preference for each soil depth. Ellipses are the 95% confidence intervals of the number of substrates used at each temperature. The axes indicate the percent variance explained. The location of the name of the substrate classes indicates the direction of axis loading (which corresponds to the r^2 and p-value in Table 5).

541 **7. Acknowledgements**

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Table 1. Characteristics of the soils used in this study. GWC= gravimetric water content; dws= dry weight soil; C= carbon; N= nitrogen. Error is represented as one standard error of the mean.

Soil Depth	Depth, cm	GWC, g H ₂ O/g dws	C, mg C/g dws	N, mg N/g dws	C:N
Organic Active Layer	2.00 ± 0.00	3.33 ± 0.58	399.45 ± 13.91	15.31 ± 0.82	26.67 ± 1.86
Mineral Active Layer	9.45 ± 0.41	1.13 ± 0.73	94.77 ± 27.42	5.45 ± 1.16	16.46 ± 0.90
Permafrost 0-5 cm	31.74 ± 0.75	1.47 ± 0.11	101.68 ± 9.59	5.55 ± 0.44	18.18 ± 0.45
Permafrost 11-15 cm	41.18 ± 1.04	1.76 ± 0.16	108.00 ± 9.89	5.78 ± 0.44	18.86 ± 0.63
Permafrost 21-25 cm	50.40 ± 1.26	1.68 ± 0.25	71.02 ± 7.78	4.26 ± 0.44	17.03 ± 0.58

Table 2

Table 2. Substrates in the EcoPlate™ (Biolog, Inc.) assay categorized by substrate classes (Zak et al., 1994). Note: We refer to the substrate groups as ‘classes’ rather than ‘guilds’ (Balser and Wixon, 2009). Technically, Glycyl-L-Glutamic acid is a dipeptide and not an amino acid, but we chose to keep the Zak et al. (1994) categories. Similarly, Tween 40 and 80 are not true polymers, however they do require extracellular decomposition. [†] indicates substances present in root exudates (Campbell et al., 1997).

Substrate Class	Substrate Name
amines	Phenylethylamine
	Putrescine
amino acid	Glycyl-L-Glutamic Acid
	L-Arginine [†]
	L-Asparagine [†]
	L-Phenylalanine [†]
	L-Serine [†]
	L-Threonine [†]
carbohydrate	D-Cellobiose
	D-Mannitol
	D-Xylose [†]
	i-Erythritol
	N-Acetyl-D-Glucosamine
	α-D-Lactose
	β-Methyl-D-Glucoside
Carboxylic acid	2-Hydroxy benzoic acid [†]
	4-Hydroxy benzoic acid
	D-Galactonic acid γ-lactone

D-Galacturonic Acid

D-Glucosaminic acid

D-Malic acid ^T

Itaconic Acid

Pyruvic acid methyl ester

α -Ketobutyric Acid

γ -Hydroxybutyric Acid

Phosphorylated chemicals

D,L- α -Glycerol Phosphate

Glucose-1-phosphate

Polymers

Glycogen

Tween 40

Tween 80

α -Cyclodextrin

Table 3. R^2 and p-values for linear regressions between growth on substrate classes and CAP 1 and 2 from db-RDA in Fig. 2, where substrate-specific growth rate was the response variable and soil depth was the factor class.

Effect	1 °C		10 °C		20 °C	
	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2
amines/amides	0.56***	ns	0.69***	ns	0.66***	0.09 ⁺
amino acids	0.18**	0.33**	0.85***	ns	0.71***	ns
carbohydrates	ns	ns	0.71***	ns	0.62***	ns
carboxylic acids	0.36***	0.25**	0.76***	ns	0.75***	ns
miscellaneous	0.37***	ns	0.54***	ns	0.66***	ns
polymers	0.46***	ns	0.26**	ns	0.34***	0.10*

⁺p<0.1

*p<0.05

**p<0.01

***p<0.0001

ns, not significant

Table 4. R^2 and p-values for linear regressions between growth on substrate classes and CAP 1 and 2 from db-RDA in Fig. 3, where r was the response variable and incubation temperature was the factor class.

Effect	Organic		Mineral		Permafrost 0-5 cm		Permafrost 11-15 cm		Permafrost 21-25 cm	
	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2
amines/amides	0.83***	ns	0.67***	0.24*	0.49***	0.21*	0.55***	0.19*	0.54***	ns
amino acids	0.87***	ns	0.64**	0.27*	0.60***	0.19*	0.45**	0.25**	0.27**	0.45**
carbohydrates	0.70***	0.18*	0.67***	ns	0.41**	0.30**	0.52***	ns	0.39**	0.22*
carboxylic acids	0.77***	ns	0.72***	ns	0.77***	ns	0.50***	ns	0.61***	ns
miscellaneous	0.56***	0.30**	0.41**	ns	0.25**	ns	ns	0.25**	ns	ns
polymer	0.48**	ns	ns	0.26*	0.56***	ns	0.60***	ns	0.36**	ns

*p<0.05

**p<0.01

***p<0.0001

ns, not significant

Table 5

Table 5. R² and p-values for linear regressions between growth on substrate classes and CAP 1 and 2 from db-RDA shown in Fig. 5, where substrate-use richness was the response variable and incubation temperature was the factor class.

Effect	Organic		Mineral		Permafrost 0-5		Permafrost 11-15		Permafrost 21-25	
					cm		cm		cm	
	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2	CAP 1	CAP 2
amines/amides	0.63***	ns	0.40**	0.40**	0.87***	ns	0.80***	ns	0.86***	0.15 ⁺
amino acids	0.84***	ns	0.67***	0.37**	0.91***	ns	0.90***	ns	0.83***	0.20 ⁺
carbohydrates	0.74***	ns	0.73***	0.19 ⁺	0.85***	ns	0.94***	ns	0.87***	0.19*
carboxylic acids	0.84***	0.36**	0.77***	0.21 ⁺	0.74***	0.13 ⁺	0.80***	ns	0.70***	0.37**
miscellaneous	0.54**	0.30**	0.32*	ns	0.28**	0.16*	0.21*	0.12 ⁺	ns	ns
polymer	0.59***	ns	ns	0.64**	0.65***	0.11 ⁺	0.79***	ns	0.54***	0.40**

⁺p<0.1

*p<0.05

**p<0.01

***p<0.0001

ns, not significant

Table 6

Table 6. The percentage of samples within a soil depth category that degrade the substrates within a class at each temperature. The number of substrates in each class is noted in the parentheses next to the substrate class names and the number of samples included in each soil depth is noted in the parentheses next to the soil depth. Carbohydrates are called ‘carbs’, carboxylic acids are called ‘carbox acid’, and phosphorylated chemicals are called ‘phos chem’.

		Substrate Class					
Soil Depth		amides (2)	amino acids (6)	carbs (7)	carbox acid (10)	polymers (4)	phos chem (2)
1 °C	Organic (8)	63	69	76	66	73	52
	Mineral (6)	19	6	6	3	17	6
	Permafrost 0-5 cm (9)	6	2	4	4	2	2
	Permafrost 10-15 cm (9)	4	11	8	5	5	4
	Permafrost 20-25 cm (9)	2	7	4	7	5	0
10 °C	Organic (8)	90	94	92	81	92	65
	Mineral (6)	36	51	42	34	19	17
	Permafrost 0-5 cm (9)	78	77	72	44	47	15
	Permafrost 10-15 cm (9)	69	77	71	54	47	17
	Permafrost 20-25 cm (9)	63	56	54	36	28	0
20 °C	Organic (8)	98	100	96	92	96	88
	Mineral (6)	67	72	52	55	36	31
	Permafrost 0-5 cm (9)	93	89	89	68	61	39
	Permafrost 10-15 cm (9)	76	90	90	69	56	33
	Permafrost 20-25 cm (9)	81	75	76	53	56	20

Figure 1

Figure 2

Figure 3
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Figure 4

Figure 5