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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 and activity at cold temperatures. Rather, 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 increase the functional capabilities of permafrost microbes to decompose the C stored in those soils.

1. Introduction

Previously-frozen stores of organic carbon (C) are subject to decomposition due to Arctic warming and associated permafrost thawing (Hinzman et al., 2005; Osterkamp, 2007; Schuur et al., 2008; Harden et al., 2012). 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

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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 (Rivkina et al., 2000; Santrucková et al., 2003; Ernakovich, 2014), 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 (Rivkina et al., 2000; Bakermans et al., 2003), 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 m^2. 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 5 cm 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 to 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