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A cross-seasonal comparison of active and total bacterial community composition in Arctic tundra soil using bromodeoxyuridine labeling

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ABSTRACT

Arctic soil microorganisms remain active at ecologically relevant rates in frozen soils. We used bromodeoxyuridine (BrdU) labeling and terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA gene amplicons to examine active bacterial communities in two Alaskan tundra soils collected in summer and winter of 2005. Active community T-RFLP profiles were compared to total community profiles to determine if active bacteria were a subset of the total community. In shrub soils, active bacteria communities differed in composition between summer and winter, and winter-active bacterial taxa were not detected in the total community, suggesting that they are likely rare within the overall community. In contrast, tussock tundra soil contained more bacterial taxa that were active in both summer and winter and also represented a large portion of the total community. Using *in silico* digest of a sequence library from this site, we attempted to identify the dominant organisms in our samples. Our previous research suggested that the total microbial community was stable throughout the year, but this new study suggests that the active community is more dynamic seasonally. In general, only a subset of the total community was growing at a given time. This temporal niche partitioning may contribute to the high diversity of microbial communities in soils. Understanding which taxa contribute to microbial function under different conditions is the next frontier in microbial ecology and linking composition to biogeochemical cycling.

1. Introduction

In Arctic tundra soils, microorganisms remain active even as temperatures drop below freezing, with respiration continuing at substantial rates down to −10 °C (Mikan et al., 2002). However, CO_2 production does not necessarily indicate that microbes are growing. We have previously shown that at −2 °C, both bacteria and fungi produce new phospholipid fatty acids (PLFAs) and that bacteria synthesize DNA, strong indicators for both growth and activity (McMahon et al., 2009). However, we do not yet know which organisms are growing during the long cold winters in arctic tundra soil or how that growth relates to overall community composition and function.

Microbial activity in arctic tundra is not simply slower in winter—it is different. For example, in Alaskan tussock tundra soils, microbes mineralize N in winter but immobilize it in summer (Weintraub and Schimel, 2005). Such seasonal changes in microbial activity could occur through physiological shifts within individual organisms, changes in substrate availability, or shifts in the composition of the active microbial community. Physiological shifts within individuals would result in an apparently stable active community, i.e. the same organisms would be active under different conditions. Previous work suggests this might be the case—the composition of Arctic tundra microbial communities appears relatively stable across seasons at coarse taxonomic resolution; for example, the balance of *Proteobacteria* vs. *Acidobacteria* in clone libraries doesn't change substantially (Wallenstein et al., 2007). However, it is possible that the presence of a large but inactive pool of organisms masks shifts in the composition of the active community when community analyses are based on total DNA. It is also possible that important community shifts occur at fine taxonomic resolution, shifts that might still affect the functioning of the community. Distinguishing between these possibilities requires evaluating whether the active bacterial community shifts seasonally and whether bacteria that grow in frozen soils are different from those that grow during the summer and from those that comprise the total microbial community.

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Examining the active portion of a bacterial community is challenging because it requires a technique that identifies recently synthesized cells, usually through the incorporation of a label. Many studies have used the stable isotope ^{13}C to examine production of membrane phospholipid fatty acids (PLFA-SIP) and DNA (DNA-SIP). PLFA analysis relies on the fact that different functional groups of microorganisms produce different PLFAs, which can thus be considered biomarkers for those groups (Cavigelli et al., 1995). Unfortunately, few, if any of those biomarkers are truly unique and the groups are very broad (e.g. Gram(+) bacteria) (Zelles, 1999). This level of resolution can answer questions of substrate use in broad groups (Abraham et al., 1998; Malosso et al., 2004; McMahon et al., 2005; Zak and Kling, 2006). However, some questions require the greater taxonomic resolution provided by DNA-based methods, motivating the use of DNA-SIP. After incubation with ^{13}C-labeled substrates, newly synthesized "heavy" DNA is separated by density-gradient ultracentrifugation. This method has worked very well for organisms that use specific compounds such as methane (e.g. Cebron et al., 2007; Han et al., 2009; Hutchens et al., 2004; Moussard et al., 2009), as DNA separates into fairly discreet bands during centrifugation. However, studies examining the entire bacterial community using universal substrates are still rare, due to the technical challenge of the smear of DNA produced with this approach (reviewed by Dumont and Murrell, 2005; Friedrich, 2006; Uhlik et al., 2009).

An alternative DNA probing method that involves labeling DNA with a thymidine analog, 5-bromo-3-deoxyuridine (BrdU) avoids the problems of both PLFA- and DNA-SIP (Allison et al., 2007; Artursson et al., 2005; Artursson and Jansson, 2003). In this method, BrdU is added to an environmental sample and after incubation, DNA is extracted and BrdU-labeled DNA is separated from non-labeled DNA using immunocapture (Borneman, 1999; Urbach et al., 1999). Labeled DNA, derived only from actively growing organisms, can then analyzed by standard molecular methods including T-RFLP and qPCR (Allison et al., 2007; Artursson et al., 2005; Edlund and Jansson, 2008). In BrdU-labeling microorganisms do not have to metabolize the added substrate to become labeled; if they are growing on any substrate(s) they may still assimilate BrdU, while BrdU does not appear to act as a growth substrate on its own (D. Roux-Michollet, pers. comm.). Second, BrdU-labeling may be advantageous when the active organisms are numerically rare. Because PCR is biased towards the most numerous sequences in a sample, rare sequences may be difficult to detect. With immunocapture, the interference of highly abundant, but inactive organisms is removed. Thus, when active organisms are minor members of the community, their sequences may not be detected in total community analysis but may be detected in active community analysis. One potential disadvantage of this technique is that all taxa may not incorporate the label with equal efficiency. However, a recent study found that at least 43 of 58 major bacterial phyla incorporated BrdU into their DNA, including the 14 major lineages found in soil microbial communities (E. Bodie, pers. comm.).

Our primary objective was to test the hypothesis that in both tussock and shrub soils, different taxa would be active in the winter and summer. A secondary objective was to test the hypothesis that winter-active bacteria would be minor members of the overall total soil community, whereas a large proportion of the total soil community would be active in the summer. To test these hypotheses, we added BrdU to soils from two tundra communities (tussock and shrub tundra) under ambient winter and summer conditions and assessed which bacteria assimilated BrdU into their DNA—i.e. which were active. We used non-metric multidimensional scaling (NMS) to analyze T-RFLP fingerprints from active and total bacterial communities.

2. Materials and methods

2.1. Site & soil description

Samples for this study were collected from shrub and tussock tundra near Toolik Field Station (68°38′N, 149°39′W) in the North Slope region of Alaska. These tundra types are dominated by different vegetation, which in turn drives different subsurface conditions including soil structure, C pools & N availability. Shrub tundra, which is composed primarily of dwarf birch (*Betula nana* L.) and willow species (*Salix* sp.), contains a small, highly labile C pool likely derived primarily from root exudates with the remainder of the soil C inputs coming from lignin- and cellulose-rich woody detritus. Shrub tundra soil is classified as a loamy-skeletal, mixed active gelic Aquaturbel. In contrast to shrub tundra, tussock tundra soil receives much of its C in the form of dead sedge roots from the *Eriophorum vaginatum* L. plants that form tussocks. Tussocks contain roots that may be decades old, indicating that they are only moderately decomposable by microbes. Tussock soil is classified as a loamy, mixed, Typic Aquaturbel.

2.2. Sample collection

Soil was collected in May, June, August and November 2005. These sampling dates represented late winter, early summer, late summer and early winter, respectively. When soil was frozen in winter, snow was swept away by hand and a SIPRE ice auger (Jon's Machine Shop, Fairbanks, AK) was used to drill soil cores to a depth of at least 20 cm. Samples were collected from the organic soil layers in tussock and shrub tundra. Thawed early summer and late summer samples were cut by hand using a serrated knife. Eight field replicates were collected from an area approx. 100 m^2 in each tundra type; locations were chosen quasi-randomly by throwing a spade with eyes closed. However, additional criteria had to be met to ensure relatively uniform samples. In the case of tussocks, the closest tussock to the spade that was large enough to permit coring (at least 30 cm diameter) was selected. In all cases, if the auger hit a rock, another sample location was chosen.

Logistics forced a sampling regime that captured the end of one winter and the beginning of the following winter, rather than the early & late stages of the same winter. As a result, observed differences may have occurred either because of changes that occur through the winter, or because the two winters were different. Such differences could arise from different fall conditions and the rate of soil freezing. In fall 2004, soils took 35 days to drop from 1 °C to −1 °C, spending 24 days at 0 °C, whereas in fall 2005 soils froze more rapidly, taking only 17 days to drop to −1 °C and spending only 12 days at 0 °C.

2.3. Sample handling & processing

To ensure that frozen soils never thawed, samples were processed in the field at sub-zero temperatures, shipped on dry ice, and experimented on in a walk-in freezer. Obvious plant material was removed from the tops of cores using a power saw. Samples were then broken up coarsely by hand (with a hammer) and then ground further in a stainless steel Waring blender. The blender was brushed out between replicate soil samples and washed between soil types to minimize cross-contamination. This aggressive processing was required to produce the granular soil required for homogeneous substrate addition. To minimize variation due to processing, thawed samples from early summer and late summer were treated similarly to the cores from winter: a knife was used to cut most living plant material off and samples were then pulsed through the blender to homogenize them. A single soil core contained too little material to