Permafrost Meta-Omics and Climate Change

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Abstract

Permanently frozen soil, or permafrost, covers a large portion of the Earth's
terrestrial surface and represents a unique environment for cold-adapted
microorganisms. As permafrost thaws, previously protected organic mat-
ter becomes available for microbial degradation. Microbes that decompose
soil carbon produce carbon dioxide and other greenhouse gases, contribut-
ing substantially to climate change. Next-generation sequencing and other
-omics technologies offer opportunities to discover the mechanisms by which
microbial communities regulate the loss of carbon and the emission of green-
house gases from thawing permafrost regions. Analysis of nucleic acids and
proteins taken directly from permafrost-associated soils has provided new
insights into microbial communities and their functions in Arctic environ-
ments that are increasingly impacted by climate change. In this article we
review current information from various molecular -omics studies on per-
mafrost microbial ecology and explore the relevance of these insights to our
current understanding of the dynamics of permafrost loss due to climate
change.

1. INTRODUCTION

Permafrost (i.e., soil that has been frozen for at least two consecutive years) represents a habitat for microbial life at subzero temperatures (Gilichinsky et al. 2008). Approximately one quarter of Earth's surface is underlain by permafrost, which contains 25–50% of the total global soil carbon pool (Schuur et al. 2008, Tarnocai et al. 2009). Reduced microbial activity and frozen conditions largely protect this carbon from microbial decomposition, but climate change is threatening to induce large-scale permafrost thaw, thus exposing the carbon to degradation. The resulting emissions of greenhouse gases (GHGs) could produce a positive-feedback loop and significantly amplify the effects of global warming. Increasing temperatures at high latitudes, changes in precipitation patterns, and frequent fire events have already initiated widespread permafrost degradation (Schuur et al. 2015).

In addition to the increasing microbial community activity in thawed and thawing permafrost, significant microbial activity can be found within frozen permafrost itself. Despite oligotrophic conditions, freezing temperatures, low water availability, high salinity, and background radiation, viable microbes have been detected in permafrost that has been frozen for thousands to millions of years (Gilichinsky et al. 2008; Knowlton et al. 2013; Panikov 2009; Rivkina et al. 1998, 2000; Rodrigues et al. 2008; Waldrop et al. 2010; Zhang et al. 2013a). Although there is often less microbial biomass and diversity in permafrost than in overlying active layer soils, which are exposed to seasonal freeze-thaw cycles, several studies show that a variety of microbial phyla reside and are active in permafrost (Hultman et al. 2015, Jansson & Taş 2014, Rivkina et al. 2000). In this article, we review the current state of knowledge about microbial ecology both within permafrost and in the soil layers activated as permafrost thaws, with an emphasis on the use of modern high-throughput sequencing technologies to understand permafrost-associated microbial communities and their response to climate change.

2. VULNERABILITY OF PERMAFROST TO CLIMATE CHANGE

2.1. Permafrost Thaw and Greenhouse Gas Emissions

In the Arctic, surface temperatures have risen 0.6°C per decade over the last 30 years (IPCC 2013), faster than in any other region on Earth and over twice as fast as the increase in global average surface temperature. The impact of these rising temperatures on the fate of carbon in Arctic ecosystems is thus a serious concern. In the Arctic, permafrost soils contain an enormous reservoir of carbon, with estimates of 885–1,185 Pg carbon to a depth of 3 m (Schuur et al. 2015). On the one hand, increasing temperatures are predicted to stimulate plant productivity, which could enhance the capacity of Arctic soils to act as a carbon sink (Friend et al. 2014). On the other hand, a warmer climate and permafrost thaw are expected to lead to carbon loss through microbial decomposition, resulting in Arctic soils becoming a carbon source (McGuire et al. 2009). The degradation of newly available soil organic matter (SOM) produces primarily carbon dioxide (CO_2) and methane (CH_4). Many Arctic regions host permafrost that is poised just below the freezing point of water (Jafarov et al. 2012). Consequently, the microbial response to permafrost thaw, and the resulting GHG emissions, has the potential to be rapid and dramatic.

Arctic terrestrial ecosystems have been deemed responsible for up to 60% of the historical global net land-based sink for atmospheric CO_2 (Hayes et al. 2011). However, research in the past decade suggests that CO_2 uptake in this region may be weakening. Climate-carbon models have forecast increases in plant productivity and CO_2 uptake coupled with rising temperature (Qian et al. 2010). However, CO_2 release from warming-induced decomposition and combustion

in boreal forest fires may be outpacing increased CO_2 uptake due to rising temperatures. The Arctic net carbon budget depends on both the winter season and the relatively short Arctic summer (Euskirchen et al. 2012). CO_2 effluxes have been observed to persist until early December, well into the cold season (Oechel et al. 2014), suggesting that long-term warming trends may be lengthening the seasonal window of activity for microbial decomposition processes, allowing cold season soil carbon losses—primarily from the active layer—to reverse growing season plant uptake.

The extent of active layer thickness (ALT), which is often used as a proxy for permafrost thaw, has increased across the Arctic (Hayes et al. 2014). On the basis of data collected from 1970 to 2006, model simulation experiments estimate an increase in maximum ALT of 6.8 cm annually. This ALT trend has been confirmed in North America (Smith et al. 2010), Russia (Romanovsky et al. 2010), and Scandinavia (Åkerman & Johansson 2008). Increasing ALT will expose previously frozen SOM to decomposition (Zhang et al. 2013b). Given the large size of this reservoir, even small increases in GHG release could substantially augment natural emissions (Montzka et al. 2011).

Besides steadily increasing ALT, changes in the Arctic ecology are influenced by rising soil temperatures, retreating glaciers, and alterations in hydrological conditions (Hinzman et al. 2013). For example, surveys throughout the Arctic have shown high coastal erosion rates of 1.15 and 1.12 m year^{-1} in Alaska and Canada, respectively, which has introduced large amounts of long-frozen permafrost carbon directly into the marine ecosystem (Lantuit et al. 2011).

Permafrost thaw stimulates degradation of both new and old soil carbon stores, releasing carbon that had been stored for hundreds to thousands of years into the atmosphere (Pries & Schuur 2013). Increasing decomposition of previously frozen carbon, combined with the saturation of CO_2 uptake by biological processes in coming decades (Canadell et al. 2007), would result in net positive contributions to global atmospheric GHG inventories (Pries & Schuur 2013). Recent results indicate that boreal regions of North America previously acted as a net carbon sink, sequestering 37 Tg C year^{-1} from 1960 to 1986, later switching to become a net source of 27 Tg C year^{-1} (Hayes et al. 2011). Long-term incubations of permafrost soils indicate that carbon loss from thawed soils, in the form of GHG production, is highly dependent on soil conditions, particularly water content and the presence of oxygen (Elberling et al. 2013). One effect of permafrost thaw is the increased degradation of archaeological wood that has been preserved for many hundreds of years (Matthiesen et al. 2014).

Several recent studies have examined the impact of different thaw conditions on rates of permafrost carbon degradation and GHG emissions. Partial thaw during the summer caused higher SOM degradation rates due to increased oxygenation and warming (Matthiesen et al. 2014). Comparison of aerobic and anaerobic permafrost incubations from 12 different locations indicated that oxygenated permafrost released 4–10 times as much carbon per gram of soil as unoxygenated permafrost (Lee et al. 2012). However, high ice content in permafrost can lead to saturated conditions upon thaw. Under these circumstances, SOM degradation is likely to occur anaerobically, first by the use of NO_3^-, Fe(III), and SO_4^{2-} as electron acceptors and then by methanogenesis.

The extent of SOM degradation and GHG flux is also dependent on the chemical composition and redox status of the permafrost soil. Chemical analysis of Greenland soil samples showed higher levels of NH_4, but not of NO_3, in permafrost than in the active layer (Elberling et al. 2010). These intact permafrost cores were thawed, drained to field capacity, stored under aerobic conditions for seven days, and subsequently resaturated to detect N_2O fluxes. At initial thaw, N_2O production rates were 0.87 µg N h^{-1} kg^{-1}, increasing to 18 µg N h^{-1} kg^{-1} upon rewetting with original meltwater containing NH_4. However, longer-term and continued N_2O production required external nitrogen input. Further down the redox ladder, the contribution of Fe(III)

and SO_4^{2-}, acting as electron acceptors, to the degradation of permafrost organic matter is not well studied. Fe(III) reduction was shown to support bacterial growth in thawed permafrost in lab-scale incubations when additional organic acids or hydrogen was supplied to donate electrons (Zhang et al. 1999). More recently, Hultman et al. (2015) demonstrated low but detectable Fe(III) reduction capacity in the active layer and permafrost, whereas Fe(III) reduction was not detected in thermokarst bog (thawed permafrost) samples. By contrast, SO_4^{2-} reduction was detected in all three soil types but was 24 times higher in the bog. However, the individual contributions of these biogeochemical processes toward CO_2 fluxes from permafrost are yet to be resolved.

Another factor that is important for GHG production is the soil moisture content during thaw. High moisture content in thawing permafrost can stimulate CH_4 production while suppressing CH_4 oxidation by limiting oxygen availability (Tian et al. 2010). Although total carbon release may be attenuated by anaerobic conditions, emission of highly potent CH_4 may contribute more to global warming than CO_2 emissions would under aerobic conditions (Lee et al. 2012).

Other large-scale changes to the Arctic landscape due to climate change can also impact permafrost. For example, wildfires, which are increasing in the Arctic (Kasischke & Turetsky 2006), have a dramatic impact on soil conditions, increasing the ATL and causing thawing of near-surface permafrost (Johnstone et al. 2010, Nossov et al. 2013). However, the immediate release of carbon into the atmosphere may be offset over the long term by the accumulation of biochar, which is largely unavailable to microbial degradation (Lehmann & Joseph 2009). As another example, deglaciation is expected to expose more permafrost to warming and thawing, which in turn could cause increased GHG production (Behl 2011). Newly deglaciated regions have been found to support methanogenic communities, whereas microbes from older samples oxidize CH_4 (Bárcena et al. 2010).

Activation of permafrost microbes can cause consequences beyond immediate release of GHGs. Heat production from microbial metabolism of organic material can result in a potential positive-feedback mechanism (Khvorostyanov et al. 2008). Hollesen et al. (2015) showed that post-thaw microbial activity in previously frozen soil generates sufficient heat to accelerate carbon decomposition. Microbially generated heat could sustain decomposition over centuries.

In addition to carbon emission through SOM degradation, permafrost thaw can result in abiotic release of GHGs, especially CH_4. Permafrost caps large reservoirs of geological CH_4 originating from fossil hydrocarbon deposits (such as coal beds and natural gas deposits) (Collett & Dallimore 2000). Additionally, some CH_4 is locked in ice-like deposits called clathrates, also known as hydrates. These clathrates are solid crystalline compounds containing a mixture of water and CH_4 that are stable under high pressures and low temperatures (Bhattacharyya et al. 2012). Estimates of total CH_4 trapped in permafrost clathrates range widely between 5 and 400 Pg (Maslin et al. 2010, McGuire et al. 2009). CH_4 clathrate destabilization was likely triggered by large-scale climatic change events in Earth's history (Kennedy et al. 2008, Lunt et al. 2011). Under conditions created by the predicted future climate, thawing permafrost could lead to degassing of CH_4 from this subsurface storage. Anthony et al. (2012) detected CH_4 emissions from 150,000 bubble-induced open holes in lake ices. These seeps were located in lakes along the boundaries of permafrost thaw and in moraines and fjords of retreating glaciers. Anthony et al. detected the release of both ^{14}C-depleted (from old organic matter) and ^{14}C-enriched (from microbial decomposition of younger organic matter) methane. Similarly, in Antarctica, a sizable reservoir of methane clathrate (70–390 Pg) is predicted to exist under the Antarctic ice sheets (Wadham et al. 2012). In high-latitude ocean waters, methanotrophs may play a role in the consumption of methane from clathrate destabilization because of the diffusion of methane in the water (Elliott et al. 2010).

2.2. The Potential for Meta-Omics to Address Key Questions About the Fate of Permafrost

Despite many in situ GHG flux measurements and lab-scale thaw experiments, the net contribution of permafrost microbial communities to GHG emissions is not well understood (Elberling et al. 2004). Carbon decomposition and GHG emission occur through the microbial loop: enzyme production, electron transfer, carbon and nutrient assimilation, growth, and food web interactions. Therefore, methods that provide fundamental new insights into microbial loop functioning promise, when integrated with biogeochemical and hydrological approaches, to address the ultimate challenge of understanding the fate of permafrost carbon, associated GHG emissions, and climatic feedbacks.

Two central questions frame this challenge: (*a*) How much carbon will be decomposed and released into the atmosphere as a consequence of permafrost thaw? (*b*) What fraction of the carbon released will be in the form of CH_4 versus CO_2? The first question constrains overall carbon cycle responses and feedbacks, and it is important because, as reviewed above in Section 2.1, the amount of carbon stored in permafrost is exceptionally large. The second question is important because each ton of CH_4 released into the atmosphere adds as much climate forcing as 34 tons of CO_2 (IPCC 2013); the ratio of CO_2 to CH_4 decomposition will thus be a key determinant of the ultimate magnitude of feedbacks to climate from thawing permafrost.

Another key question is how cycling of nutrients and elements (e.g., nitrogen, iron, and sulfur) together mediates both overall decomposition and the fraction of decomposition resulting in methane production. For example, how do nutrient constraints on decomposition and other factors [such as redox potential and composition of terminal electron acceptors (TEAs)] determine which decomposition reactions are thermodynamically feasible?

In this review, we hypothesize that the new meta-omics technologies that are advancing microbial ecology will also advance our understanding of these questions regarding the vulnerability of permafrost and climate feedbacks. To explore this hypothesis, we first briefly review meta-omics methods (Section 3) and then discuss how their application may increase our biogeochemical understanding of the key questions outlined above (Section 4).

3. MULTI-OMICS METHODS FOR PERMAFROST COMMUNITY ANALYSIS

Although interest in permafrost microorganisms has existed for decades, only over the past several years have we been able to characterize the phylogenetic and functional microbial diversity of permafrost in depth. This is because most permafrost microbes—and most environmental microbes in general—fail to grow under controlled laboratory conditions. Methods that utilize DNA sequencing technologies can bypass the need for culturing through manipulation and analysis of DNA extracted directly from the environment. DNA-based approaches are enabled by advances in sequencing technologies that produce sequence on an unprecedented scale and at a fraction of the cost required for traditional microbiological methods. With ever-increasing affordability, advances in high-throughput sequencing offer new strategies for exploring the microbial universe at a resolution that was previously unimaginable.

Meta-omics investigations of environmental samples can be used to derive information about:

1. community composition and diversity, obtained via amplification and sequencing of a few specifically targeted marker genes (which can serve as a name tag for different organismic lineages or functions) that are extracted from communities of interest [this approach targets a gene coding for 16S ribosomal RNA (rRNA), which is ubiquitous in prokaryotic organisms];

2. total community metabolic (or functional) potential, obtained via sequencing of a large sample of genes drawn from the whole-soil pool of DNA (metagenomic sequencing) and analyzing for suites of genes coding for proteins that potentially catalyze all the metabolic reactions of a community; and

3. total community metabolic (or functional) activity as represented by those genes that have been expressed, identified by either (*a*) sequencing of all RNA that has been transcribed from DNA (metatranscriptomic sequencing) or (*b*) mass-spectrometric surveys of all proteins translated from their associated transcripts (metaproteomics).

3.1. Community Composition via Targeted 16S rRNA Gene Sequencing

The first permafrost studies to use new sequencing platforms investigated microbial community composition through 16S rRNA gene sequencing. 16S rRNA is commonly used as a phylogenetic marker gene because it is remarkably well conserved through billions of years of evolution (Tringe & Hugenholtz 2008). This conservation allows amplification and analysis from bacteria and archaea, revealing the taxonomic distribution and evolutionary relationships among microorganisms. 16S rRNA gene sequencing, developed by the microbiologist Carl Woese, was used to reveal that methanogens were not bacteria but part of a completely new domain of life, the Archaea (Woese & Fox 1977). Application of high-throughput sequencing technologies makes what was once an arduous task into a straightforward analysis, as hundreds to thousands of samples can be sequenced simultaneously (Caporaso et al. 2011). This enables differentiation of the microbial community into taxonomic units, allowing researchers to systematically investigate whether microbial communities have ecological dynamics, whether they follow ecological rules, and whether microbial ecology plays a significant role in mediating or driving biogeochemical function.

3.2. Total Community Metabolic Potential via DNA Sequencing: Metagenomics

Although 16S rRNA gene sequencing is highly informative for defining community membership, it is less suited to determining the functional potential of microbial communities. The range of chemical reactions that microbes are genetically capable of catalyzing defines their metabolic or functional potential, and this can be examined indirectly through the genetic composition of the microbiome. The technique of isolating, sequencing, and analyzing the DNA of microbial communities directly from the environment is termed metagenomics.

Metagenomics can uncover the enormous diversity of biochemical pathways, reveal previously unknown and uncharacterized pathways, and provide biological insights into complex microbial communities (Handelsman et al. 1998, Tyson et al. 2004, Venter et al. 2004). Although metagenomics offers the potential for deep insights into soil processes generally, specific technical hurdles must be overcome when extracting DNA from permafrost soil samples. Preparation of DNA for sequencing is often problematic due to low DNA yield and coprecipitation of enzymatic inhibitors during DNA extraction. Therefore, different approaches have been applied to increase the DNA yield prior to sequencing, including multiple displacement amplification (Yergeau et al. 2010), now disfavored because it significantly biases results, and a more preferred approach, emulsion polymerase chain reaction, which has successfully amplified DNA from low-yield permafrost samples (Hultman et al. 2015, Mackelprang et al. 2011). Commercial kits that require 1 ng or less of input DNA (such as the Nextera XT kit from Illumina) are increasingly available, but they may not perform well on some permafrost samples, likely due to high inhibitor concentrations (R. Mackelprang, unpublished observations).

The introduction and development of next-generation sequencing (NGS) technologies are enabling studies of more complex communities at greater depths. There are now multiple approaches for analyzing metagenomic sequences. Currently available methods are selected based on several factors: diversity and complexity of the microbial communities, sample number, amount of sequence generated, and availability of and access to computational resources.

3.3. Total Community Activity: Other -Omics Strategies

DNA-based metagenomic studies are useful for inventorying the functional potential of microbial communities. However, these studies do not reveal which genes are actively expressed and thus which potential biochemical functions are being realized at a given time. In permafrost, this distinction between metabolic potential (as indicated by genomes) and metabolic activity may be especially important because constant subzero temperatures create excellent preservation conditions and inactive or dead cells (along with their genomes) may remain in the soil for extended periods of time (Dmitriev et al. 2001, Hansen et al. 2007). To better determine which parts of a community's overall functional potential are actually being realized by microbial metabolic activity, metagenomic data can be complemented with other approaches that provide information about gene expression. Metabolic functions are performed through expression of genes that code for proteins, which catalyze biochemical reactions. A multi-omics strategy can combine information about gene composition and expression, protein production, and even metabolites. The first step in expression is transcription, during which DNA is transcribed to produce complementary sequences of RNA. Metatranscriptomics, the direct sequencing of representative samples of all RNA in an environment, is the approach used to determine which genes are expressed.

Ongoing studies have successfully applied metatranscriptomics to permafrost, revealing the metabolic strategies utilized by the active microorganisms in a changing environment (Hultman et al. 2015). However, other attempts at RNA extraction from ancient permafrost, particularly Pleistocene permafrost, have not been successful (R. Mackelprang, N. Taş, and C.S. Jacobsen, unpublished observations), suggesting that if gene expression does occur in these samples, it is low. It appears that metatranscriptomics will be a useful tool for assessing microbial activity in only a subset of permafrost samples, but it may be especially useful in identifying emerging patterns of microbial activity in newly activated soils as permafrost thaws (Coolen & Orsi 2015, Tveit et al. 2015).

The abundance of messenger RNA (mRNA) is not always a predictor of protein activity due to differences in mRNA stability, turnover, posttranscriptional regulation, and translation. Metaproteomics, however, reveals proteins that are actually present in environmental samples and provides a potentially more robust snapshot of microbial metabolism as it actually occurs. A metaproteome is typically generated using a shotgun approach that starts with the digestion of all extracted proteins into peptides, which are analyzed by mass spectrometry (Pan & Banfield 2014). Spectra can be searched against metagenome sequence and/or public databases to identify proteins (Chourey et al. 2010, Nicora et al. 2013).

In the literature, use of a multi-omics-based approach to study permafrost microbial communities is limited. Mondav et al. (2014) combined metagenomics and focused proteomic data to characterize the importance of a dominant methanogen in methane production in thawing permafrost. Tveit et al. (2015) combined metagenomics, metatranscriptomics, and targeted metabolic profiling of permafrost communities to elucidate the temperature dependence of methane metabolism therein. Hultman et al. (2015) were the first to combine all three types of meta-omics information (metagenomics, metatranscriptomics, and metaproteomics) in a single study of thawing permafrost. Substantial strides have been made over the last decade, and we expect to

see an increasing number of studies that apply multi-omics strategies to permafrost microbial communities.

4. APPLICATION OF MULTI-OMICS STRATEGIES TO PERMAFROST ENVIRONMENTS

Multi-omics studies are uncovering the range of microbial community functions in permafrost-associated soils. These studies show that communities may utilize a highly diverse and complex set of biochemical processes involved in carbon processing, organic matter decomposition, methane generation and oxidation, and nitrogen cycling (Hultman et al. 2015, Lipson et al. 2013, Mackelprang et al. 2011, Mondav et al. 2014, Taş et al. 2014, Yergeau et al. 2010) (**Figure 1**).

We return in this section to the two central questions (discussed in Section 2.2) that determine the fate of carbon and the feedback to climate change from thawing permafrost: the amount of carbon that may be released and the fraction that will be released as CH_4. We begin with the methane question, as methane cycling is related in a straightforward manner to a relatively small group of microorganisms.

4.1. Methane Production and Oxidation

Large uncertainties remain in estimates of the proportion of permafrost carbon that will be released as methane in the next 100 years. This uncertainty is manifest in Earth system models that simulate methane cycling, which, having no predictive basis for simulating the fraction of anaerobic decomposition that produces methane, typically prescribe this critical dynamic parameter as a fixed fraction (Wania et al. 2013). In this section, we first review the basics of microbial methane production and consumption and then turn to the question of controls on the fraction of permafrost carbon likely to be metabolized to methane, including a review of the important role of methane oxidation.

Methane production occurs in anaerobic habitats such as wetlands, which constitute the largest natural source of methane emissions to the atmosphere (IPCC 2013). Thawing permafrost wetlands across the Arctic are expected to be an increasingly important methane source as climate change accelerates. This feedback is particularly important in regions where permafrost thaw causes the land surface to collapse (Johansson et al. 2006). Water inundates the land surface and creates carbon-rich anoxic wetlands, an ideal habitat for methane-generating organisms.

Biogenic methane production is carried out solely by methanogens, a specialized polyphyletic group of archaea. Methanotrophs, aerobic methane consumers that are capable of utilizing methane as a sole energy source, belong to the classes Gammaproteobacteria (type I) and Alphaproteobacteria (type II) of the phylum Proteobacteria and to a lesser extent to the phylum Verrucomicrobia (Chistoserdova et al. 2009, Kolb 2009, Op den Camp et al. 2009). Organisms capable of anaerobic oxidation of methane are closely related to methanogenic archaea and are thought to oxidize methane through the reversal of methanogenesis (Knittel & Boetius 2009). Methanogenesis and methanogens in Arctic soils have been studied in permafrost for decades (see, e.g., Khalil & Rasmussen 1989; Kvenvolden & Lorenson 1993; Nozhevnikova et al. 2001; Rasmussen et al. 1993; Rivkina et al. 1998, 2002, 2007), and are closely correlated with vegetation productivity (Updegraff et al. 2001, Whiting & Chanton 1993), which provides the fuel for methanogen metabolism. Application of NGS strategies to permafrost has increased and refined our understanding of these methanogens. Though no comprehensive study exists to explain the soil biogeochemical factors that influence methanogen community structure, distribution, phylogeny,
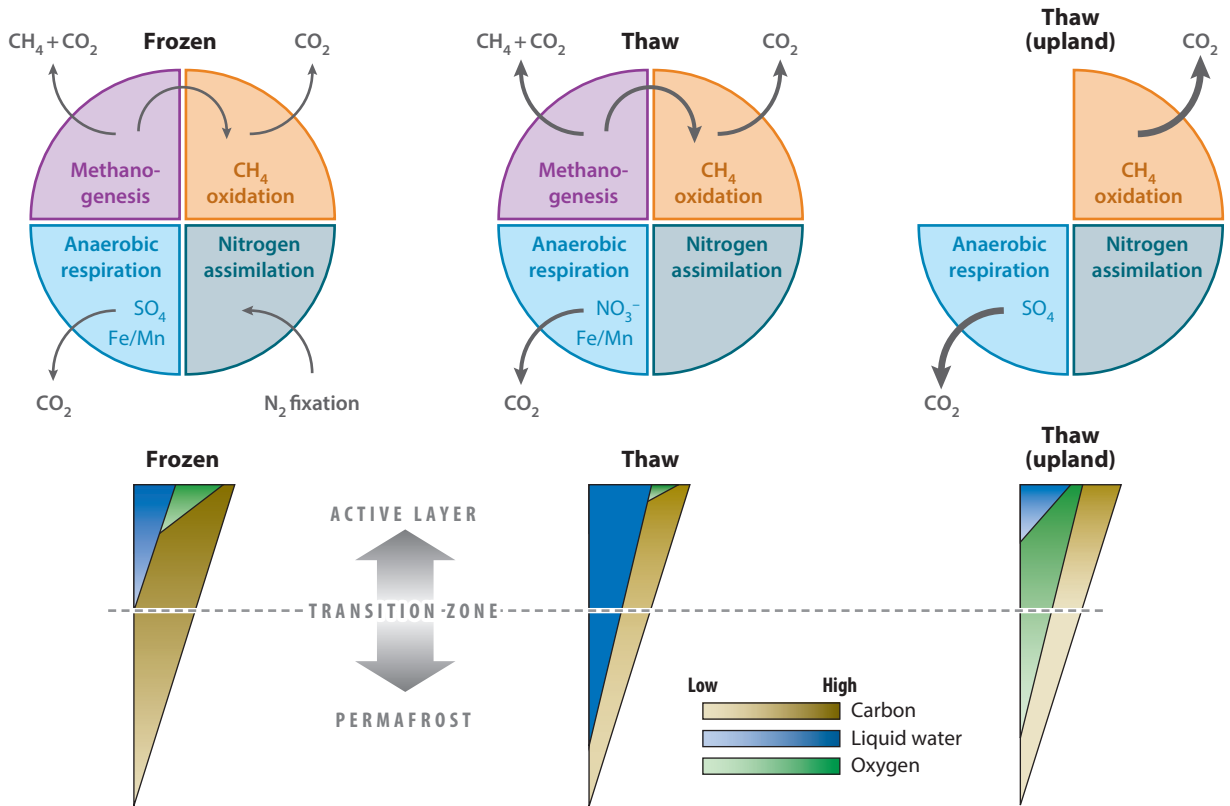
Figure 1

Permafrost thaw impacts soil properties and microbial metabolic potential differently at lowland and highland elevations. The permafrost is overlain by a seasonally thawed active layer that contains high amounts of carbon (*brown*) and liquid water (*dark blue*) but low oxygen (*green*). The bold gray dashed line indicates the surface of the permafrost table, which is sometimes referred to as the transition zone. Depending on the geographical location, permafrost can contain large amounts of organic carbon or be mineral dominated (Schuur et al. 2015). In lowlands (or in areas with poor drainage), thaw causes more liquid water to become available in (former) permafrost layers, and the active layer can also become saturated due to surface inundation. This results in faster depletion of oxygen in the active layer and development of anaerobic conditions. Increases in temperature and availability of liquid water promote microbial processes and result in degradation of permafrost carbon stores. At high elevations permafrost thaw can result in drainage of the soil moisture, carbon, and other nutrients, and may originate from heat generated by wildfires, which further depletes moisture in the active layer. Water drainage and lack of ice can increase soil porosity, allowing oxygen penetration into deep soils. Metagenomic studies conducted under these thaw scenarios suggest strong microbial response to the changing environment. Genes for the utilization of many terminal electron acceptors in respiratory processes—both aerobic and anaerobic—were detected in active layer soils. In comparison, frozen permafrost showed reduced genetic potential for aerobic and anaerobic respiration, nitrogen assimilation, methane production, and methane oxidation. -Omics studies showed that many of these processes are highly expressed and active when permafrost thaws (Hultman et al. 2015). In contrast, at high elevations, oxygenation of deep soils promotes aerobic metabolism (Taş et al. 2014).

and abundance clearly are not uniform between sites (Hultman et al. 2015, Mackelprang et al. 2011, Mondav et al. 2014, Rivkina et al. 2007).

As might be expected, methanogenesis and methanogen abundance are greater in thawed bogs and fens, which emit large amounts of methane, than in low or nonemitting habitats such as intact permafrost (Hultman et al. 2015, Mondav et al. 2014). In bog samples, genes, transcripts, and proteins involved in methanogenesis were abundant in metagenomes, metatranscriptomes, and

metaproteomes coinciding with high levels of bog methane production; however, they were absent in permafrost and overlying active layer soils with no observable methane production (Hultman et al. 2015). Similarly, across a thaw gradient, methanogens and methanogenesis genes were more abundant in fully thawed fens than in partially thawed bogs, and methanogens and methanogenesis were extremely limited at locations with intact permafrost (Mondav et al. 2014).

Although thawed bogs and fens generate the most methane, methanogenesis is known to occur in frozen permafrost, causing methane to accumulate over millennia (Rivkina et al. 2002). Much of the methane does not diffuse upward and is therefore sequestered away from the atmosphere. Thaw could liberate and reintroduce trapped methane into the modern carbon cycle (Rivkina et al. 2007).

Meta-omics tools for microbial analysis may help address an outstanding question of methane biogeochemistry: What regulates the amount of carbon that is released to the atmosphere as methane? Two key factors must be understood to answer this question: the regulation of the ratio of the methanogenesis end products CO_2 and CH_4 in anaerobic environments and the fate of the produced methane as it is transported to the atmosphere through aerobic zones, where it may be subject to oxidation by methanotrophs.

In anaerobic environments, following the depletion of alternate inorganic TEAs (such as nitrate, sulfate, or iron), methanogenesis dominates, producing CH_4 and CO_2 in an expected stoichiometric ratio of 1:1, according to the methanogenesis reaction $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$. This equation represents the net effect of both major methane production pathways, acetoclastic and hydrogenotrophic (Conrad 1999).

However, a longstanding puzzle of biogeochemistry is the cause of the low production of CH_4 relative to CO_2 observed in many wetlands, including permafrost wetlands. Wetland CO_2:CH_4 production ratios are often much greater than one and vary over several orders of magnitude across different wetland systems; particularly high values are seen in soils from sphagnum bogs (Bridgham et al. 2013). This phenomenon is confirmed in controlled anaerobic incubations of thawing permafrost soils, in which alternate inorganic TEAs are known to be low (Hodgkins et al. 2014, 2015). Hypothesized explanations for this puzzle include the presence of organic TEAs (Bridgham et al. 1998), the buildup of fermentation by-products such as acetate (which, intriguingly, is often not consumed by acetoclastic methanogens in bogs) (Duddleston et al. 2002, Hodgkins et al. 2014, Keller & Bridgham 2007), and the presence of phenols or aromatic substances that may have an antibiotic or toxic effect on microbes (Bridgham et al. 2013, Hines et al. 2008, Ye et al. 2012).

All of these hypotheses implicate interactions among microbial communities, vegetation communities, and the associated biogeochemistry. We should thus expect that greater knowledge about how microbial community ecology and metabolism change with respect to the hypothesized correlates of CO_2:CH_4 production in organic matter (including potentially antibiotic compounds and organic TEAs) will lead to fundamental discoveries about the regulation of methane production. Intriguingly, a recent study finds evidence that microbial community composition correlates to CH_4:CO_2 production ratios in anaerobic environments (McCalley et al. 2014).

The fate of the methane produced by methanogens depends ultimately on how much of it is oxidized to CO_2 by aerobic or anaerobic methane oxidation. Aerobic methane oxidation (thought to be much more important than anaerobic oxidation in terrestrial environments) is conducted by methanotrophic bacteria, which may consume 40–70% of gross wetland CH_4 production globally (Megonigal et al. 2003). Methanotrophs thus play an important role in regulating the net amount of CH_4 emitted to the atmosphere at the ecosystem scale (Liebner et al. 2008, Wagner 2008). Though directly accomplished by microbes, methane oxidation in ecosystems depends significantly on microbial interaction with vegetation and hydrology, which influence CH_4 transport from the

soil to the atmosphere (Bridgham et al. 2013, Neumann et al. 2016). Methane that escapes to the atmosphere by ebullition (bubbles) or by passing through the aerenchyma of plants, instead of diffusing through an aerobic zone of soil, bypasses methanotroph communities and is less oxidized. Thus, studies that seek to explain the production and fate of methane will integrate microbial methods with plant ecology and hydrology.

Though vertical soil profiles are frequently depicted as dividing neatly into anaerobic zones overlain by aerobic zones, real soil systems are often more complex, with aerobic microsites found within what would typically be characterized as anaerobic zones, and vice versa. Understanding the pattern of these zones may be critical to determining the net result of methane production and oxidation, as exemplified by a field-based study of a gradient of different soils, from anaerobic flooded soils that produce methane to dry soils dominated by methane oxidation (Jørgensen et al. 2015). Interestingly, effective indicators of this microsite heterogeneity may be given most directly by microbial data. For example, in the thaw experiments described above (Mackelprang et al. 2011), methanotrophs (detected in the metagenomic data) rapidly consumed much of the methane despite anaerobic headspace, likely obtaining the necessary oxygen from permafrost water or aerobic microsites. In another study, methane-containing horizons were found to alternate with non-methane-containing strata (Rivkina et al. 2007), and methanogen abundance can vary dramatically between highly similar samples from the same location. The proportions of methanogens within the total archaeal communities of four directly adjacent cores from the Cold Regions Research and Engineering Laboratory (CRREL) permafrost tunnel were 0.9%, 14.2%, 76.4%, and 15.0% (R. Mackelprang, unpublished data). One possible explanation for this difference in abundance may be the presence of other alternative TEAs such as Fe(III) (Lipson et al. 2013), which inhibits methanogens (Roden & Wetzel 1996).

In some soils, CH_4 flux can be entirely dominated by methane oxidation, and in such cases [e.g., CH_4 consumption in the Canadian High Arctic, reported by Lau et al. (2015)], sites become sinks for atmospheric CH_4. Metagenomic sequencing of samples from the Canadian site revealed that ~0.7% of all sequences were from methanotrophic taxa and that abundance decreased with depth. Transcripts and peptides from the metatranscriptome and metaproteome originated from key genes involved in the CH_4 oxidation pathway, demonstrating that methanotrophic taxa were active.

As thaw occurs, both methane production and consumption dynamics are likely to emerge from interactions among microbial, vegetation, and hydrological components. By integrating new sequence-based microbial information with the vegetation and hydrological information, significant progress will likely be made in understanding the details of this regulation.

4.2. Degradation of Permafrost Carbon

The full range of carbon degradation pathways (both anaerobic and aerobic) encompassed by microbial metabolism is much more complex and interlocking than the few pathways controlling the decomposition of organic matter to methane. Methanogenesis and methanotrophy are carried out by a small set of specific taxa, and thus the presence of these organisms can be used as a proxy for functional potential; by contrast, taxonomy is often a poor predictor of the capacity for carbon metabolism. The number of exploitable substrates is considerable, as is the number of metabolic strategies for carbon processing. Genetic conservation for degradation of carbon polymers is low compared with conservation for methane-related genes, there is broad substrate specificity, and the number of copies of carbon-processing genes in a genome is variable.

A diversity of key genes involved in carbon degradation has been found in Arctic soils. For example, Yergeau et al. (2010) found genes involved in starch, lignocellulose, chitin, and trehalose

degradation in both the active layer and permafrost. A study of the active layers of two Arctic peat locations included an in-depth analysis of the genetic potential to degrade plant polymers, which compose the bulk of peat carbon (Tveit et al. 2012). The researchers identified 73 CAZy glycoside hydrolase families. Oligosaccharide-degrading families were most abundant, followed by debranching enzyme and (endohemi)cellulase families, which were also observed frequently. Fermentation was identified as a common microbial process in an Alaskan permafrost metagenome in which genes involved in the butanol pathway were highly represented (Lipson et al. 2013).

In some cases the abundance of carbon-related genes can be correlated to changes in environmental conditions and parent soil chemistry. Mackelprang et al. (2011) found that genes involved in cellulose degradation and transport, sugar utilization and transport, and chitin degradation changed significantly after transition from a frozen to a thawed state. However, the direction of the change—whether the genes became more or less abundant—was different between replicate cores. A slightly higher level of dissolved organic carbon and higher carbon density were observed in one of the cores, which were correlated with a greater number of carbon-processing genes in that core. Despite the difference in genes involved in the processing of specific carbon substrates, the NADH dehydrogenase complex (part of the electron transport chain) and subunits E1 and E2 of pyruvate dehydrogenase (converts pyruvate to acetyl-Coenzyme A, which can then be used in the TCA cycle) increased in abundance, suggesting an increase in respiratory processes.

In contrast, a metagenomic study investigating soil microbes at the site of the 2004 Boundary Fire in Alaska found few differences in carbon cycle genes between adjacent burned and control sites (Taş et al. 2014). Corroborating the metagenomic data, there were no significant differences in measurements of carbohydrate active enzymes between the former permafrost layers that had been thawed by the fire and the unburned control soil at the same depth. The differences between the results of Mackelprang et al. (2011) and those of Taş et al. (2014) may be due to topology. The Boundary Fire occurred on sloping terrain, resulting in significant drainage of carbon and moisture, whereas Mackelprang et al. (2011) studied a lowland site with poor drainage.

4.3. Nitrogen Cycle

Several studies have investigated different processes involved in the cycling of nitrogen in permafrost. The potential of permafrost microbes to fix N_2 was first observed in a metagenomic study targeting permafrost (Yergeau et al. 2010). A key gene encoding part of the nitrogenase complex (*nifH*), which is responsible for converting N_2 to ammonia, was highly abundant in both the active layer and permafrost. It was also found that nitrogen fixation genes were highly abundant in frozen permafrost, but they decreased significantly after short-term thaw (Mackelprang et al. 2011). By contrast, after thaw, genes involved in nitrate reduction, denitrification, and ammonification increased in abundance. It was hypothesized that the frozen conditions in permafrost sequester biologically available nitrogen, making nitrogen fixation necessary to obtain nitrogenous organic molecules. Upon thaw, other sources of nitrogen become available for assimilation and for use as TEAs. In a study of a naturally thawed permafrost gradient (Hultman et al. 2015), *narG* (encoding nitrate reductase) expression was detected in permafrost. However, denitrification proteins were not detected, which is consistent with low observed denitrification rates. Interestingly, they found that genes encoding both glutamine synthetase (*glnA*) and glutamate synthase (*gltB*, *gltD*, and *gltS*) were transcribed and translated. Taken together, these results suggest that cells were poised to assimilate nitrogen in permafrost.

Permafrost thaw due to wildfire also impacts nitrogen utilization pathways (Taş et al. 2014). In burned subsurface soils, nitrogen assimilation, denitrification, assimilatory nitrate reduction, and dissimilatory nitrate reduction genes were significantly more abundant than in permafrost.

Nitrite and nitrous oxide reductases were less abundant in the burned subsurface. Nitrogen fixation and nitrification genes were present in both burned and intact soils but not significantly different between them. The observation of higher genetic potential for the reduction of nitrogen compounds in permafrost compared to the burn-affected subsurface may be due to preferential use of oxygen as a TEA. At high elevations, water drainage after thaw can increase soil porosity, allowing diffusion of oxygen to deeper soil levels.

4.4. Iron Reduction

Two recent studies indicate that iron reduction is an important process in anoxic permafrost soil. In one study, the high abundance of genes involved in iron reduction suggested that it was the dominant anaerobic pathway in the active layer and permafrost (Lipson et al. 2013). In a study using multi-omics approaches and process measurements, multiple lines of evidence for iron metabolism were found in permafrost (Hultman et al. 2015). In addition, by binning metagenomic sequence data, the authors assembled a novel draft genome with close sequence similarity to *Acidimicrobium ferrooxidans*, which is able to reduce iron under anoxic conditions. The draft genome contained iron transport genes and iron uptake and reduction cytochromes. Using the proteome data they found 58 proteins in permafrost that matched another iron reducer, *Rhodoferax ferrireducens.* These data suggest that iron reduction can serve as a survival strategy for microorganisms in mineral permafrost soils.

4.5. Sulfur Cycle

The extent to which the oxidation and reduction of sulfur compounds occurs in permafrost is not well understood. The potential for sulfur metabolism is clearly present in permafrost microbes. A sulfur-reducing bacterium was isolated from ancient permafrost (Vatsurina et al. 2008), and 16S rRNA gene sequences from sulfate-reducing and sulfur-oxidizing bacteria were found in permafrost (Hansen et al. 2007, Steven et al. 2007). Taxonomic assignment of metagenomic sequences to sulfate reducers and the presence of genes involved in sulfate reduction also revealed that microbes that use sulfate as a TEA are present in permafrost (Chauhan et al. 2014, Lipson et al. 2013).

Studies tracking sulfate reduction potential and rates provide evidence that this process is sensitive to environmental perturbations. Seven years after the 2004 Boundary Fire, genes for sulfite reduction and sulfur oxidation were present at all depths in both fire-impacted and control samples. Genes for sulfate reduction were significantly more abundant in fire-thawed deep soil layers than in intact permafrost (Taş et al. 2014). At the thaw gradient site near Fairbanks, Alaska, sulfate reduction rates were higher in thermokarst bog samples compared to permafrost and active layer soils (Hultman et al. 2015). Transcripts from genes involved in sulfate reduction were also found in the bog. The metatranscriptome-to-metagenome ratios of sulfate reducers were high, suggesting they were active. In permafrost, sulfate reduction rates were negligible, key genes in the pathway were low in abundance, and no sulfate reduction–related transcripts were found in the metatranscriptome. Taken together, these data suggest that redox conditions become more favorable for sulfate reduction after thaw, at least at the sites sampled to date.

4.6. Stress Response

Permafrost microorganisms must survive oligotrophic conditions, freezing temperatures, low water availability, high salinity, and background radiation (Rodrigues & Tiedje 2008). These

conditions affect membrane fluidity, biochemical reaction rates, substrate transport, the conformation of DNA and proteins, and ice crystal formation (Varin et al. 2012). Many organisms employ a suite of strategies to counter these physical and chemical stresses (Jansson & Taş 2014). These adaptations have been studied primarily in species isolated directly from permafrost. For example, the *Psychrobacter arcticus* genome contains a wax ester synthase gene and extracellular polymeric substance (EPS) production genes, which may play roles in membrane fluidity and water retention, respectively (Ayala-Del-Río et al. 2010). *P. arcticus* proteins show reduced use of proline and arginine, which allows increasing flexibility at low temperatures. *Planococcus halocryophilus* also has a suite of genes encoding proteins known to be involved in cold adaptation, such as chaperones, cold-shock proteins, osmotic stress proteins, and temperature-related translation factors (Mykytczuk et al. 2013).

Studies focusing on individual species do not address whether cold-adaptation genes are more abundant in permafrost communities than in temperate communities. The lifestyles of bacteria and archaea involve more exposure to the environment than plants and animals experience. Therefore, they have hard-wired responses (i.e., stress response and adaptation genes) to adapt to a changing environment. Cells respond to abrupt change by modulating the expression of genes that contribute to physiological and metabolic adjustments. This is true of mesophilic organisms as well as psychrophiles. Whether permafrost microbes adapt to cold primarily through regulation of a suite of genes similar to those of mesophilic communities or they have a greater number of more diverse cold-adaptation mechanisms is a question that is being actively investigated.

Hultman et al. (2015) directly addressed the question of protein abundance through comparison of active layer, permafrost, and bog soils and found high numbers of cold-shock proteins in permafrost. Cold-shock proteins were also found in the active layer but with lower abundance. Bog samples had the lowest number of cold-tolerance and other stress-related proteins. In a separate study, wildfire and soil depth had no apparent effect on stress-response genes (Taş et al. 2014).

4.7. From Community Composition to Genome-Enabled Functional Community Ecology

The past decade's revolution in high-throughput low-cost DNA sequencing technologies has provided researchers with a new frontier in genomics research. Access to microbial genomes from environmental samples enables improved understanding of the global distribution of phylogenetic lineages and metabolic potentials. Yet meta-omics tools applied to environmental samples provide a community-centric view of microbial populations. A systems-level understanding of biogeochemical, ecological, and evolutionary processes requires community- and species-level information. The development of analysis methods is paving the way for the reconstruction of large genome fragments and near-complete genomes from short-read metagenome sequences.

4.7.1. Microbial community composition and diversity.
An increasing number of 16S rRNA gene surveys from permafrost and active layer samples are becoming available; they demonstrate that permafrost regions house a highly diverse array of microorganisms and that community composition varies dramatically across the Arctic (Jansson & Taş 2014). In this section, we discuss some important trends. Unlike in some other environments that are considered extreme, the bacterial diversity in permafrost is generally higher than the archaeal diversity. Of particular note is that comparisons between studies reveal substantial differences in bacterial taxonomy across the Arctic. For example, Acidobacteria were highly abundant in Swedish permafrost but were nearly nonexistent in Canadian High Arctic permafrost and some Alaskan permafrost samples. Chloroflexi

represented approximately one quarter of community members in Alaskan samples but were not found in Canadian, Swedish, or Antarctic samples (Jansson & Taş 2014). These differences are not surprising given what we know about other soils, in which community composition varies depending on soil biogeochemistry (Fierer et al. 2012). A survey of 88 temperate soils revealed that soil pH was the best predictor of community composition and diversity (Lauber et al. 2009). Similarly, a survey of nonpermafrost Arctic soils showed that community composition was dependent on pH (Chu et al. 2010). However, these striking differences in bacterial composition and diversity among different permafrost locations might be due to stark differences in permafrost chemistry, mineralogy, and ice content. In addition, other factors not relevant to temperate soils may influence community structure. For example, permafrost age appears to shape taxonomy and diversity (Willerslev et al. 2004). Currently, no cross-sectional studies targeting permafrost have been published. Data are limited to comparisons between studies with different DNA extraction and sequencing methods, some of which have limited data on soil chemistry. We therefore do not know what factors influence microbial community composition in permafrost. Because permafrost samples are more difficult to collect than temperate soil samples, a comprehensive cross-sectional study may require a coordinated community effort. The results of such a study should help determine the environmental drivers of microbial community composition as well as the species composition that is characteristic of permafrost under a given set of environmental conditions.

4.7.2. Genome-enabled functional ecology: assembling genomes from metagenomes. 16S rRNA gene surveys (as discussed in Section 4.7.1) enable microbial taxonomic identification and power the study of microbial ecology, but in such studies each microbial taxon includes only the limited genetic information contained in the 16S rRNA gene marker rather than the rich suite of functional information contained in the whole genome of an organism. Metagenomes contain such information for the whole community, but we would like to integrate our understanding of the community ecology of diverse organisms with the distinct suite of functions contained by each taxon. The large-scale assembly of genomes from metagenomes (Brown et al. 2015, Imelfort et al. 2014) will enable the linking of distinct members of ecological assemblages to their genomically determined metabolic potential. In this section we review the nascent research on assembling population genomes from metagenomes in permafrost systems.

Metagenomic sequences can be assembled into overlapping reads to generate longer sequences known as contigs. Large contigs can be further processed to construct individual genomes. This process—called binning—is done by computationally grouping contigs into genome clusters. Binning results in so-called draft genomes, which are not as accurate or complete as finished genomes generated from the sequencing of microbial isolates. In an initial effort to assemble a genome from a permafrost metagenome, Mackelprang et al. (2011) found evidence in Alaskan lowland organic soil of a novel methanogen that is related to members of Methanomicrobia. In a later study a near-complete genome with substantial sequence similarity was assembled from the metagenome of the Stordalen Mire in Northern Sweden (Mondav et al. 2014). This newly described organism, *Candidatus* Methanoflorens stordalenmirensis, is highly abundant at sites in the initial stages of permafrost thaw and contains genes for hydrogenotrophic methanogenesis that associated proteomic data showed were highly expressed (Mondav et al. 2014). The strong association between the abundance of this phylotype and the presence of methane carbon isotopes indicative of hydrogenotrophic methanogenesis (McCalley et al. 2014), together with the widespread distribution of this phylotype in Sweden, Alaska, and other high-methane-flux locations around the world, suggests that it may be an important mediator of methane-based positive feedback to climate.

Recently, Hultman et al. (2015) binned three methanogen genomes from a thermokarst bog whose sequences were very different from those of the genomes assembled by Mondav et al. (2014)

and Mackelprang et al. (2011). The closest known relatives to the Hultman et al. methanogens were only 70%, 47%, and 43% identical at the DNA sequence level, indicating that they probably represent previously undescribed methanogen genomes. One of the genomes contained metabolic potential for hydrogenotrophic methanogenesis, whereas another genome had 70% sequence identity to an acetoclastic methanogen, suggesting this community utilizes both main pathways for methanogenesis.

In addition to the three methanogen genomes described above, Hultman et al. (2015) assembled 11 draft genomes from active layer, permafrost, and bog metagenomic sequences. Many of these represent novel species. Genomes were assembled from two novel Chloroflexi whose closest genetic relatives were members of the *Anaerolinea* genus. 16S rRNA gene sequences from these taxa were highly abundant in these samples and in other permafrost studies (Liebner et al. 2008, Mackelprang et al. 2011), suggesting these are common permafrost community members. Other draft genomes were from Proteobacteria, Spirochaetes, Gemmatimonadales, and Actinobacteria.

5. QUANTIFYING AND MODELING ARCTIC SOIL MICROBIAL PROCESSES IN THE META-OMICS ERA

The ability to predict responses of permafrost regions to a changing climate is limited. In the Arctic, both numerical and analytical models are used to predict the impacts of permafrost thaw on land surface responses (Riseborough et al. 2008), but most do not consider permafrost and microbial mechanisms in detail. Microbial decomposition processes are often represented as constant variables (i.e., biomass or decomposition rate) based on SOM turnover times (Manzoni & Porporato 2009), which does not reflect the dynamic nature of microbe-environment interactions and microbial activity. The mechanisms of carbon decomposition and GHG production in thawing permafrost operate primarily through microbial actions such as enzyme production, electron transfer, carbon and nutrient assimilation, growth, and food web interactions. Thus, the ultimate fate of permafrost carbon depends critically upon microbial activity, diversity, abundance, and metabolic capacity for degrading and oxidizing carbon. The extent to which global warming will induce GHG emission can be determined only by understanding the microbial communities within the permafrost and how they respond to thaw. Additionally, dynamic responses to permafrost thaw, including changes in soil moisture (Subin et al. 2012), vegetation dynamics (Sitch et al. 2003), and disturbances (Thonicke et al. 2010), have not yet been linked to microbial responses.

Today, applications of NGS techniques and other -omics approaches provide us with the ability to fill in these missing links and to understand microbial functions and responses to climate change. Current global climate models connect several land, ocean, and atmospheric submodels to predict the net flux of carbon from Arctic ecosystems (Friedlingstein et al. 2014). Coupling of many submodels results in complex models with hundreds of parameters and processes defining ecosystem feedbacks. To resolve this complexity, models are often reduced to small sets of parameters that represent fundamental biological and physical processes. As a result, the predictive power of the current climate models relies on representation of relevant processes and availability of accurate and complete data from experimental and field measurements for use as parameters.

Current global climate models must tackle many uncertainties concerning the mechanisms regulating carbon cycling in terrestrial ecosystems and the response of these mechanisms to global change (Koven et al. 2013). For example, the majority of terrestrial carbon cycling models still rely solely on SOM content as the main predictor of decomposition rates. Yet carbon and climate models are increasingly expanding their capabilities to represent complex biogeochemistry in soils (Melton et al. 2013, Meng et al. 2015, Wieder et al. 2013). For example, highly specialized

biogeochemical processes, such as CH_4 cycling, were successfully integrated into the Community Land Model (CLM) (Lawrence et al. 2011, Riley et al. 2011). Through incorporation of all components of the CH_4 cycle (CH_4 production, oxidation, aerenchyma transport, ebullition, aqueous and gaseous diffusion, and fractional inundation), this model predicted an approximately 20% increase in CH_4 emissions over the course of this century. This is lower than previous model estimates that did not consider better drainage caused by thawing permafrost (Riley et al. 2011). However, evaluation of multiple large-scale wetland CH_4 models showed disagreements among the predictions of different modeling approaches (Melton et al. 2013). Although one cause of these disagreements is the lack of appropriate data for model parameterization, another major issue is the missing or insufficient representation of critical microbial processes that contribute to observed GHG fluxes. Inclusion of measures of basic microbial metabolism, such as Michaelis–Menten kinetics or microbial growth efficiency, are already allowing better representation of soil carbon dynamics (Wieder et al. 2013).

Microbial responses to global change can influence the extent to which ecosystems form positive or negative feedbacks on global climate (Allison & Martiny 2008). Therefore, it is critical to generate suitable data and to further develop methodologies that include microbial processes in climate models. At the microbial scale, a number of models have been developed to capture the biological mechanisms involved in soil carbon turnover based on first- and second-order dynamics with direct microbial control over decomposition in temperate climate regions, but such models have yet to be scaled up to the global level (Todd-Brown et al. 2012) and adapted to Arctic ecosystems. Functional traits—characteristics of an organism that are linked with its fitness or performance (McGill et al. 2006)—can be used to understand and predict the community-level responses of microorganisms by taking variations in microbial community composition and metabolic potential into account (Green et al. 2008). The use of trait-based modeling approaches that parameterize specific traits (i.e., transcript abundance, ribosomal copy number, or metabolite content) to represent the diversity and activity of microorganisms across ecosystem gradients has recently generated interest (Allison 2012, Bouskill et al. 2012, Green et al. 2008). Using this approach, researchers have predicted heterotrophic activity (Hall et al. 2008), litter decomposition (Allison 2012), and nitrogen cycling (Bouskill et al. 2012) rates in temperate ecosystems, holding promise for applications to permafrost.

Predicting the metabolic responses in a microbial community from metagenomic data is one of the greatest challenges in microbial ecology today (Myrold et al. 2014). Although metagenomics can be used to generate hypotheses about microbial metabolism and lifestyle, to date no applications connect the metabolic pathways represented in metagenomes to prediction of GHG fluxes. This challenge is mainly due to the enormous diversity and complexity in microbial communities. Moreover, permafrost microbial communities and their functions differ from location to location. Consequently, the intermediates and products of microbial metabolism, the metabolites, constitute a huge spectrum of compounds that cannot be easily detected, quantified, or predicted. A primary goal of current research is to establish what can be inferred from metagenomic data and determine what additional experimental and computational methods are necessary for complete understanding of microbial responses (Hanemaaijer et al. 2015). A potential area for integration of metagenomic data with climate models emerges from synthetic biology and metabolic engineering fields. Genome-scale models provide the necessary elements to translate sequence information from metagenomes into a set of possible metabolic reactions involving substrate utilization and their resulting products (metabolites and fluxes) (Price et al. 2004). An indicator of a response to environmental change on the molecular level is the alteration of fluxes within metabolic networks. For example, a change in substrate availability results in modification of fluxes through metabolic pathways that are related to utilization of the substrate (Balázsi et al. 2011). These metabolic

changes can provide information about cellular physiology and responses to perturbations. Application of these modeling approaches offers the potential to incorporate multi-omics data into predictive microbial community–level models in permafrost environments.

6. CONCLUSIONS

Application of high-throughput sequencing and other -omics technologies is enabling in-depth studies of permafrost microbial communities and is providing high-resolution information about community composition and function in a variety of permafrost locations. However, permafrost is highly heterogeneous, and the impacts of thaw differ dramatically depending on geography, biochemistry, and microbial residents. Future research will benefit greatly from a better understanding of the physiology of microbes in different permafrost soils. Further, we have only begun to understand the microbial mechanisms controlling GHG emissions. Metagenomics must be coupled with enhanced measurements of geochemistry and microbial processes to develop a comprehensive understanding of microbial function and activity in permafrost. Predictive understanding will require information generated by both laboratory-based experiments and long-term in situ studies. In the near future, it is imperative for knowledge generated by metagenomics to be incorporated into climate models to fully integrate microbiology, geochemistry, geophysics, and hydrology for a better representation of Arctic ecosystems.

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