2. Functional Response of Microbial Communities to Permafrost Thaw

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2.1 Introduction

Permafrost soils underlie one quarter of the land surface in the northern hemisphere and contain vast stores of carbon and nitrogen [1]. Because permafrost is frozen by definition and has been for hundreds, tens of thousands, or even millions of years, it has not been thought of as a particularly hospitable environment for microbial activity. Over the past several decades this understanding has shifted, and we now know that microbial communities in permafrost soils are active, in part because the temperature of permafrost is not too extreme. We also know that in the coming century models predict that large areas of permafrost will thaw. Permafrost thaw imposes large changes on the carbon cycle and will potentially releasing 12 to 112 Pg of carbon back to the atmosphere by 2100 [2,3], and much of this could be ancient permafrost carbon [4]. Increased Arctic temperatures have already initiated large carbon losses from thawing permafrost [5-7]. This is coincident with observations of increased greenhouse gas fluxes and lateral DOC fluxes [8,9] that outweigh potential increases in plant productivity [10,11]. Much of this increase is due to the phase change from ice to water, warmer soils, and the presence of labile organics and nitrogen in the newly thawed environment [12,13] flowing through the metabolic engine that is the soil microbial community.

Although we have some understanding of the soil and ecosystem response to permafrost thaw, less is known about the functional response of the microbial community. Functional response can be defined as rates of respiration, methanogenesis, and methane oxidation, temperature and moisture responses, ATP production, and growth post-thaw. At the molecular level, much more detailed functional responses can be elucidated including changes in functional gene abundance, transcript abundance, or protein abundance, as well as changes in the abundance of rRNA gene sequences which describe shifts in archaeal, bacterial or fungal community composition. We review the literature for functional responses in both in lab and *in situ* conditions. At the molecular level, there is a body of work that evaluates community structural (i.e. 16S rRNA gene and ITS2 amplicon sequencing) response to thaw, and other studies that track functional response to surface soil warming. However, there are few that evaluate functional response of permafrost microbes to thaw. Other chapters cover amplicon-based studies and surface soil studies but do not capture the transition that occurs when permafrost microorganisms that are long-adapted to life at sub-zero

temperatures cross the freezing point threshold. We therefore focus the molecular-based component of this chapter on the smaller body of work compares functional-level differences between intact and thawed permafrost. Understanding functional shifts in response to thaw will help elucidate the mechanisms controlling decomposition and greenhouse gas fluxes in this impending global ecosystem transformation.

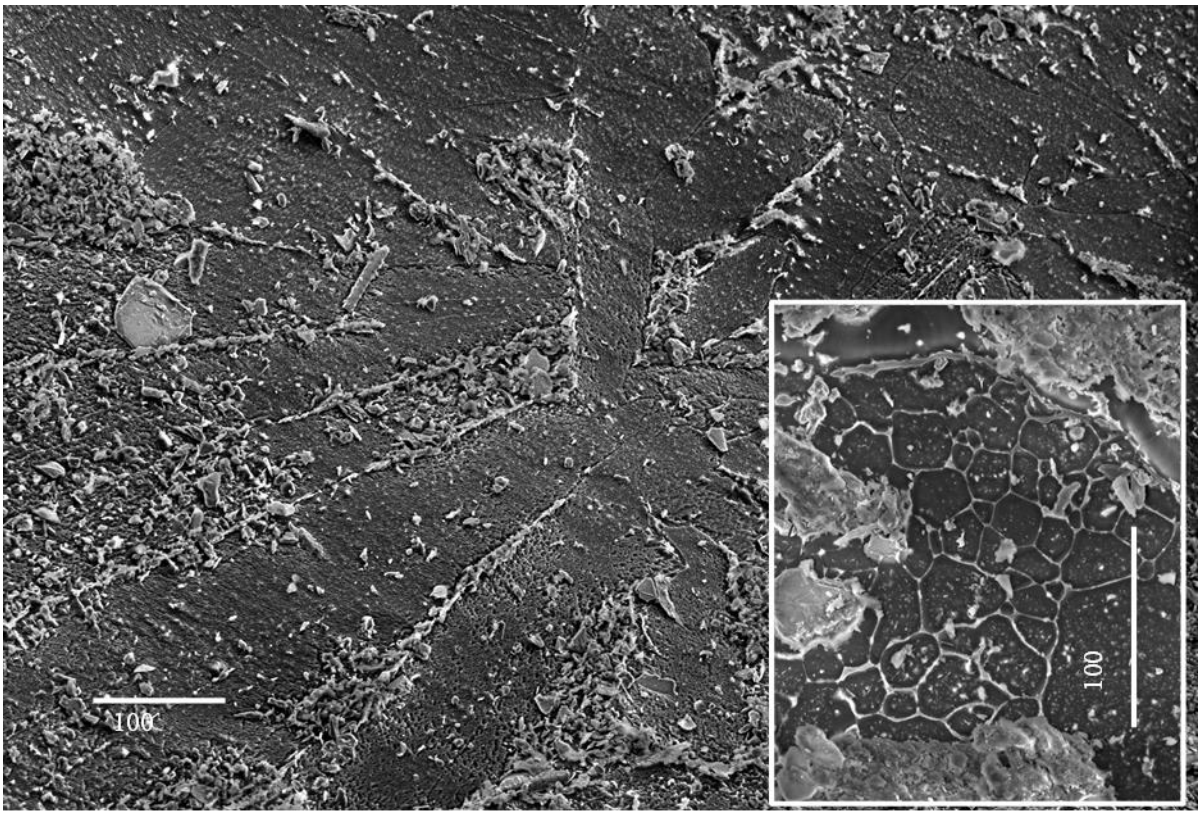

Figure 1. Scanning electron microscopy (SEM) of permafrost samples from Interior Alaska. Embedded image shows fractures within the permafrost where the mix of organics, minerals, and microbes are concentrated as permafrost forms. The dark material is ice. Inset is a plane view of a fracture fill which has peeled away here showing 'patterned ground' of ice in miniature. Microbial activity likely persists along these fractures in areas of presumably high salinity and sediment concentrated during ice formation. (Photo courtesy of Marjorie Schulz and Kristen Manies)

2.2 Activity in Permafrost

Permafrost is a challenging environment for microbial communities. As temperatures drop below freezing, frozen water constricts activity to water films where diffusion of substrates, nutrients, and waste products is limited [14]. Figure 1 shows an image of permafrost soil spanned by cracks that have squeezed out their internal constituents. These constituents are likely a mixture

of organics, clay minerals, and microbial cells. These cracks may also contain higher concentrations of salts than the surrounding ice, lowering the freezing point of water, but increasing osmotic stress for microbial communities. Microbial communities in permafrost face additional challenges, including low thermal energy [15], high viscosity [16], protein denaturation and loss of cell membrane fluidity impacting the transport of nutrients [17].

Microbial metabolic activity does not cease when soil temperatures drop below 0 °C. Evidence for microbial activity in frozen permafrost soils comes through incubation experiments, aspartic acid (Asp) racemization measurements [18], growth assays, microscopy, and immunological approaches [19]. For incubation experiments, samples are placed into airtight jars and changes in headspace CO_2 are monitored over time. Although most studies show microbial activity from 0 to -8 °C or so, some studies have observed microbial activity down to as low as -39 °C [14,20-23]. D-Asp/L-Asp ratios from intact cells in ancient permafrost (up to 1.1 Ma) suggests that they are viable and metabolically active [18]. To measure microbial activity at these very cold temperatures, ^{14}C labeled substrates had to be used [23,24]. Methanotrophic activity, likely a lower detectable rate than respiration, has also been detected below freezing using $^{14}CH_4$ in 1.8-3 million years old permafrost samples from Siberia [25]. Methane fluxes from permafrost, however, seem to show higher spatial variation, more complex dynamics, and surprisingly low fluxes compared to CO_2 from the same soils [26,27]. Several incubation studies show that there is a lag in CH_4 production from frozen soils, that there is low or negligible CH_4 from permafrost, and methane production from permafrost seems lower in more ancient permafrost deposits [26,28-31]. This could indicate a very low abundance of methanogens in permafrost soils, low availability of substrates such as acetate or CO_2 for methanogenesis, physical stress, or other microbes are outcompeting methanogens for available substrates. However, the temperature response of CH_4 may be higher than for CO_2 [28,32], indicating that the low CH_4 flux in permafrost soils may have more to do with limited substrate availability than low biomass numbers.

Respiration rates from frozen permafrost have been shown to be higher than frozen active layer soils incubated at the same temperature [29,33,34] indicating that permafrost microbial communities may have developed unique adaptations that have allowed them to be more active in frozen conditions. One such adaptation may be the higher production of ATP in microorganisms in frozen environments compared to warmer environments [35]. Under frozen conditions, microbes increase ATP production, possibly to maintain reaction rates as temperatures drop. This

pattern could also occur if permafrost contains higher concentrations of water-soluble organic carbon (WSOC). In many cases, permafrost has higher concentrations of WSOC than seasonally thawed soils [9,33], and WSOC is correlated to respiration rates from frozen soils [33]. Several internal molecular mechanisms allow microbial communities in permafrost to be more active than their active layer counterparts under frozen conditions and are described below, but increased lability of organic matter within permafrost may add to this putative adaptive response.

In the field, studies of microbial activity in frozen soils are mostly limited to the comparison of surface soils in summer and winter, not permafrost. But studies show that microbial activity continues through wintertime often under the insulating blanket of snow that keep surface soils just below freezing [36-40]. Dissimilatory iron reduction and methanogenesis are shown to be active part of permafrost microbial metabolism [41] but to our knowledge, there is as yet no *in situ* measurement of microbial activity in permafrost soils. Enrichment cultures and isolates from different permafrost habitats have given us additional knowledge about the types of microorganisms in permafrost soils and their strategies for survival [42]. The majority of permafrost isolates are from Bacterial phyla, *Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* [43-45]. Many of these isolates can form spores [24,46] or have high-GC content that may reduce DNA damage due to cold, high salt and high radiation in permafrost [47]. For example, six bacterial isolates of the genus *Carnobacterium* (*Firmicutes*) obtained from ancient Siberian permafrost (6000-8000 y) grow at 0°C under low pressure (7 mbar) and CO_2-enriched anoxic conditions [48]. Studies of permafrost isolates showed that cells lower their metabolic rates to maintain survival during low temperatures. [^{3}H]leucine and [^{3}H]thymidine incorporation studies done with the Siberian permafrost isolate, *Psychrobacter cryohalolentis*, showed that cells were able to synthesize DNA and proteins (1 to 16 proteins per day) at −15°C [35]. In another study, permafrost isolate Arthrobacter sp. 9-2 was shown to incorporate ^{14}C-ethanol during growth at temperatures as low as -17 °C [34]. Similar incorporation rates were also detected for some other permafrost isolates and enriched co-cultures [49,50], suggesting that even with slow metabolic rates it is possible to compensate for the DNA damage and protein denaturation that occurs under low temperatures [35].

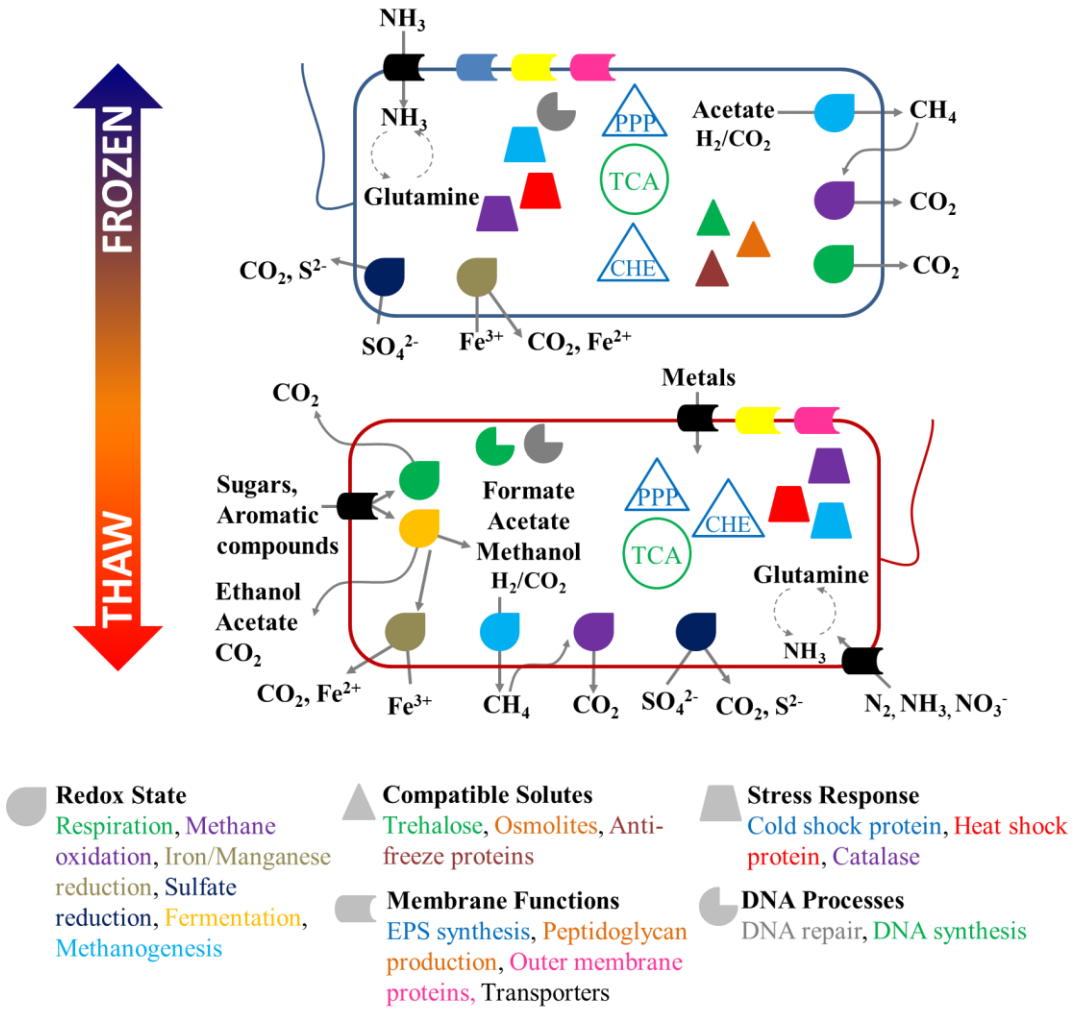

Figure 2. Active microbial processes observed in intact and thawed permafrost. Metabolic models represent wide variety of functions detected in pure culture, metagenome, metatranscriptome and metaproteome studies [41,51-61]. Cell morphology is arbitrarily displayed. Pathways; PPP: Pentose Phosphate Pathway, TCA: Citric Acid Cycle, CHE: Bacterial Chemotaxis

2.3 Response to thaw

Changes in the rate of CO_2 flux as permafrost soils warm and thaw is an important relationship to understand and model in global change research [62]. The Q10 value of microbial respiration, defined as the change in rate per 10 °C change in temperature, above freezing often is around 2.0 [63]. But the Q10 of frozen soils can be much larger, due to extracellular barriers to diffusion and osmotic stress in the frozen state [14,29,37]. Some researchers have found simple exponential increases in activity as soils cross 0°C mark [34,39,64], whereas others have found strong step changes in activity [14]. Given that permafrost soils often will remain just below the

freezing point of water for long periods of time before undergoing phase change, and high Q10s just below freezing indicate rapid changes with minimal warming, it is important that we understand the rates and controls on microbial activity at this critical point.

Functional response to thaw can be examined in greater detail by tracking changes in the abundance and types of genes, transcripts, and proteins (Figure 2). Genes show the potential of microbial populations to carry out certain functions, while transcripts show genes that are actively being expressed, and proteins show transcripts that have been translated and should be the most direct observation of the microbial cell carrying out a process. Each of these types of observations are measurements at different levels of resolution. Changes in functional gene abundance can occur over hours, days, months, and years and may reflect the long-term adjustment of the microbial community to the new environment. Proteins and transcripts can change over minutes to hours and may dominate the initial thaw response because they can change more rapidly relative to the time required for cell division and changes in community structure. Despite the importance of measuring change at all three levels of resolution—genes, transcripts, and proteins—such studies are rare. Permafrost is characterized by low biomass and activity, making RNA and protein extraction difficult. To our knowledge only two studies have successfully obtained RNA and/or protein from intact permafrost, and then only in relatively young permafrost samples [41,53].

Laboratory-based incubation experiments show that the functional thaw response measured at the level of genes [51], transcripts [53], and enzyme activity [65] is robust and rapid—on the order of days to weeks—despite the low incubation temperatures. The phase change of water, which can cause orders of magnitude level changes in activity and metabolic processes, likely explains the quick response. Suggesting a greater reliance on respiratory process for ATP production after thaw, Mackelprang et al (2011) [51] found that genes encoding components of the respiratory electron transport chain and enzymes that link the breakdown of organic carbon to the tricarboxylic acid cycle increased in relative abundance. Indicating a general increase in microbial activity and growth, Coolen and Orsi (2015) [53] found that transcripts involved in translation, biogenesis, and ribosomal structure were more abundant in thawed permafrost compared with frozen samples after short-term (11 days) incubation.

In the field, even slight temperature increases (~1.1°C) can trigger shifts in gene relative abundance [66]. Genes changing the most indicate that soil redox state and energy acquisition drives adaptation to thawed conditions. After five years of warming, methanogenesis, sulfate

reduction, and dissimilatory nitrate reduction genes increased in relative abundance while cytochrome *c* oxidase genes decreased at the active layer/permafrost transition zone [66].

Changes in respiratory functions where microbes break down organic matter and transfer electrons to an inorganic terminal electron acceptor generate large amounts of CO_2 and are particularly relevant to the climate change equation. The genomic potential to use a variety of terminal electron acceptors has been found in pristine permafrost and includes genes involved in denitrification [41,51,55,67-69], sulfur & sulfate reduction [54,55,68], and iron reduction [41,55]. Together, these data suggest that the functional reservoir in permafrost will enable communities to rapidly take advantage of higher redox conditions after thaw.

Permafrost thaw that results in soil saturation creates anaerobic conditions, which necessitates alternative terminal electron acceptors or fermentation for energy production. Nitrate and nitrite may be particularly important in thawed permafrost. Permafrost nitrogen concentration can be high [70] and thaw increases nitrogen availability [12,71-73]. N cycling organisms are abundant in intact permafrost [19] and laboratory and field studies show that nitrogen metabolism genes increase in relative abundance after thaw [51,66]. In a short-term laboratory incubation experiment, Mackelprang et al (2011) [51] observed increases in genes for multiple components of complete denitrification pathway where nitrate is reduced eventually generating N_2. Specifically, the nitrate reductase (*nar*), nitric oxide reductase (*nor*), and nitrous oxide reductase (*nos*) suite of genes increased relative to frozen controls. They also observed increases in respiratory nitrite ammonification (*nrfAC*) genes. At the permafrost-active layer boundary, Johnson et al (2019) [66] observed increases in dissimilatory nitrate reduction to ammonia genes (but not in *norB* or *nosZ* genes) after five years of warming. Tas et al (2014) [52] found that nitrate reduction genes (nar) were more abundant in wildfire-thawed upland permafrost compared with unthawed intact permafrost. However, the other genes in the denitrification pathway decreased after thaw. This suggests that nitrate may not be completely reduced to N_2 and may instead produce N_2O, a potent greenhouse gas. Comparing intact permafrost with a thermokarst bog along a thaw gradient, Hultman et al (2015) [41] found that the relative abundance of nitrate reductase (*narG*) genes and transcripts were greater in permafrost. However, denitrification and nitrate reduction rates were greater in bog samples. This discrepancy is likely due to the use of relative abundance—the high abundance in permafrost indicates the importance of denitrification relative to other processes rather than overall process rates.

One of the primary factors contributing to the functional response to thaw is the availability and quality of carbon substrates [9,74] and it appears that the abundance of genes and transcripts are altered based on carbon chemistry. Coolen and Orsi (2015) [53] found that transcripts for an ABC type sugar transporter, 6-phosphogluconate dehydrogenase (from the pentose phosphate pathway), a sugar isomerase, and pyruvate formate lyase activating enzyme (fermentation) were more abundant in thawed permafrost. They also found an increase in transcripts from a gene encoding aminopeptidase C (involved extracellular protein degradation) suggesting that labile proteins may be a C and N sources after thaw. As evidence for rapid degradation of plant-derived cellulose, Coolen et al (2011) [65] measured extracellular enzyme activities and found that beta-glucosidase activity was highest in thawed permafrost compared to pristine soils. Leucine aminopeptidase was initially active post-thaw but decreased, suggesting that labile proteins or polypeptides were initially available but used rapidly. Mackelprang et al (2011) [51] also found evidence for increased use of labile carbon, although the specific substrates targeted by changing genes differed between samples.

In contrast to permafrost thaw in lowlands, thaw in uplands can result in warmer and drier soils due to the loss of the insulating cover of moss and the loss of permafrost that limits the downward movement of water. In an upland soil seven years after fire, Tas et al (2014) [52] found no overall increases in carbon-processing genes. Few genes (specifically those involved in galactose metabolism) were less abundant in thawed soils. Further analysis of these data showed that while there were few differences at the community level, individual members may adapt to disturbance conditions by altering their genomes [54]. Through assembling metagenomic sequence data and binning contigs into draft genomes, they were able to compare the genomes of two individual community members in pristine and fire-impacted soils. Adaptation occurred by altering 2-3% of their genomes resulting in the loss of genes involved in carbohydrate transport and metabolism, amino acid transport and metabolism, transcriptional regulation, and nutrient transport. The reduction in carbon metabolism related gene might reflect the lower carbon content soils post-thaw.

Much of the variation in CH_4 fluxes from permafrost landscape is due to the moisture regime and plant community present post thaw [75]. In lowlands, carbon-rich anoxic methane producing bogs and fens can form following thaw, ground subsidence, and water inundation [76,77], whereas thaw occurring in uplands can result in drier soils and net CH_4 uptake due to CH_4

oxidation. Compared to intact permafrost and the active layer, newly-forming thermokarst bog and fen features contain high levels of methanogenesis-related genes, transcripts, and proteins that indicate capacities for hydrogenotrophic and acetoclastic methanogenesis as well as the ability to grow on formate, methanol, and methylamine [41,78,79]. It is clear that both hydrogenotrophic and acetoclastic methanogens play integral roles in CH_4 emissions in thawed soils [80], but that the specific type depends on soil chemistry, environmental conditions, and the paleoenvironment during permafrost formation [81]. Some evidence suggests that thaw causes a shift from hydrogenotrophic towards acetoclastic methanogenesis [66,78]. However, other studies have shown a decline in the ratio of acetoclastic to hydrogenotrophic methanogenesis across gradients of permafrost degradation in bogs and palsa peatlands [82,83]. Holm et al (2020) [81] found that acetoclastic methanogens dominated Eemian permafrost (formed under higher temperatures and precipitation) during thaw whereas other Pleistocene and Holocene permafrost samples were dominated by hydrogenotrophic methanogens. Using porewater isotopes of dissolved CO_2 and CH_4**,** Neumann et al. (2016) [83] modeled microbial respiration, methanogenesis, methane oxidation, and acetogenesis at the edge and center of a thermokarst bog. At the edge of the bog, where permafrost thaw was taking place, microbial respiration, methanogenesis (both acetoclastic and hydrogenotrophic), acetogenesis, and methane oxidation all were higher than the older part of the bog, indicating that freshly released organics and/or N from permafrost soils could dramatically fuel microbial activity in situ. Five years of experimental warming at the natural thaw-front increased the relative abundance of methanogenesis genes, particularly those involved in methane production from acetate [66]. In a lab experiment of tussock tundra permafrost, Coolen and Orsi (2015) [53] found that transcripts involved in acetoclastic methanogenesis increased after 11 days of thaw. They also found that acetogenesis transcripts (but not transcripts for acetogenic fermentation) were expressed after thaw suggesting that acetogenic bacteria are active and producing acetate post-thaw.

Methanotrophs play a critical role in net CH_4 flux from permafrost ecosystems. Methane that is not oxidized by methanotrophs can be released into the atmosphere. Thus, methanotrophs may mitigate CH_4 emissions from thawing permafrost. In the field, genes for aerobic methanotrophy are present in the active layer of permafrost affected soils [41,84,85], and both aerobic and anaerobic methane oxidation occurs in thermokarst wetlands where subsurface methane concentrations are high [83,86]. In submarine permafrost, anaerobic methane oxidizers

likely mitigate methane release when thawed [87]. In uplands, where methane production is very low, genes for methanogenesis and methane oxidation are detectable but low, and they may be reduced after thaw [52]. In the lab, Mackelprang et al (2011) [51] found that thaw yielded a burst of trapped CH_4, which decreased within seven days as methane monooxygenase (*pmoA*) —a key genetic indicator of methanotrophy—increased. This was despite incubation with anaerobic headspace, suggesting that oxygen necessary for methanotrophy originated from permafrost water or aerobic microsites in the soil. The methyl coenzyme-M reductase alpha subunit (*mcrA*) gene, which catalyzes the last step in methanogenesis did not change in abundance. In contrast, Coolen et al (2015) [53] found that methanogenesis (but not methane oxidation) transcripts increased after just 11 days of thaw. We expect that future work will enable us to better utilize genomic data to directly link gene and transcript abundance to processes that control net methane fluxes from soils.

Future directions/Discussion

Our understanding of the functional consequences of permafrost thaw on microbial communities is clearly still in its infancy. Although several studies have investigated changes in the composition of soil microbial communities following thaw, very few have investigated microbial communities at the level of functional -omics. Several gaps in knowledge revolve around an incomplete understanding of the response and role of other domains of life to thaw, most especially fungi and viruses. We also do not know the extent to which, or under what circumstances, changes in community composition or functional gene abundance guarantees a change in biogeochemical function. The common result we observe is that microbial communities are responding to the unique physical, chemical, and substrate-accessibility conditions present within their microenvironment. Importantly, most work is conducted as a lab assay, and microbial communities in the field may not respond as they do in incubation studies because microorganisms can immigrate into newly thawed environments, the external supply of organic material or TEAs may change, and plant interactions occur whereas they are decoupled from these interactions in the lab. And although most effort is focused on how microbial responses affect CO_2 and CH_4 flux, we still have little understanding of the role microbes play in affecting plant responses post thaw through mechanisms such as nutrient mineralization, symbioses, and pathogenic interactions.

At the field scale, permafrost thaw occurs in many different environments in many different forms [76], thus limiting our ability to generalize results until many more sites have been

examined. Each geographic location has a very different ecological and disturbance history affecting the types of microorganisms that may be entrained in permafrost and thereby affecting how they may respond post thaw [88]. We have primarily focused on soil or wetland microbial communities in this article and have avoided lake, sediment, marine, and riverine microbial community responses to thaw. Most permafrost thaw experiments in the field are space for time substitution experiments. Chronosequences or other gradient studies offer the ability to look at processes over large temporal and spatial scales [5,7,89], but can be limited by incomplete knowledge of the history of the different sites. Field soil warming manipulations can be useful in understanding specific microbial responses to warming but they are typically limited to surface soils [90-94]. Only one study currently exists that tracks genetic changes at the active layer-permafrost transition [66]. This may in part be due to technical constraints of heating deep soils, but techniques such as snow manipulations [40,95] or water manipulations [96] result in subtle yet important permafrost manipulations that help us to understand system change [66]. It is important that microbial ecologists collaborate with field experimentalists to best understand microbial linkages to changing biogeochemical processes.

Understanding how microbial communities respond to thaw and coupling thaw response to greenhouse gas emissions and/or plant community dynamics is important for understanding system responses. It will require a systems-level approach to investigate microbial community functional processes in the thawing permafrost over multiple spatial and temporal scales.

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