Chapter 8
Microbial Life in Permafrost

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Abstract Permafrost is a hostile environment that harbors a diverse and active microbial community. Next generation sequencing studies have demonstrated a wide diversity of microorganisms present in Arctic, Antarctic and high altitude permafrost soils. In situ activity of these microorganisms has been demonstrated through multiple lines of evidence. Radiolabeled studies and stable isotope probing have established that active respiration and DNA replication occur in permafrost soils under frozen conditions. Furthermore, microorganisms capable of subzero growth have been isolated from permafrost samples. These isolates have adapted to the permafrost environment through a multitude of molecular changes, such as increased expression of cold shock and metabolite transport proteins, reduced fatty acid saturation in the membrane, and presence of temperature specific isozymes. Recent studies have focused on permafrost thaw due to anthropogenic climate change. The subsequent thaw of frozen organic carbon stores in permafrost is thought to increase microbial activity and emissions of greenhouse gases to the atmosphere. As the permafrost thaws, the microbial community changes in terms of diversity and functional potential in response to warmer temperatures, and increased carbon and water availability.

Contents

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8.1 Introduction

Permafrost represents a large and extremely challenging environment for microbial life, covering 27% of the terrestrial surface on Earth, in which microorganism must cope with multiple environmental stressors (Goordial et al. 2013). Freezing temperatures, low kinetic energy, and low water and carbon availability limit microbial growth (Nikrad et al. 2016). Despite this, permafrost harbors a diverse microbial community that is viable and metabolically active. Furthermore, there has been a surge of interest into the diversity and activity of permafrost microorganisms as the permafrost thaws due to anthropogenic climate change.

Permafrost microorganisms are generally characterized as cryophiles, organisms that can sustain growth and reproduction at low temperatures ranging from −17 °C to +10 °C (D'Amico et al. 2006; De Maayer et al. 2014). In addition to permanently subfreezing temperatures, any microbial life within permafrost must be able to survive the often oligotrophic conditions, background radiation on geological timescales, and limited liquid water activity; any liquid water present in permafrost is thought to exist in saline brine veins or in special saline niche environments such as cryopegs (Gilichinsky et al. 2003). As such, any organisms which are able to survive these conditions are often polyextremophilic. While many microorganisms are either non-viable or dormant under these harsh conditions, there is clear evidence that globally, diverse and abundant microbial communities within permafrost can be viable and active in situ. In addition to in situ measurements indicative of microbial growth, psychrophilic organisms have been successfully isolated from permafrost environments, with subzero growth observed in the laboratory. This chapter discusses the diversity of microorganisms found in permafrost globally, as well as evidence for viability and activity within the permafrost environment. Finally, we discuss the issue of climate change and how microbial communities in permafrost are expected to respond, with an emphasis on methane dynamics based on research to date.

8.2 Permafrost Environments

Permafrost, defined as ground material that is at $\leq 0\,^{\circ}C$ for two or more consecutive years (van Everdingen 1998), is found primarily in the Arctic, sub-Arctic, and Antarctic regions, as well as in alpine regions (for example in the Qinghai-Tibet Plateau, South America, and Sweden) (Bockheim and Munroe 2014). Perennially frozen permafrost is most often overlaid by a seasonally thawed active layer, the depth of which is dependent on air temperatures, moisture content, vegetation, and snow cover (Tarnocai 1980). Permafrost can be hundreds of meters thick (e.g., over 500 m in Siberia), while the active layers can range between a few centimeters to several meters in depth (Tarnocai 1980). A transition zone exists between permafrost and the active layer, which acts as a temperature buffer and fluctuates between being seasonally frozen and perennially frozen over decadal time scales (Shur et al. 2005). Permafrost is a heterogeneous environment due to frost heave, front sorting, and cryoturbation, processes which disrupt and mix different soil horizons, sometimes transferring organic carbon from surface to deeper layers and often creating patterned ground (van Everdingen 1998; Hugelius and Kuhry 2009).

Permafrost soils can also contain different geomorphological features such as ice-wedges, taliks, cryopegs, massive ground ice, frost boils, thermokarst lakes, organic accumulations, and broken soil horizons, each providing a unique habitat for microbial growth (Fig. 8.1). Ice-wedge polygons are formed when the frozen ground contracts during the cold winter months and cracks the surface, dividing it into polygonal blocks (Kerfoot 1972). During the spring, these cracks are filled with snow melt water, which freezes and forms ice-wedges. As the temperature rises, the ground between the cracks expands and elevates, forming the polygon center (Kerfoot 1972). The ice-wedges are overlaid with active layer soil, creating the polygon trough (Shur et al. 2005). Over subsequent years of freeze and thaw cycles,

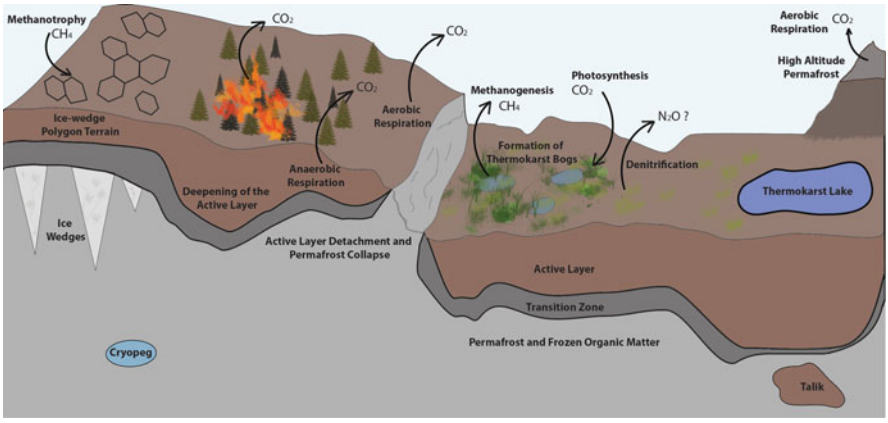

Fig. 8.1 Schematic diagram of permafrost, including microbial and abiotic processes involved in permafrost thaw and GHG emissions

the ice-wedges grow, resulting in high centered polygon surrounded by lower troughs. Taliks are unfrozen masses of ground soil found within the permafrost; often they are located under lakes due to the water's ability to transfer heat (Shur and Jorgenson 2007). Cryopegs are supercooled groundwater brine lenses that remain liquid at below 0 °C due to their high salt content (Gilichinsky et al. 2003). Along with a very steep temperature gradient, soluble nutrients, such as nitrogen, phosphorus, calcium, magnesium, and potassium, can also potentially form a gradient in the soil. As the nutrients are solubilized in meltwater, they move along the thermal gradient, enriching the top frozen permafrost layer (Kokelj and Burn 2005; Tarnocai 2009). This results in the upper layer of permafrost becoming a sink of soluble materials and nutrients during permafrost formation and can then conversely become a source of these nutrients during permafrost degradation (Kokelj and Burn 2005).

Arctic permafrost overall is a heterogeneous environment with both mineral and organic soils. The polar deserts of High Arctic permafrost tend to harbor mineral permafrost, without an organic layer over the mineral horizons (Steven et al. 2006; Shur and Jorgenson 2007; Tarnocai et al. 2009). However, the Arctic does contain large stores of organic carbon frozen in permafrost. Pleistocene Yedoma permafrost deposits in the North East Siberia are rich in organic carbon and were formed by windblown dust and sediment deposits that were subsequently frozen during the glacial age (Zimov et al. 2006; Tarnocai 2009; Vonk et al. 2013). These deposits contain large ice-wedges and well-preserved organic material, which constitutes roughly one-third of organic carbon stored in permafrost globally (Vonk et al. 2013). Due to the cold climate during the formation of the deposits, the organic carbon is well preserved and readily available for biological degradation (Schuur et al. 2008; Vonk et al. 2013). A prevalent feature of the Yedoma permafrost deposits is the high water content present in the form of ice veins and large ice-wedges that account for ~50% of the soil content (Vonk et al. 2013). Due to the high ice content, the Yedoma permafrost deposits are highly susceptible to permafrost degradation due to climate change (Vonk et al. 2013). Permafrost peatlands, primarily in the southern Arctic and sub-Arctic regions, also hold large reservoirs of soil organic carbon. The permafrost deposits in these areas initially developed as fens and bogs that contained woody plants, mosses, and sages (Routh et al. 2014). These perennially frozen peatlands are vulnerable to climate warming in the Arctic and sub-Arctic regions; the melting of ice in the permafrost will lead to degradation of the peatlands and biodegradation of the currently sequestered carbon (Tarnocai 2006).

Antarctic permafrost is less studied than its Arctic counterpart; however, Antarctic permafrost represents a much more extreme environment in terms of the combined freezing temperatures, aridity, and oligotrophy. The Antarctic contains 37% of the world's permafrost (Bockheim and Hall 2002), though compared to Arctic and alpine permafrost, relatively little is known about Antarctic permafrost communities (Goordial and Whyte 2014). The majority of the Antarctic continent is snow and ice covered, with only 0.35% of exposed ground on which permafrost occurs (Campbell and Claridge 2009). The McMurdo Dry Valleys' polar desert

represents the largest ice-free area in the Antarctic and has been the focus of most permafrost studies to date (Gilichinsky et al. 2007; Goordial et al. 2016). Trough-like depressions may be underlain by sand wedges instead of ice, though ice veins and ice lenses may also be found within sand wedge structures (Bockheim et al. 2009). The McMurdo Dry Valleys receive very low annual precipitation and is the only known place on Earth where dry permafrost is found (defined as permafrost which contains less than <3% water by mass) which forms from sublimation of ice-cemented permafrost over time (Bockheim et al. 2007). Ice-cemented permafrost is found primarily on the coastal areas and younger surfaces where the geography facilitates drainage and therefore results in wetter soils. Dry permafrost is found overlaying ice-cemented permafrost and is found at higher elevations and in older inland arid areas (Campbell and Claridge 2006). In some higher elevation valleys, there is an absence of any active layer that rises above 0 °C seasonally (Marinova et al. 2013). The majority of the water in the permafrost is frozen and the possibility for brine veins or thin films of water depends on temperature, solute concentration, and distance from the coast. Coastal and lower elevation valleys receive higher salt influx and have higher soil chlorine concentrations, as well as have a larger number of thaw days throughout the year (Goordial et al. 2016). As a result, higher elevation valleys, such as University Valley, do not have sufficient solute concentrations or temperatures to form thin films of water for more than a few hours a year (Goordial et al. 2016).

In addition to the poles, permafrost is also present at high altitudes. The largest amount of high altitude (alpine) permafrost is located in China (Ran et al. 2012) with the Tibetan plateau being the largest alpine permafrost region (Chen et al. 2016). Temperate mountain permafrost soils have well-drained coarse sediments, steep slopes that have higher spatial and geothermal variability, more variable snow distribution, lower influence of vegetation, and warmer mean annual temperatures compared to Arctic and Antarctic permafrost (Haeberli and Gruber 2009; Frey et al. 2016). Furthermore, incoming solar radiation is moderated by the slope and shading (Etzelmüller 2013). In more coastal areas, alpine permafrost tends to be located above the tree line; however in continental areas, forests may promote permafrost development (Etzelmüller 2013). Recently, high altitude permafrost has garnered more attention since, in the last decade, monitoring of alpine permafrost has shown warming across the globe, but particularly in colder regions, with unknown consequences on geotechnical stability (Haeberli and Gruber 2009; Etzelmüller 2013).

8.3 Microbial Diversity and Abundance in Permafrost

Despite being a hostile environment, permafrost does harbor a microbial community with 10^5–10^9 cells g^{-1} in Arctic permafrost, 10^{10}–10^8 in alpine permafrost, and a lower amount of 10^3–10^6 cells g^{-1} in Antarctic permafrost (Vishnivetskaya et al. 2006; Gilichinsky et al. 2007; Hansen et al. 2007; Steven et al. 2007; Blanco et al. 2012; Hu et al. 2015; Goordial et al. 2016). Across studies in alpine and polar

regions, the dominant groups present in permafrost soils tend to be Actinobacteria, Bacteroidetes, Proteobacteria (Alpha- and Beta-primarily but also Delta- and Gamma-Proteobacteria), Firmicutes, Chloroflexi, and Acidobacteria (Steven et al. 2008; Yergeau et al. 2010; Wilhelm et al. 2011; Deng et al. 2015; Stackhouse et al. 2015) (Table 8.1). The fermentative members of Chloroflexi and Bacterioidetes increase with soil depth indicating anaerobic carbon degradation in permafrost (Deng et al. 2015). Archaea and fungi are present, though 200–1000 times lower in abundance (Yergeau et al. 2010; Stackhouse et al. 2015; Frey et al. 2016). Abundant archaeal groups in permafrost are often related to halophilic archaea, part of Euryarchaeota, likely due to the brine veins thought to host active microbial life within permafrost soils (Steven et al. 2007). However, Crenarchaeota appear to dominate acidic wetland permafrost (Wilhelm et al. 2011). Under frozen permafrost conditions, based on RNA data, the most transcriptionally active organisms appear to be the same as the dominant phyla: Proteobacteria, Firmicutes, Acidobacteria, and Actinobacteria, as well as Euryarchaeota and ascomycetous fungi (Coolen and Orsi 2015) (Table 8.1). Furthermore, permafrost contains active sulfate and Iron (III) reducers, nitrifying bacteria, methanotrophs, and methanogens (Yergeau et al. 2010; Stackhouse et al. 2015). This is reflected in the presence of genes, transcripts, and proteins involved in sulfate reduction, Iron(III) reduction, nitrogen cycle, methanogenesis, and methane oxidation found in the permafrost (Yergeau et al. 2010; Mackelprang et al. 2011; Hultman et al. 2015). Functional groups of bacteria in high altitude permafrost also include ammonia-oxidizing bacteria and archaea, methane-oxidizing bacteria, nitrifying bacteria, nitrogen-fixing rhizobial symbiont bacteria, sulfur- and sulfate-reducing bacteria, and thiosulfate-oxidizing bacteria (Wu et al. 2012; Zhang et al. 2009; Yun et al. 2014; Hu et al. 2016). A unique study of the northern slope of Mount Everest showed an interesting comparison of ammonia oxidizers above and below 5800 m a.s.l.: the soil above this threshold is permafrost and is characterized by increasingly colder temperatures, stronger radiation, lower oxygen concentration, and lower nutrients with increase in altitude (Zhang et al. 2009). The increase in altitude also decreased the abundance of both archaeal and bacterial ammonia oxidizers; however, at lower elevation, the soils were dominated by archaeal ammonia oxidizers, but were replaced by bacterial ammonia oxidizers in the higher altitude permafrost soils (Zhang et al. 2009). Overall, caution should be used when taking the results of higher altitude permafrost microbial abundance and function studies at face value, since authors in these papers often confusingly refer to active layer soils as permafrost.

Overall, permafrost contains genes involved in response to stressors, pathogenicity, toxicity, degradation of carbon compounds, methanogenesis, methane oxidation, denitrification, nitrogen fixation, ammonia assimilation, and sulfate reduction (Yergeau et al. 2010; Hultman et al. 2015) and have shown presence of proteins involved in chemotaxis and motility (Hultman et al. 2015). This result is also supported by the isolation of motile permafrost microbes from brine veins containing flagella (Shcherbakova et al. 2005). Compared to the overlaying active layer, the permafrost contains lower abundance of transporter proteins and transcripts, but higher presence of cold-shock proteins and other stress response genes

Table 8.1. Table outlining the microbial members of permafrost community present, active, and cultivable in Arctic, Antarctic and in high altitude permafrost

Permafrost microbial member	(sub)Arctic				Antarctic				High altitude			
	Present[a]	In situ active[b]	Cultivable isolates	References	Present[a]	In situ active[b]	Cultivable isolates	References	Present[a]	In situ active[b]	Cultivable isolates	References
Proteobacteria	+	+	+	1–15	+		+	16–19	+		+	20–24
Alpha	+	+	+	1, 4, 9, 13, 14, 15	+		+	16, 17	+		+	20–23
Beta	+	+	+	1, 4, 9, 12, 14, 15	+		+	16, 17	+		+	20–23
Gamma	+		+	3, 4, 9–15	+		+	16, 17, 25	+		+	20–23
Delta	+			3,4,6,9,13–15					+			20, 21
Firmicutes	+	+	+	1,3,5-9, 11,13,14,26,27	+		+	16-18	+			20–23
Acidobacteria	+	+		1, 2, 5, 7, 8, 14, 15	+			19	+			21, 24
Actinobacteria	+	+	+	1-3, 5, 7, 8, 9, 12-15, 28	+		+	16, 17, 19	+			20–24
Chloroflexi	+	+		6, 7, 13–15	+			19	+			21, 24
Gemmatimonadetes	+			2, 4, 7, 14, 15	+			19	+			24
Verrucomicrobia	+	+		2, 7, 14, 15	+			19	+			21, 24
Bacteroidetes	+		+	1, 3-7, 9, 13–15	+			16, 17, 19	+		+	22–24, 29
Planctomycetes	+	+		4, 7	+			19	+			21, 24
OP11 (Microgenomates)	+			7					+			24
OP5 (Caldiserica)									+			24
OD1 (Parcubacteria)									+			24
TM7 (Saccharibacteria)	+			9					+			24
GN02 (Gracilibacteria)									+			24
AD3 candidate division	+			15								
Spirochaetes	+	+		7								
Chlorobi	+	+		7								

(continued)

Table 8.1. (continued)

Permafrost microbial member	(sub)Arctic				Antarctic				High altitude			
	Present[a]	In situ active[b]	Cultivable isolates	References	Present[a]	In situ active[b]	Cultivable isolates	References	Present[a]	In situ active[b]	Cultivable isolates	References
Cyanobacteria									+			21
Nitrospirae									+			21, 24
Euryarchaeota	+	+	+	1, 3, 4, 7, 8, 30–32, 34	+			17	+			20, 33
Crenarchaeota	+			2, 4					+			33
Thaumarchaeota	+			34					+			20
Bathyarchaeota	+			34								
Woesearchaeota	+			34								

[a]DNA based evidence
[b]RNA based evidence

1, Yergeau et al. (2010); 2, Wilhelm et al. (2011); 3, Steven et al. (2008); 4, Steven et al. (2007); 5, Stackhouse et al. (2015); 6, Deng et al. (2015); 7, Hultman et al. (2015); 8, Coolen and Orsi (2015); 9, Hansen et al. (2007); 10, Bakermans et al. (2003); 11, Ponder et al. (2005); 12, Panikov and Sizova (2007); 13, Gittel et al. (2014a); 14, Gittel et al. (2014b); 15, Taş et al. (2014); 16, Gilichinsky et al. (2007); 17, Goordial et al. (2016); 18, Tamppari et al. (2012); 19, Bakermans et al. (2014); 20, Hu et al. (2016); 21, (Yun et al. (2014); 22, Bai et al. (2006); 23, Zhang et al. (2007); 24, Frey et al. (2016); 25, Kim et al. (2012); 26, Shcherbakova et al. (2005); 27, Mykytczuk et al. (2013); 28, Finster et al. (2009); 29, Zhao et al. (2011); 30, (Rivkina et al. (2007); 31, Shcherbakova et al. (2011); 32, Mackelprang et al. (2011); 33, Wei et al. (2014); 34, Shcherbakova et al. (2016)

(Yergeau et al. 2010; Mackelprang et al. 2011; Hultman et al. 2015). Microbiota in mineral permafrost horizons have lower carbon degradation capacity compared to organisms from organic active layers; this can be due to lower taxonomic diversity in oligotrophic conditions found in the permafrost (Ernakovich and Wallenstein, 2015; Hultman et al. 2015). However, the permafrost environment does contain genes for degradation of sugar alcohols; aminosugars; mono-, di-, and oligosaccharides; starch; lignocellulose; chitin; trehalose; and cellulose (Yergeau et al. 2010; Hultman et al. 2015). Furthermore, Actinobacteria is often a dominant bacterial phyla present in permafrost soils (Table 8.1). This phyla is known to contain members that are facultative anaerobic degraders of complex soil organic matter and are adapted to low carbon availability. In addition, permafrost soils have demonstrated potential enzymatic activities of hydrolytic (cellobiohydrolase, endochitinase, *N*-acetylglucosaminidase, and leucine aminopeptidase) and oxidative (phenoloxidase and peroxidase) enzymes (Gittel et al. 2014a, b).

Compared to permafrost soils, ice-wedges and ground ice are relatively low in diversity and abundance of microorganisms (Steven et al. 2008), they reflect the community of the surrounding permafrost soils and include members of Proteobacteria, Actinobacteria, Acidobacteria, Bacterioidetes, and Firmicutes (Steven et al. 2008; Wilhelm et al. 2012).These are habitable cryoenvironments which show evidence of in situ heterotrophic activity based on occluded gas measurements (Katayama et al. 2007; Lacelle et al. 2011; Wilhelm et al. 2012) and contain cultivable microbiota, despite low viable cell counts (Gilichinsky et al. 1995; Katayama et al. 2007; Lacelle et al. 2011). Soil particles suspended in the ice-wedges and brine veins are thought to serve as refugia for microorganisms and protect the cells from ice crystals.

Specifically in the Antarctic, the microbial community consists of Proteobacteria (Gammaproteobacteria) (Gilichinsky et al. 2007) (as well as members of *Nitrospina*, green non-sulfur bacteria and relatives, *Fibrobacter*, *Acidobacterium*, and the Flexibacter–Cytophaga–Bacteroides group) and low levels of anaerobes including denitrifying bacteria, methanogens, and sulfate reducers. Culturable isolates belonged to Alpha-, Beta-, and Gamma-Proteobacteria; Actinobacteria; Firmicutes; and Methylobacterium; as well as mycelial fungi and yeast groups (Gilichinsky et al. 2007). Interestingly, unicellular green algae including *Mychonastes* sp. (Chlorellaceae), *Chlorococcum* sp. (Chlorococcaceae), and *Chlorella* sp. (Chlorellaceae) have also been isolated from Antarctic permafrost (Gilichinsky et al. 2007). The McMurdo Dry Valleys' permafrost ranges from cold and dry in the coastal Taylor Valley (TV; subxerous; 1–75 mm precipitation; summer temperatures sometimes above 0 °C in the Valley) to extremely cold, extremely dry, and highly oligotrophic in high inland University Valley (UV; ultraxerous; average summer temperature −5 °C in the Valley; 0.01−0.05% total carbon, undetectable to 0.09% total nitrogen) (Tamppari et al. 2012; Goordial et al. 2016). The diversity of the microbiota is also reflected between the two valleys. The wetter TV permafrost contains a higher abundance of microbes compared to the drier and colder UV (Tamppari et al. 2012). For example, TV permafrost showed presence of both anaerobic and aerobic microbial groups, whereas in the UV

permafrost the anaerobic microbial groups were undetected based on PLFA data; however, later sequencing data did show presence of anaerobes in UV permafrost as well; this is likely due to TV permafrost being "wetter" and therefore potentially having anoxic conditions near the permafrost table (Tamppari et al. 2012; Goordial et al. 2016). Furthermore, the dominant microbial groups in TV are Proteobacteria, sulfate-reducing bacteria, anaerobic metal reducers, Acidobacteria, Gemmatimonadetes, and Firmicutes, whereas in UV the dominant groups are Gamma- and Beta-Proteobacteria, though Firmicutes, Actinobacteria (including Actinomycetes), and Bacteroidetes were also present (Tamppari et al. 2012; Bakermans et al. 2014; Goordial et al. 2016).

The permafrost microbial community in high elevation permafrost appears to be dominated by bacterial phyla of Proteobacteria and Actinobacteria and in one case Patescibacteria superphyla (Hu et al. 2016; Frey et al. 2016), although Actinobacteria, Proteobacteria, and Chloroflexi dominate high altitude wetland permafrost (Yun et al. 2014). Arachaeal diversity is dominated by Thaumarchaeota (Hu et al. 2016). Culturable isolates in high altitude permafrost belong to Arthrobacter; Pseudomonas; Alpha-, Beta-, and Gamma-Proteobacteria; Firmicutes; and CFB (Bai et al. 2006; Zhang et al. 2007; Hu et al. 2015). Interestingly, the majority of the phylotypes of bacterial and archaeal origin were less than 97% similar to previously isolated strains (Hu et al. 2016). Frey et al. (2016) conducted an elegant study on alpine permafrost and found that the permafrost compared to the nearby non-permafrost soils was highly enriched in uncultured bacteria. The uncultured members were part of candidate phyla OD1 (proposed Parcubacteria), TM7 (Saccharibacteria), GN02 (Gracilibacteria), OP11 (Microgenomates), OP5 (Caldiserica), SR1, MVP-21, WS5, and Kazan-3B-28. The candidate phyla OD1, TM7, GN02, and OP11 are part of a proposed superphylum Patescibacteria (Frey et al. 2016). Members of Patescibacteria have very limited biosynthesis abilities of key macronutrients and are hypothesized to lead a semi parasitic or symbiotic lifestyle by attaching to the surface of other cells. These bacteria can be characterized by their low C–G content, small genome size, and are found in anoxic environments (Frey et al. 2016). Such features may embed a selective advantage to this superphylum in the harsh, oxygen-limited permafrost environment.

8.4 Are They Livin' or Just Chillin'?

It may be intuitive to imagine that the microbes in the harsh frozen permafrost environments are in a state of dormancy and stasis. However, microbes in permafrost are biologically active. The active layer of the permafrost does boast higher microbial activity and microbial diversity; however, despite the harsh permafrost conditions, active microbes are still found within the permafrost (Yergeau et al. 2010; Coolen and Orsi 2015; Mackelprang et al. 2016). There are several complimentary lines of evidence for this: subzero respiration under aerobic and anaerobic

conditions measured, isotope incorporation and mineralization under frozen conditions in permafrost soils, isolation of permafrost microbes capable of subzero growth, and evidence from RNA based studies

8.4.1 Soil Respiration and Laboratory Incorporation Studies

Respiration and subsequent release of gases has been measured in frozen soils in situ and in laboratory studies. Some of the early evidence that microbes are living and are active in permafrost soils came from measurements of CO_2 and CH_4 release to the atmosphere from wintertime frozen tundra and peat bog soils (Fahnestock et al. 1999; Panikov and Dedysh 2000; Elberling and Brandt 2003). However, these in situ soil respiration studies should be interpreted with caution since the gas emissions could be due to releases of trapped gas. Laboratory experiments using permafrost soils have also demonstrated respiration at freezing temperatures as low as −2 °C (Michaelson and Ping 2003), −4 °C (Larsen et al. 2002), −16 °C (Panikov and Dedysh 2000), −18 °C (Elberling and Brandt 2003), −39 °C (controversial) (Panikov et al. 2006), and in Antarctic soils down to −5 °C (Bakermans et al. 2014). Overall, respiration in frozen soils is dependent on water availability, temperature, and carbon content of the soils (Panikov and Dedysh 2000; Michaelson and Ping 2003; Öquist et al. 2009).

Respiration and heterotrophic activity under subzero conditions on environmental permafrost samples has also been shown in studies using highly sensitive radiolabeling microbial activity assays. For example, respiration in permafrost soils, active layer soil, and ice-wedges has been demonstrated using C^{14} labeled glucose and acetate as substrates (Rivkina et al. 2000; Steven et al. 2008; Wilhelm et al. 2012). The briny habitat existing within cryopegs is also thought to host active microorganisms as activity down to −15 °C was demonstrated via uptake of C^{14} labeled glucose (Gilichinsky et al. 2003). Massive ground ice on the other hand was not found to host an actively mineralizing community (Steven et al. 2008). Similarly, the highly unique permafrost in the high elevation Dry Valleys do not appear to respire under frozen conditions in laboratory or in situ, as demonstrated in University Valley soils (Goordial et al. 2016). In this instance, the combination of cold temperature (mean annual temp −23 °C with no days above freezing), aridity (<10 mm snow melt equivalent per year), and low salinity to facilitate brine veins within the permafrost (Goordial et al. 2016) all contribute to an inactive community. In contrast, the permafrost in the more coastal, and therefore wetter and warmer, Taylor Valley of McMurdo Dry Valleys does show active respiration at subzero conditions (Bakermans et al. 2014). These incorporation studies highlight environmental samples and conditions which favor active microbial life—namely the presence of liquid water, facilitated through the presence of solutes or salt. While these studies show microbial activity under ambient permafrost conditions, they do not show active DNA replication and growth, and do not reveal which microbial community members are active in the subzero permafrost environment.

8.4.2 Stable Isotope Probing in Permafrost Soils

Stable isotope probing (SIP) is used to label the DNA of actively dividing microbes. In an elegantly designed study, Tuorto et al. (2014) were able to demonstrate microbial genome replication in permafrost soils in temperatures ranging from 0 °C to −20 °C using C^{13}-acetate. The active community members that were able to perform DNA replication in frozen permafrost conditions were part of Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and Proteobacteria phyla and were distantly subzero related to isolated psychrophilic strains that are able to grow at subzero temperatures (Tuorto et al. 2014). Firmicutes were not detected by SIP in this study, suggesting that spore-forming members of this phyla are dormant and non-metabolically active in an ambient permafrost environment, and their near ubiquitous detection in molecular studies may be due to the increased longevity of spores. Some members of the permafrost community were actively growing across all temperatures, whereas some were limited to specific niche temperature ranges; for example, uncultured members of Actinobacteria and Proteobacteria were only able to synthesize DNA between −9 °C and −20 °C. This could be due to changes in solute concentrations and available water, permitting only the growth of microorganisms that are adapted to very specific niche conditions (Tuorto et al. 2014).

8.4.3 RNA/DNA Ratios

Ratios of RNA/DNA can be used to infer metabolic activity of the community or a particular group of microbes. Microbial community members with higher RNA/DNA ratios are thought to be more metabolically active in the environment, as the active microbes would be synthesizing higher amounts of RNA per cell compared to inactive ones, while the DNA content would remain static regardless of activity (Eriksson et al. 2001; Blazewicz et al. 2013). Hultman et al. (2015) looked at the ratios of RNA transcripts in metatranscriptomes (MT) to the DNA in metagenomes (MG) of permafrost soils to determine that the most active groups of microbes in permafrost were Proteobacteria, Acidobacteria, and Firmicutes, suggesting that these members of the community were more acclimated to life at subzero temperatures (Hultman et al. 2015). Furthermore, the study showed that permafrost had a higher ration of methanogenesis and methane oxidation transcripts (RNA) to genes (DNA) than the active layer, suggesting that the permafrost had relatively higher ratio of active methanogens and methanotrophs compared to the active layer. However, the active layer appeared to have a higher RNA/DNA ratio of *nif* genes, suggesting that it harbors a more active community of nitrogen fixers (Hultman et al. 2015). Schostag et al. (2015) also performed a DNA and RNA based analysis though only on the active layer of permafrost; their study spanned the winter and the summer season allowing the comparison of the same soils in frozen

and thawed conditions. The copy number of rRNA genes and transcripts did not fluctuate between the two seasons, suggesting that a similar relative abundance of microbes continue to be active during the winter with the soil temperatures below −10 °C (Schostag et al. 2015).

8.4.4 Subzero Growth of Permafrost Isolates

Further evidence that microbes in permafrost are active is the ability of isolated permafrost microorganisms to grow at below freezing temperatures. There have been many permafrost isolates, mainly belonging to the Firmicutes, Actinobacteria, Proteobacteria, and Bacterioidetes phyla (Goordial et al. 2016; Jansson and Taş 2014). However, few are able to sustain subzero growth and those that do have evolved key adaptive strategies. For example, *Psychrobacter cryopegella* is able to grow down to −10 °C and up to 28 °C, with maximum growth at 22 °C. It was isolated from saline cryopegs buried within 40,000 years old Siberian permafrost (Bakermans et al. 2003). The authors demonstrated that growth yield of the isolate peaked at 4 °C; at this temperature, the microbial cells needed the least amount of RNA and proteins to divide. At freezing temperatures, the isolate likely needed to produce more cold acclimation and cold shock proteins as well as initiate other cold adaptive changes; above this temperature, the isolate needed to produce more RNA and proteins due to higher turnover and degradation rate caused by higher temperatures (Bakermans and Nealson 2004). Other Siberian permafrost isolates include a Gram-positive *Exiguobacterium sibiricum* and a Gram-negative *Psychrobacter* sp. 273-4. These isolates were able to grow at −2.5 °C and are thought to cope with the subzero temperatures by lowering fatty acid saturation and chain length in their membranes, changing the composition of exopolysaccharides, and increasing their ice-nucleation activity which is thought to reduce damaging intracellular ice accumulation (Ponder et al. 2005). An obligate anaerobic, spore forming, bacterial isolate that was capable of subzero growth was isolated from a water brine from the Kolyma Lowland region; *Clostridium algoriphilum* grows down to −5 °C, with optimal growth at +5 °C (Shcherbakova et al. 2005). From Alaskan permafrost, Panikov and Sizova (2007) isolated bacterial and fungal members capable of subzero growth. The bacterial isolates *Pseudomonas* sp. 3-2005 and *Arthrobacter* sp. 9-2 grew at temperatures down to −17 °C; *Polaromonas* sp. strain *hydrogenovorans* grew at −1 °C. The fungal members *Leucosporidium* spp. MS-1, -2 and *Geomyces* spp. FMCC-1, -2, -3, -4 were able to grow down to −35 °C; *Mrakia* sp. MS-2 grew down to −12 °C. However, in these isolates, growth between −18 °C and −35 °C was transient and ceased after three weeks, though normal growth dynamics were sustained in subzero growth above −18 °C (Panikov and Sizova 2007). An Antarctic *Psychrobacter* sp. PAMC 21119 isolate capable of subzero growth was isolated from permafrost soil on Barton Peninsula (Kim et al. 2012). The microbe is able to grow down to at least −5 °C, partially due to an increase in production of proteins involved in metabolite transport, proper protein

folding, and membrane fluidity (Koh et al. 2016). Two other Antarctic isolates (*Rhodotorula* and *Rhodococcus* spp.) are also able to grow at −10 °C and −5 °C, respectively, and were isolated from extremely dry University Valley Antarctic permafrost (Goordial et al. 2016).

Another bacterial permafrost isolate capable of subzero growth of note is *Planococcus halocryophilus* Or1; it was isolated from high Arctic permafrost and is able to grow down to −15 °C and sustain low level of metabolic activity down to −25 °C (Mykytczuk et al. 2013). Cells grown under the colder temperatures counter-intuitively contained higher levels of saturated fatty acids over branched ones and developed a crust of dense nodular material (Mykytczuk et al. 2013). The genome of the isolate showed adaptations to cold and osmotic stress including cold shock proteins, chaperones, genes involved in regulation, repair mechanisms, osmolyte uptake, and membrane alteration. Genome redundancy in *P. halocryophilus* would also suggest the presence of isozymes that may be adapted to specific temperatures (Mykytczuk et al. 2013). However, despite our ability to culture subzero permafrost isolates, it appears that the uncultivable native consortia of permafrost microorganisms are more adapted to subzero temperatures compared to individual permafrost members currently isolated, based on comparisons of growth yield and utilization of C^{14} labeled ethanol between permafrost isolates and permafrost soils (Panikov and Sizova 2007).

8.4.5 DNA Repair

Another piece of evidence that microorganisms are likely alive, active, and growing in the permafrost environment is their need and ability to repair DNA damage from background ionizing radiation. Natural background radiation can damage a cell's DNA over long geological timescales, such as those encountered in permafrost (Johnson et al. 2007). Completely dormant and inactive bacteria frozen in permafrost would continue to accumulate DNA damage and eventually lose viability; thus any viable microbes recovered from an ancient permafrost environment would need to have been at least minimally metabolically active in order to repair DNA damage during their tenure in the subzero permafrost (Price and Sowers 2004). Unchecked DNA damage will cross link the DNA and/or reduce it into 100 bp fragments within 100,000 to 1 million years in frozen conditions (Poinar et al. 1996; Hansen et al. 2006). To date, there have been several permafrost bacteria isolated from ancient permafrost; for example, *Exiguobacterium sibiricum* was isolated and cultured at subzero temperatures from a depth of 43.6 m from a 2–3 million years old Siberian permafrost (Ponder et al. 2005). Furthermore, experiments on *Psychrobacter cryohalolentis* K5 and *P. arcticus* 273-4 showed that the microbes are able to grow under −15 °C conditions while simultaneously exposed to ionizing radiation (Amato et al. 2010). These microbes were metabolically active, as demonstrated by [^{3}H] thymidine incorporation, and showed that microbes were able to sustain enough metabolic activity to repair DNA damage in the permafrost environment

(Amato et al. 2010). Indeed, RNA sequencing of permafrost under frozen and thawed conditions has also demonstrated a relative overexpression of genes involved in DNA repair mechanisms in frozen permafrost soils (Coolen and Orsi 2015). In addition, Johnson et al. (2007) demonstrated bacterial survival for at least half a million years in sealed permafrost. The authors amplified a long 4 Kbp DNA fragment from ancient permafrost samples (400,000–500,000 years). The fragment was 20-fold longer than ancient DNA from dead plant/animal samples (max amplicon lengths of 100–500 bp) of a similar age, suggesting that an active DNA repair mechanism must have been present to yield such a large DNA fragment from the ancient permafrost (Johnson et al. 2007). Sequence diversity greatly decreased with permafrost age, suggesting select few microorganisms are able to sustain long-term survival in permafrost (Johnson et al. 2007). In younger permafrost (5000–30,000 years), endospore forming bacterial members were shown to accumulate DNA damage; in older permafrost samples (400,000–600,000 years), there was no presence of bacteria with capacity for dormancy; instead, members related to non-spore forming *Arthrobacter* (Actinobacteria) were dominant. In addition, authors were able to show active respiration in these older permafrost samples at ambient permafrost temperatures. Together, these results suggest that microbes in ancient permafrost can sustain viability by maintaining low levels of metabolic activity and DNA repair; bacterial members that are capable of this strategy may outperform bacterial members capable of dormancy in very old permafrost environments (Johnson et al. 2007).

8.5 Live Microbes or Ancient DNA

The advent of next generation sequencing technologies has greatly propelled the study of microbial diversity, giving us a wealth of information and greater insight into the microbial processes that take place in permafrost environments. However, one uncertainty of studying microbial life and ecology of permafrost through novel molecular means is whether the nucleic acids that are isolated from the permafrost represent the current active microbial community in the ground, DNA within cryopreserved cells, spores, or just extracellular DNA and RNA molecules (i.e., eDNA) adsorbed to soil particles (Willerslev et al. 2004a, b; Pietramellara et al. 2009). Do nucleic acids extracted from permafrost represent a frozen snapshot of past life or does it represent current life adapted to a harsh environment? Dry conditions, low temperatures, and salinity promote the persistence of nucleic acids in the environment, with the rate of DNA degradation decreasing by a level of magnitude for every 10 °C drop in temperature (Smith et al. 2001; Willerslev et al. 2004a; Hebsgaard and Willerslev 2009). However, rates of nucleic acid degradation under different environmental factors are not well understood (Hebsgaard and Willerslev 2009), and permafrost conditions are thought to be favorable for long-term microbial and nucleic acid persistence (Johnson et al. 2007). Though DNA fragments (100–500 bp) may not persist in colder

environments for more than 10^5 years (Lindahl 1993; Briggs and Summons 2014). DNA in the environment, resting cells, and endospores degrade over time because of chemical hydrolysis and oxidation, eventually becoming non-viable and non-amplifiable due to lack of active DNA repair. However, even minimally metabolically active cells may retain a functioning DNA repair mechanism and persist in the permafrost environment over longer time periods (Price and Sowers 2004; Johnson et al. 2007).

Finally, contamination can never be ruled out, when working with such low quantities and quality of nucleic acids, as in the case of permafrost, the sensitivity of PCR to contaminants becomes problematic. In addition, when we are studying the current microbial community in the permafrost soils, extracellular eDNA from dead cells can obscure the microbial diversity recovered (Carini et al. 2016). On the other hand, when trying to use ancient nucleic acids as fossil molecules for paleodiversity studies and to reconstruct past ecosystems, there is risk of currently active microbes interfering with the results (Bellemain et al. 2013; Briggs and Summons 2014). Bellemain et al. (2013) run into this problem in their fungal paleodiversity study of two Siberian Pleistocene aged permafrost samples. The authors used permafrost samples to infer past ecology and environment of the area and through metabarcoding detected presence of plant-associated fungal taxa and fungal insect pathogens; based on this, they concluded that these fungi were an active component of the Pleistocene environments. However, psychrophilic and psychrotolerant fungal taxa were also detected in their molecular analysis, suggesting presence of metabolically active taxa in the permafrost that were potentially interfering with an accurate paleo-reconstruction (Bellemain et al. 2013). So how is it possible to differentiate between old nucleic acids preserved in the environment and a potentially metabolically active microbial community?

8.5.1 Strategies for Differentiating Between Old Biomarkers and an Active Microbial Community

As DNA degrades over time, it is increasingly hard to amplify long stretches of preserved DNA. Designing primers that flank large portions of 16S or other genes of interest is one way to ensure that only recent DNA is amplified (Johnson et al. 2007). RNA molecules on the other hand are more susceptible to degradations and do not persist in the environment as free molecules like DNA, due to their single stranded nature allowing direct cleavage of the phosphodiester bonds. Because of this, it is thought that molecular reconstruction of current microbial communities through RNA extractions and sequencing is more reliable. However, RNA extractions are notoriously difficult in permafrost soils; this is either due to low biomass in the samples or lack of actual RNA molecules altogether. The latter would imply lack of active cells. It is prudent to note that some studies have been able to extract or show evidence of long-term RNA preservation in permafrost, ice, snow, and

other environments aged 50–140,000 years; for now, these studies are limited to viral and plant RNA, though this does not exclude the possibility of microbial and fungal RNA preservation (Guy 2014). Contamination may be an even bigger hurdle in permafrost RNA studies compared to DNA ones, due to ubiquitous prevalence of RNases. Another option is the propidium monoazide (PMA) treatment, which is able to differentiate between live cells' DNA and eDNA/DNA within dead cells. PMA binds to DNA and inhibits the PCR reaction; however, it is not able to penetrate intact membranes of live cells (Bae and Wuertz 2009). Therefore, permafrost studies that aim to describe the current viable microbial community can use PMA treatment on their samples (Yergeau et al. 2010). However, these studies are limited to amplicon sequencing or any design that requires a PCR amplification step in the protocol. The advantage of this is that differences between the "live" and total DNA can be used to elucidate the portion of DNA that is environmental and preserved in the permafrost. Though ancient DNA would provide us with the most comprehensive phylogenetic information of past microbial communities, other biomolecules can be more persistent in the environment and may serve better for paleodiversity studies (Briggs and Summons 2014).

8.6 Warming Climate and Permafrost

Permafrost contains large amounts of frozen ancient carbon stores, in the range of 25–50% of the total soil organic carbon (Tarnocai et al. 2009). These pools are currently mostly inaccessible to microbial metabolism (Mackelprang et al. 2016). However, as the climate warms due to anthropogenic climate change, these pools of frozen carbon thawing are becoming available for heterotrophic microbial decomposition (Schuur et al. 2015). As the carbon pools are degraded, the microbes release greenhouse gases (GHG; CO_2, CH_4, and N_2O) into the atmosphere which can potentially increase the rate of climate change via a positive GHG feedback loop (Marushchak et al. 2011; Graham et al. 2012). Auxiliary effects of climate change such as wildfires further increase permafrost degradation and active layer deepening (Taş et al. 2014) (Fig. 8.1). Active layer detachment and permafrost collapse due to thawing further expose formerly buried permafrost and also increase microbial activity and degradation of previously unavailable soil organic matter (Pautler et al. 2010).

In laboratory warming experiments, permafrost thaw induced an increased CO_2 production in both Arctic and high altitude permafrost soils (Stackhouse et al. 2015; Mu et al. 2016). While carbon emissions from permafrost at subzero temperatures are present, rates of CH_4 and CO_2 emissions generally significantly increase with permafrost thaw under aerobic and anaerobic conditions (Song et al. 2014). Furthermore, N_2O emissions have been reported in laboratory permafrost core melting experiments (Elberling et al. 2010). Interestingly, the initial permafrost thaw released minimal N_2O; however, a cycle of drying and rewetting with the meltwater induced high rates of N_2O production in wetland permafrost (Elberling et al. 2010).

Understanding the extent of ancient carbon degradation and resulting GHG emissions from permafrost is challenging due to different factors that affect the thaw of permafrost and the heterogeneity of permafrost environments, such as vegetation cover, latitude, soil composition, hydrology and geology of the area, precipitation, types of organic carbon stored, C:N ratios, and permafrost depth (Chen et al. 2016). Indeed, deeper permafrost soils upon thaw may release less CO_2 due to these older soils containing higher proportions of recalcitrant carbon and lower enzyme (amylase and cellulose) concentrations and activity (Song et al. 2014). Thus, characterizing the current microbial community and activity in permafrost and understanding how that will shift with permafrost thaw and deepening of the active layer is one of the key current questions of environmental microbiology and the study of permafrost life. Indeed, studies that not only consider the current microbial biodiversity of permafrost, but that directly explore the changes associated with permafrost thaw by looking at environments that are currently undergoing this transition or by directly thawing permafrost samples in the laboratory, are starting to provide us with real insight into what the microbial community, its metabolic potential, and its effects on further GHG production may be, once permafrost thaws.

Coolen and Orsi (2015) looked at the transcriptional response of a microbial community to permafrost thaw in a moist acidic Arctic tundra location. Thawing permafrost conditions stimulated the growth of Firmicutes, Bacteroidetes, Euryarchaeota, Chloroflexi, Crenarchaeota, and ascomycetous fungi (Coolen and Orsi 2015). However, other studies reported that permafrost melt increased abundance of Actinobacteria upon thawing short term (Mackelprang et al. 2011) and long term (Deng et al. 2015). In mineral permafrost horizons, thaw increased the abundance of Actinomycetales that degrade complex recalcitrant carbon sources, as well as *Chitinophaga* (a chitinolytic genus) and Sphingomonadales (degraders of aromatics compounds) (Deng et al. 2015). Gene expression upon thaw tended towards overexpression of genes involved in amino acid transport and metabolism; energy production; and DNA repair, replication, and recombination (Coolen and Orsi 2015). However, genes encoding for biofilm formation, virulence, and horizontal gene transfer were higher expressed under frozen conditions compared to thawed soil (Coolen and Orsi 2015). Biofilm formation in the frozen soils could be restricted to the liquid brine vein microhabitat that surrounds the frozen permafrost soil particles (Gilichinsky et al. 2003; Coolen and Orsi 2015). As the permafrost thaws, genes that code for translation, ribosomal structure, and biogenesis are upregulated, as well as genes involved in extracellular protein degradation, anaerobic metabolism, and the uptake, transport, and degradation of carbohydrates and are thus, likely contributing to permafrost soil organic carbon degradation. However, genes coding for hydrolases responsible for the cleavage of complex carbon polymers into C1 and C2 substrates were expressed in both frozen and thawed permafrost (Coolen and Orsi 2015).

Wildfires in upland Boreal Alaska stimulate near surface permafrost thaw; this thaw can shift the microbial community in the soils (Taş et al. 2014). Taş et al. (2014) conducted a novel study looking at the effects of fire and subsequent permafrost thaw on the microbial community and its metabolic potential for further

GHG fluxes. The fire burned the majority of the top organic layer, thawed the permafrost to at least 1m in depth and lowered the C, N, DOC, and moisture content of the soils, but increased the pH and percentage of aromaticity. While fire and the subsequent thawing permafrost had a negative impact on the abundance of Verrumicrobia and Chloroflexi, it had a positive impact on the abundance of candidate phyla AD5, which was one of the most abundance phyla in the deeper thawed and frozen permafrost soils. This is likely due to lowered C, N, and moisture content and a higher pH, as the abundance of AD5 was also correlated to these soil parameters (Taş et al. 2014). It is likely that this candidate phyla, with no known culturable isolates, thrives in nutrient and moisture poor environments, as it was shown to increase in abundance with both depth and fire disturbance. Furthermore, the permafrost thawed soils had a different functional potential compared to intact permafrost. The thawed permafrost contained more genes for the hydrogenotrophic methanogenesis compared to aceticlastic methanogenesis in intact permafrost (Taş et al. 2014). However, regardless of the metabolic pathways, anaerobic incubations of permafrost soils demonstrated an overall reduction in CH_4 production in burned soils; this is potentially due to a reduced moisture content and thus reduced microbial activity and anaerobic niches (Taş et al. 2014). Nitrogen cycling genes were overall more abundant in the thawed permafrost compared to intact permafrost, including genes for nitrate assimilation and denitrification. However, genes coding for nitrite and nitrous oxide reductases were lower in the thawed permafrost soils; this suggests a potential for incomplete denitrification and release of N_2O upon thaw (Elberling et al. 2010; Taş et al. 2014).

Effects of Antarctic permafrost thaw on the microbial community and GHG emissions have not been studied. However, Buelow et al. (2016) have looked at the potential effects of permafrost thaw on McMurdo Dry Valley soils by simulating addition of water and organic matter to the arid Antarctic topsoils. Soils were dominated by Actinobacteria, Firmicutes, and Proteobacteria phyla (Buelow et al. 2016). Water and organic matter supplements increased bacterial abundance but decreased diversity suggesting that the region may experience loss of endemic dry-adapted oligotrophic taxa and replacement by generalist taxa with increasing temperatures due to climate warming (Buelow et al. 2016).

8.6.1 Methane Dynamics

Methane is ~20 times more potent that CO_2 as a GHG. Currently, it is uncertain the portion of stored organic carbon that will be released as CH_4 versus CO_2 (Mackelprang et al. 2016). The flux of methane from the soils is governed by the equilibrium of methanotrophs and methanogens. Methanogens are anaerobic archaea (part of Euryarchaeota) that are responsible for the biogenic production of CH_4. Methanotrophs are characterized by their ability to oxidize CH_4 and assimilate it as organic carbon (Hanson and Hanson 1996), belonging to the phyla Verrucomicrobia and Proteobacteria and are further classified as either

Type I methanotrophs belonging to the Gamaproteobacteria or Type II methanotrophs belonging to the Alphaproteobacteria (Conrad 2007). Furthermore, anaerobic oxidation of CH_4 is also possible via reverse methanogenesis by a group of ANME archaea related to methanogens (Knittel and Boetius 2009). Currently, wetland areas of the Arctic that are acting as methane sources and upland polar desert soils are acting as methane sinks (Christiansen et al. 2015; Lau et al. 2015). It is thought the emissions of CH_4 in peat and wetlands are currently offset by CH_4 uptake in upland dryer soils (Emmerton et al. 2014; Christiansen et al. 2015). However, this may not hold up as the climate warms and permafrost degrades. As the permafrost thaws, it not only introduces organic carbon into the deepening active layer, but also causes land surface collapse, changes in soil hydrology, and the formation of thermokarst bogs and wetlands with anoxic conditions (Johansson et al. 2006; Graham et al. 2012). These anoxic conditions promote the growth of methanogens and are favorable conditions for CH_4 production. So the question arises: what is the potential of permafrost to act as a CH_4 sink or source once it thaws?

Permafrost does contain an active community of methanotrophs and methanogens (Yergeau et al. 2010; Mackelprang et al. 2011; Allan et al. 2014; Deng et al. 2015), though there are conflicting results in terms of diversity of these organisms and no consensus as to how their abundance will be affected with permafrost thaw. Overall, the permafrost methanogen community is dominated by Methanococcales and Methanomicrobiales in mineral permafrost and *Methanosarcina*, *Methanoregula*, and *Methanobacterium* in methane containing permafrost (Allan et al. 2014; Shcherbakova et al. 2016). In contrast, active layer soil microbial community is dominated by Methanobacteriales, Methanosarcinales, Methanocellaceae, and Methanomicrobiaceae (Ganzert et al. 2007; Barbier et al. 2012). Methanogens' diversity and abundance increases with soil depth in permafrost, likely due to the anaerobic conditions in the subsurface (Stackhouse et al. 2015; Shcherbakova et al. 2016). Furthermore, methanogens increase in abundance and diversity with permafrost thaw and creation of wetlands; this may unfortunately increase the ratio of CH_4 to CO_2 flux from microbial decomposition of stored organic matter (Allan et al. 2014; McCalley et al. 2014). By looking at habitats at different stages of permafrost thaw, Hultman et al. (2015) observed higher abundance of methanogens in the thawed sites, possibly suggesting that even if the current permafrost soil do not harbor a large number of methanogens, they can be colonized by these organisms as the permafrost thaws. In addition to abundance, the thaw also stimulates methanogen activity, likely due to increase in temperature (Allan et al. 2014); this is reflected in a higher abundance of *mcrA* gene transcripts, a gene that catalyzes the final methanogenesis step (Coolen and Orsi 2015). Furthermore, upon thaw there is an increase in transcripts of the *fhs* gene which is part of the acetogenic fermentation pathway; this corresponded with an increase in the genes involved in the acetoclassic methanogenesis by *Methanosarcina barkeri* (able to utilize C1 and C2 compounds including acetate) (Coolen and Orsi 2015). This supported a previous finding by McCalley et al. (2014), who demonstrated that increased CH_4 emissions due to peatland permafrost thaw are associated with a switch from a hydrogenotrophic to acetoclastic methanogenesis. This was also reflected in the shift of dominant novel methanogen taxa from

Methanoflorens stordalenmirensis in partially thawed sites to members of *Methanosaeta* genus in fully thawed sites (McCalley et al. 2014; Mondav et al. 2014). However, this is in contrast to two studies that found higher abundance of hydrogenotrophic Methanobacteria members in the upper permafrost horizons compared to acetoclastic Methanomicrobia members in the lower permafrost horizons (Barbier et al. 2012; Deng et al. 2015). This could have been also due to a shift from organic more aerobic upper soils to more anoxic mineral lower permafrost soils (Mondav et al. 2014; Deng et al. 2015). In addition to thawing permafrost, there is evidence that methanogenesis is able to occur in frozen permafrost as well (Rivkina et al. 2007; Yergeau et al. 2010). When methane is not able to diffuse through the frozen soil, permafrost ends up accumulating methane that may be released rapidly into the atmosphere as the permafrost thaws (Rivkina et al. 2007; Mackelprang et al. 2011).

Whether permafrost thaw will have a significant impact on release of CH_4 into the atmosphere is also dependent on the response of methanotrophs. Permafrost and active layer soils harbor Type I, Type II, and Verrucomicrobia methanotrophs; however, these organisms are not ubiquitous across all permafrost soils (Yergeau et al. 2010; Christiansen et al. 2015, Stackhouse et al. 2015). The relative abundance of methanotrophs decreases with depth, possibly due to an increasingly anoxic environment (Stackhouse et al. 2015). The dominant methanotroph organisms in permafrost soils are members of Methylococcaceae, Methylocystaceae, and *Methylocapsa*, *Methylocella*, and *Methylacidiphilum* (Verrucomicrobia) (Yergeau et al. 2010; Deng et al. 2015; Stackhouse et al. 2015). Currently, organic rich permafrost and permafrost affected soils act as a methane source and mineral permafrost soils act as a methane sink, possibly due to the activity of high affinity methanotrophs (Christiansen et al. 2015; Lau et al. 2015). Though many studies predict increases in CH_4 releases with permafrost thaw (Mackelprang et al. 2016), Emmerton et al. (2014) suggests that the future changes in temperature and soil moisture content may increase CH_4 consumption in mineral well-drained permafrost affected soils, but at the same time will have minimal effects on increasing CH_4 production (Emmerton et al. 2014).

8.7 Conclusions

Despite permafrost being an inhospitable environment, it harbors an active microbial community in the Arctic, Antarctic, and at high altitudes. Next generation sequencing and -omics techniques have allowed us to fully grasp the diversity of microbial life in permafrost. This unique environment has also provided us with many microbial isolates that help us understand the physiological limits of life and the necessary adaptations needed to survive in such an extreme environment. In the more recent years, with the warming climate, it has become imperative to determine the effects of permafrost degradation on the microbial community. Understanding how the current microbial community is responding to this warming and subsequent GHG release is the focus of many current permafrost studies.

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