Permafrost and Periglacial Processes

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Microbial Ecology of Permafrost Soils: Populations, Processes, and Perspectives

Mark P. Waldrop[1] | Jessica Gilman Ernakovich[2] | Tatiana A. Vishnivetskaya[3] | Sean R. Schaefer[4] | Rachel Mackelprang[5] | Jiri Barta[6] | Joy M. O'Brien[7] | Matthias Winkel[8] | Robyn A. Barbato[9] | Liam Heffernan[10] | Mary-Cathrine Leewis[11] | Rebecca E. Hewitt[12] | Jenni Hultman[13] | Yanchen Sun[14] | Christina Biasi[15,16] | James A. Bradley[17,18] | Susanne Liebner[19] | Michael P. Ricketts[20] | Mario E. Muscarella[21] | Ursel Schütte[22] | Fumnanya Abuah[3] | Emily Whalen[23] | Ina Timling[22] | Carolina Voigt[24] | Neslihan Taş[25] | Karen G. Lloyd[3] | Henri M. P. Siljanen[26] | Elizaveta M. Rivkina[27] | Jana Voříšková[28] | Jing Tao[29] | Renxing Liang[30] | Zhen Li[31] | Jay T. Lennon[32] | T. C. Onstott[33,†]

Correspondence: Mark P. Waldrop (mwaldrop@usgs.gov)

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ABSTRACT

Permafrost microbial research has flourished in the past decades, due in part to improvements in sampling and molecular techniques, but also the increased focus on the permafrost greenhouse gas feedback to climate change and other ecological processes in high latitude and alpine permafrost soils. Permafrost microorganisms are adapted to these extreme environments and remain active at low temperatures and when resources are limited. They are also an important component of global elemental cycles as they regulate organic matter turnover and greenhouse gas production, particularly as permafrost thaws. Here we review the permafrost microbiology literature coupled with an exploration of its historical aspects, with a particular focus on a new understanding advanced by molecular biology techniques. We further identify knowledge gaps and ways forward to improve our understanding of microbial contributions to ecosystem biogeochemistry of permafrost-affected systems.

1 | Background and Justification

Permafrost-affected soils can be found in a wide variety of ecosystems including forests, shrublands, grasslands, and wetlands [1] and encompass an enormous diversity of soil types with varying physicochemical properties, landscape-level features, and permafrost disturbance history. While permafrost itself is defined as ground that has been frozen for two or more consecutive years [2], the collective term "permafrost-affected soils" describes landscapes that include permafrost, an active layer (seasonally frozen soil above the permafrost table), and degraded permafrost (no longer frozen year-round). As with all soils, permafrost varies widely in texture, structure, and chemical composition including organic matter quantity and quality. Permafrost at higher latitudes is distributed continuously towards the North Pole and discontinuously to sporadically towards its southern extent [3]; it can also be found in alpine environments including the Tibetan Plateau. Although they remain frozen, permafrost temperatures do fluctuate, influenced by local topography, climate, and the insulating effects of surface cover [4].

The first documented studies of microorganisms in permafrost date back to the early 20th century in Siberia [5]. From the first detection of bacteria in frozen mammoth and surrounding permafrost from Siberia until today, permafrost microbiology research has come a long way (Figure 1). This figure represents a visual display that scientific interest in studying life forms preserved inside permafrost has been increasing each year. Application of different bacteriological, microscopic, sequencing, and bioinformatic approaches resulted in uncovering novel taxonomic, functional, and metabolic diversity that is highlighted in the major milestones (Figure 1). A boom in

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For affiliations refer to page 8.

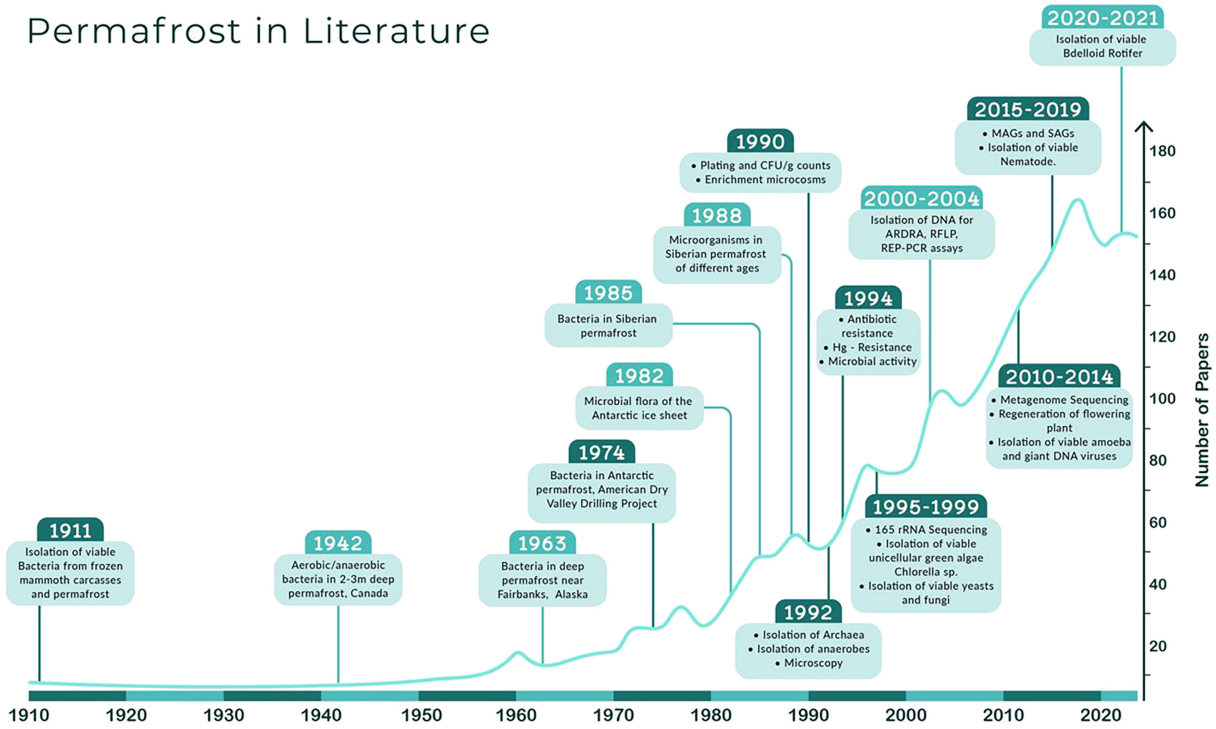

FIGURE 1 | Development of permafrost microbiology research for the last century. The number of papers reporting research of different aspects of microbial life and survivability in permafrost was obtained by keyword search in the Web of Science (webofscience.com; accessed August 2023). From the 1980s until his death in 2012, research in the field of permafrost microbiology, both in the Arctic and Antarctic, was associated with the name of David Gilichinsky. Papers for each of these milestones are listed in Appendix 1. [Colour figure can be viewed at wileyonlinelibrary.com]

permafrost microbiology research occurred between 1980 and 2000 highlighted by works of David Gilichinsky and other researchers who applied classical bacteriological methods to different permafrost sediments in northeastern Siberia. The review paper by Gilichinsky and Wagener [5] describes the history of uncovering microbial life in permafrost.

Since 2000, there has been a surge in permafrost microbiology research. Presently, we understand that, in simplified terms, permafrost hosts a wide variety of microbial taxa that are capable of surviving and thriving in unique ways, while carrying out processes that are critical to ecosystem biogeochemistry, especially when permafrost soils thaw. Despite the research surge, many open questions remain regarding the distribution and activity of permafrost microorganisms, and how they may affect soil processes, especially the permafrost carbon feedback.

Arctic warming is occurring more rapidly than elsewhere on the planet, and widespread permafrost thaw is expected to occur this century [6]. Permafrost thaw is one of the most concerning climate-forcing events occurring globally, as it causes numerous cascading shifts in the soil environment such as changes in soil temperatures, water content, redox processes, and nutrient availability that influences the taxonomic composition and activity of the soil microbiome [7–9]. All these changes directly contribute to greenhouse gas production, impacting the climate. Recent estimates suggest that thawing permafrost could release between 5% and 15% of the permafrost soil carbon pool, or around 146–160 Pg C, primarily in the form of microbially derived CO_2 and CH_4 by 2100 [10]. In additional, though currently less quantified, N_2O may also be released from these regions produced by microbial nitrification and denitrification processes [11], further intensifying the feedback between permafrost thaw and climate change.

Variation in the soil microbiome can affect soil processes in unpredictable ways [9, 12]. Northern ecosystems are shifting from carbon sinks to carbon sources, with high flux rates of CO_2, CH_4, and N_2O from soils to the atmosphere [13]. High variability around these mean flux rates [13] indicates that much more needs to be done to constrain the magnitude of the permafrost carbon feedback. More needs to be learned about the microbiology of intact (i.e., unthawed) permafrost, and how microbial communities, their taxonomic composition and activities, change following thaw. Because permafrost encompasses an enormous diversity of soil types, ice contents, ages, and physicochemical properties (Figure 2), care must be taken to limit extrapolation from individual studies mentioned in this review, as pattern and process may differ among locations due to the inherent permafrost variability.

2 | The Microbiology of Intact Permafrost

Most of the published data on the microbial biomass abundance in permafrost and active layer soils originate from Siberia [14–17] with some reports from Svalbard [18] and the Canadian high Arctic [19, 20]. They are based upon a variety of techniques including fluorescent DNA and protein stain direct counts [16, 18, 21], fluorescent in situ hybridization (FISH) [15], intact lipid analyses [22], and quantitative PCR [23–25]. Microbial abundance and diversity in permafrost affected soils is often

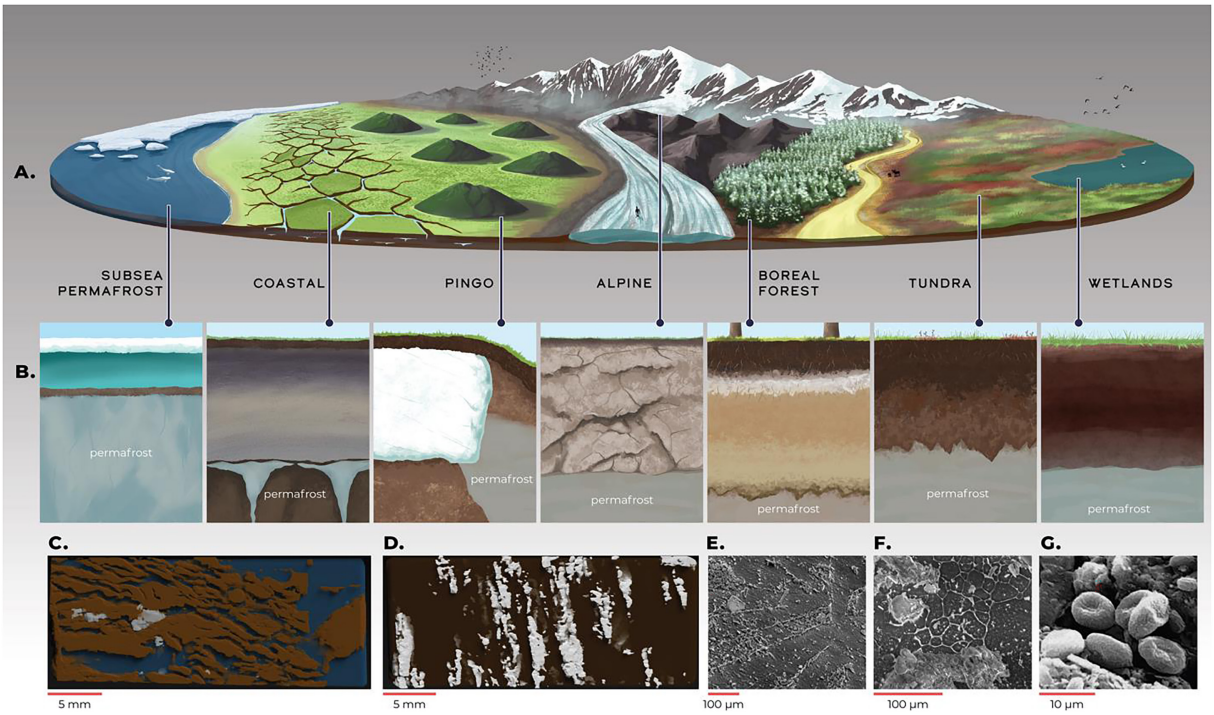

FIGURE 2 | Permafrost encompasses a diversity of landforms (panel A), which have implications for life at the microbial scale (panel B). Different landforms contain different chemical, mineralogical, and climatic features that affect the structure of microbial communities and their activity in permafrost. For example, permafrost could be a carbon rich frozen peatland or an iron rich mineral upland; this has implications for the diversity, identity, and abundance of microorganisms in permafrost and how they may respond to thaw. At the micron scale, where microbes reside, different textures and organic matter chemistries, among other factors, give rise to variation in microbial communities. (C) X-ray computed tomography (CT) scan of loess permafrost from above the Cold Regions Research and Engineering Lab (CRREL) Permafrost Tunnel in Fox, Alaska, (Photograph by Nathan Blais, University of New Hampshire, August 2021, sample source: Tom Douglas, Cold Regions Research and Engineering Lab; used with permission). In these post-processed images, soil is brown, ice is blue, and air is white. Notice the characteristic lenticular ice structure of this loess permafrost soil. (D) CT scan of permafrost from the Barrow Experimental Observatory in Utqiaġvik, Alaska, which is characterized by organic material over cryoturbated sand and silt (image source: Nathan Blais, sample source: Robyn Barbato; used with permission). In this organic permafrost soil, vertical air striations were observed. (E) Scanning electron micrograph (SEM) image of microbial cells on the surface of a permafrost particle. Lines of cells can be observed along cracks that likely were pushed out during the SEM preparation process that included deep freezing (source: K. Manies and J. Schulz, US Geological Survey). (F) SEM of a permafrost fragment showing 'patterned ground' at the micron scale (source: K. Manies and J. Schulz, US Geological Survey). (G) Microbial cells on the surface of permafrost mineral particles observed by the SEM (source: 10.1089/ast.2006.0012). [Colour figure can be viewed at wileyonlinelibrary.com]

correlated with soil nutrient availability (C & N content) and/or pH [15, 26]. Cell counts range from 10^7 to 10^9 cells/g with abundance generally declining with depth [20]. Cell counts within ground ice are much less, ranging from 10^4 to 10^5 cells/g [20]. Quantitative PCR, lipid, and FISH analyses indicate that Bacteria are the dominant domain followed by Eukaryotes and Archaea as a distant third [15, 22, 27, 28].

The prokaryotic community in permafrost soils comprises a diverse suite of bacteria and archaea capable of a multitude of metabolic pathways. Proteobacteria and Actinobacteria frequently dominate the permafrost community, and variation in the relative abundance of Acidobacteria, Bacteriodetes, Chloroflexi, and Firmicutes is frequently observed [8, 26, 29–31].

2.1 | Methane

Methanogens, archaea that produce methane, have been detected in deep permafrost (> 2 m) [32–35] and near-surface permafrost [8, 23, 25, 36, 37]. Methanogens from permafrost-affected soils can be active at subzero temperatures [22], but acetoclastic and hydrogenotrophic methanogens exhibit different temperature niches. Evidence exists that acetoclastic methanogens have lower temperature optima than hydrogenotrophic and methylotrophic communities [36], but this does not necessarily translate to predictable changes in the relative abundance of these groups with increasing temperature or permafrost thaw [7–9]. Increasing water availability due to permafrost thaw has been shown to trigger an increase in archaeal taxa such as *Methanoflorens* that are adapted to fluctuating water availability [38, 39] or *Methanothrix* and *Methanoregula* that are associated with wet conditions [38, 40]. In some permafrost areas, such as permafrost peatlands, methane fluxes can increase significantly, with rates shifting from 0.3 g CH_4-C m^{-2} $year^{-1}$ in permafrost peat to 3.9 g CH_4-C m^{-2} $year^{-1}$ in bogs and up to 19.2 g CH_4-C m^{-2} $year^{-1}$ in unfrozen fens along a thawing chronosequence [41].

Methane oxidation consumes a fraction of CH_4 produced in soils before it enters the atmosphere, thus mediating CH_4 emissions. In drier environments with low CH_4 concentrations, such as uplands, methanotrophs may also consume CH_4 from the

atmosphere to fulfill the C and energy needs [42]. Aerobic methanotrophic communities have been detected in active layer soils [43, 44], surface permafrost [25], and deep permafrost [45], with aerobic methane oxidation measurements by gas chromatography from slurries [43, 46], stable isotope probing [47] and radiotracer incubations [45], implying that O_2 must diffuse somewhat readily through the soil profile. A few studies also measured dissolved oxygen in the pore water of active layers [46]. Research has shown that the relative abundance of transcripts and proteins for methane oxidation varies between permafrost and active layers, indicating differences in microbial activity between these soil layers [48]. Methanotrophs can also thrive in the water column or in association with mosses [49] contributing to CH_4 consumption in permafrost environments [50]. In permafrost-affected soils, aerobic methanotrophs are mainly related to type Ia (*Methylobacter*, *Methylomonas*, *Methylomicrobium*, and *Methylosarcina*) and type IIb (*Methylocystis* and *Methylosinus*) methanotrophs [51]. However, the detection of unculturable methanotrophs, such as the Upland Soil Cluster (USC) α and γ, and rare organisms like *Methylococcus*, *Methylocaldum*, *Methylocapsa*, and *Methylocella*, using sequencing technologies is changing our understanding of the diversity and function of this group [51]. For example, metabolically active USC α have been detected in mineral cryosols, i.e., permafrost affected soils [52, 53] that are typically a sink for atmospheric CH_4 [54–56]. Aerobic methanotrophs are classified into groups by their CO_2 fixation pathways and affinity towards CH_4, where low-affinity methanotrophs typically operate in environments with high soil CH_4 availability and high-affinity methanotrophs at near ambient CH_4 concentrations. *Methylocystic* sp. SC2 and uncultured USC α and γ possess the high-affinity form of the particulate methane monoxygenase, allowing them to remove CH_4 at low (i.e., near atmospheric) concentrations [42]. Aerobic methanotrophs can survive in permafrost and perform methane oxidation at subzero temperatures (−5°C) [45]; however, higher temperatures promote methane oxidation [47, 52] to comparably high rates as measured for temperate wetlands [57]. Higher temperatures can also lead to a shift in the active community from type I to type II methanotrophs [57]. But besides the methane affinity and temperature influence on community shifts, not much is known about the driving factors that influence the structure and function of aerobic methanotrophic communities in permafrost.

Methane is also oxidized under anaerobic conditions in permafrost soils, generally at slower rates, but little is known about the diversity, function, and ecology of this group [58]. Anaerobic methane oxidation is thought to be performed by anaerobic methanotrophs (ANME) with close phylogenetic similarity to methanogens [59]. In terrestrial environments, the methanogen-related ANME *Methanoperedenaceae* is one of the most prominent representatives that have been detected frequently in permafrost [35, 60]. *Methanoperedenaceae* were recently linked to AOM in thawing deep submarine permafrost [58] and have also been shown to be active in deep thermokarst lakes [61]. However, these studies did not provide information on the distribution, niche, metabolic capacity, and potential syntrophic partners involved in the AOM. In addition, relatively little is known about their potential response to disturbance and climate change factors or their contribution to CH_4 cycling in thawing permafrost environments.

2.2 | Nitrogen Cycling Microorganisms

Recent research has also substantially advanced our understanding of the microbial involvement in nitrogen cycling in permafrost environments, particularly with the discovery of N_2O emission hotspots, where fluxes can reach up to 6 mg N_2O m^2 day^{-1} (comparable to those in fertilized soils) in permafrost regions [62–64]. Generally, nitrifier and denitrifier populations in the active layer and permafrost soils are abundant and similar in size to other soils [25, 66–67]. Gross ammonification and nitrification rates in active layers are, contrary to previous beliefs, of similar magnitude as observed in temperate and tropical systems, as highlighted in a recent synthesis [68]. Microbial nitrification has been identified as a key process in the nitrogen cycle in permafrost-affected environments, controlling the availability of dissolved inorganic nitrogen, which is a substrate for plant growth and denitrification, a process that produces N_2O [69]. In permafrost soils with low pH (2.8–4.0) and low organic carbon, ammonia-oxidizing archaea (AOA) dominate over ammonia-oxidizing bacteria (AOB) despite the latter's high abundance [70]. In N_2O hotspots of permafrost environments, new nitrate reducers have been isolated [70]. In these hotspots, *NosZ*, the gene catalyzing the reduction of N_2O to N_2, occurred at low frequencies (relative to narG, a gene coding for nitrate reductase). Sixty percent of *NosZ* were only distantly related to *NosZ* of cultured microorganisms indicating a new, specific, and acid-tolerant denitrifier community capable of N_2O reduction in these hotspots. Generally, the ratio of *nirS* + *nirK* (which catalyze the reduction of nitrite to nitric oxide) to *NosZ* correlates to N_2O reduction and N_2O emissions in permafrost-affected soils [63, 64], which can differ among soil types [64]. Most of what is known about N cycling microorganisms, however, is from active layer and/or recently thawed permafrost, and less is known about abundance or activity in intact permafrost.

2.3 | Fungi

Permafrost is a habitat for psychrophilic and psychrotrophic fungi that function as cryoprotected paleo-archives of fungi that were present in ancient ecosystems [71–73]. The extreme environment likely led to the development of extremotolerant fungi with high adaptive potential [24, 71–73]. Diversity measures range from 80 species based on culturing measures [71–73] to a Chao1 index of ~700 taxa based on sequencing with the Chao1 richness estimates being only slightly lower than the richness in the active layer [24]. Extremophilic fungi include a majority of known lifestyles including cold-adapted yeasts, filamentous, micromycetes, darkly pigmented fungi, lichenized fungi, ectomycorrhizal fungi, endophytic fungi, pathogens, and saprotrophs [73, 74]. Similar to bacterial communities in soils, most of the fungal taxa are rare and only few are present in proportions > 1% [24]. The few studies that have assessed fungal communities in permafrost include locations from the Arctic (Alaska, Canada [24], and Siberia [23, 74]), Antarctic (terrestrial [72] and maritime [75]), European alpine [76], Tibetan plateau [77], and North Eastern China [78]. Varsadiya et al. [24] carried out one of the more comprehensive studies on fungal communities in permafrost in Canadian Arctic cryosols and noted that fungal community composition and

the fungal guilds changed with depth. Permafrost had ~1000 fold lower fungal abundance based on gene copy numbers compared with the active layer. Permafrost samples contained lower proportions of the root-associated genus *Meliniomyces*, but increased in proportions of *Naganishia*, a genus known to have high resistance to UV radiation [79], *Piloderma*, and the ubiquitous saprotrophic yeast *Rhodotorula* [80]. With regard to fungal guilds, permafrost contained increased proportions of ectomycorrhizal fungi and wood saprotrophs, comparable proportions of plant pathogens and soil saprotrophs, but decreased proportions of root endophytes [24].

Although most major fungal phyla are present in permafrost, Ascomycota and Basidiomycota are the most dominant, followed by Chytridiomycota and Mucoromycota [24, 71–74, 76, 81]. There is no evidence of arbuscular mycorrhizal fungi (AMF) being present in intact permafrost. However, this may be due to primer pair selection during PCR reaction or because the Internal Transcribed Spacer (ITS) region commonly used to describe fungal communities is less suitable to describe AMF communities. Many AMF remain undescribed and are highly underrepresented and sequences are often poorly curated in the ITS database [82] for describing fungal communities in permafrost. This is partly due to primer selection: No AMF sequences were detected in the active layer of intact and thawed permafrost soils in Alaskan peatlands when sequencing the ITS region, but a diverse AMF community was detected when targeting the large subunit (LSU) region [83]. As with prokaryotes, the abundance of fungi in permafrost is lower than in the active layer [23, 71, 81]; however, fungal rRNA transcripts in permafrost can increase to levels comparable to those found in active layers upon thaw [84].

2.4 | Pathogens

Potentially pathogenic organisms are also entrained in permafrost [85, 86], resulting in much media attention [87]. Because there are possible risks to global public health, more research could enhance understanding of pathogen behavior in intact and thawing permafrost. Pathogens could affect humans, animals, and plants, though the pathogens present and the risks to human health from thawing permafrost are likely similar to those posed by non-permafrost soils [88, 89]. Pathogens likely are treatable with modern antibiotics as the bacterial pathogens that may be emerging out of thawing permafrost are likely naive to modern antibiotics, reducing the risk of widespread disease outbreaks. More pressing concerns stem from increased expansion and winter survival of disease vectors' range [90], which is exacerbated by health disparities between Indigenous and non-Indigenous people [91].

A central question for risk assessment is whether intact viruses survive decades to millennia entrained in permafrost. Attempts, however, to isolate infectious smallpox and 1918 flu viruses and to recover long nucleic acid fragments (as a proxy for intact virus) have failed [92, 93], suggesting that survival of these human-infectious viruses may be limited in permafrost and that concerns about their release to a susceptible and vulnerable populace may not be as warranted.

Survival of viruses that infect non-human hosts is also important for wildlife managers. Most viruses identified in cold soils infect bacterial hosts and do not pose a direct risk to human health [94, 95], so those targeting non-human eukaryotic hosts are a more appropriate proxy. The most well-known example is the revival of "giant" viruses from ancient permafrost that infect *Acanthamoeba* (widespread amoebae) [85, 96]. In contrast to viruses such as the 1918 Flu virus, these viruses may be better adapted to harsh environments due to the formation of rugged stable capsids [97] and for their hosts to form robust cysts, resulting in resistance to extremes in temperature and pH [98, 99]. Cysts formed during infection can act as a reservoir, protecting the virus and enabling reemergence under favorable conditions.

For permafrost viruses to infect humans or for a human-infectious virus to remain intact even during thaw before encountering a susceptible host, it must undergo a host shift. While the possibility of these events cannot be entirely disregarded, the risks of such occurrences are likely low. The evolutionary distance between microeukaryotes and humans is a substantial barrier to host-shifting as successful host-shifting decreases as the phylogenetic distance between hosts increases [100]. Similar to the giant *Acanthamoeba*-infecting viruses, microeukaryotes in cold soils and their viruses have co-evolved in harsh conditions through geologic time [101], explaining why they survive in permafrost. Viruses with human hosts do not have this evolutionary history. They lack cold adaptations and a host that facilitates persistence.

Many microbes such as *Bacillus anthracis* form spores and can persist for long periods of time in inhospitable environments including permafrost. The best-described outbreak was in 2016 in Siberia. It killed thousands of reindeer, sickened dozens of humans, and killed one [102]. However, it is likely that permafrost thaw alone is not enough to cause disease, and that multiple factors have to act synergistically for a disease outbreak to occur. Environmental conditions have to be conducive for the pathogen to survive and human behavior including reduced animal and human vaccination and an increase of herd size may be key to facilitating disease spread [102].

We know little about plant pathogens in permafrost and even less about how thaw affects aspects such as their survival and being competitive against the members of the existing soil microbiome in the active layer. Recent studies on the effects of thaw on changes in microbial communities in the active layer in Interior Alaska found that the active layer of thawed sites contains higher proportions of putative plant pathogens and a lower proportions of plant beneficial microbes [83, 103]. The changes in microbial communities as a result of thaw were associated with a decrease in plant productivity of key boreal plant species, in particular mycorrhizal dependent plant species [83, 103]. The risk that plant pathogens present in permafrost pose to causing widespread mortality is unknown, but the risk from globally changing disease spread and virulence of plant pathogens may pose a higher risk to ecosystems [104, 105]. It is more likely that plant pathogens in permafrost affected soils will play a central role in regulating plant diversity, coexistence and productivity similar to what has been shown to occur in other ecosystems [106–108].

2.5 | Activity Below Freezing

Surprisingly, many microorganisms are metabolically active in permafrost [17, 109–111]. Even below zero, permafrost contains liquid water in thin brine channels surrounding soil particles [112]. Microbial activity in permafrost relies on the liquid water in these thin films to supply resources and energy. RNA and protein signatures from Alaskan permafrost together with metagenome sequences show that stress and survival genes are important for survival in permafrost [66]. However, the quantity and diversity of transcripts and peptides was several fold higher in thermokarst bog and active layer soils analyzed in the same study. The measured process rates correlated well with the molecular data, for example methanogenesis and methane oxidation in thermokarst, but in intact permafrost similar observations with the process rates were not observed, possibly due to the frozen state and low activity of permafrost microbiota. Other studies have correlated changes in the molecular composition of dissolved organic matter in permafrost with concomitant shifts in the relative gene abundance of carbohydrate-active enzyme families, hydrogenotrophic methanogenesis and genes associate with fermentation of short chain fatty acids [113]. Together, this implies that active microbial communities affect the chemistry of ancient permafrost carbon over millenia.

2.6 | Adaptations by the Permafrost Community

Permafrost microorganisms employ a suite of adaptations to counter the physical and chemical stresses for sustaining growth and survival under frozen conditions [29]. These have been studied primarily in bacteria isolated from permafrost and include increased membrane fluidity [14, 114], amino acid composition favoring protein flexibility [115], increased copy number of stress-related genes [116], production of cold and osmotic stress proteins [117], and temperature-related translation factors [118]. Although culture-based studies have demonstrated the existence of these strategies, their relevance to the survival of microbial populations in situ remains unknown. Recently emerged multiomic strategies are now enabling direct interrogation of community processes relevant to the survival of microbial populations in situ. These data show genes, proteins, and transcripts involved in stress response, survival, and maintenance [7, 8, 66, 78]. Mackelprang et al. [119] found evidence of continued adaptation to the permafrost environment through geologic time, and that community survival strategies include cell envelope synthesis and maintenance, increased reliance on horizontal gene transfer, environmental sensing, chemotaxis, stress response, and scavenging of detrital biomass. This significant diversity of microbial adaptative and metabolic strategies may reflect the diversity of permafrost parent material and age [26]. As active layers deepen and permafrost thaws, it will be important to understand the in situ community and how it may change with changing conditions.

3 | Microbial Communities and Thawing Permafrost Soils

Fungal, bacterial, archaeal, and viral propagules in permafrost may become active when physical conditions are ameliorated upon thaw [8, 73, 84]. The first papers to investigate the microbiome in thawing (lab incubated) permafrost found that active communities shift rapidly (e.g., within days) in response to thaw, as do their C and N cycling functional responses [7, 8, 28, 84]. However, the thaw-induced functional response of microorganisms varies between studies and even within studies [8]. For instance, following 7 days of thaw at 5°C, Mackelprang et al. [8] found an increase in Actinobacteria, Choloroflexi, Methanosarcinales, Methanomicrobiales, Methanomicrobia, and Methanobacterales. Coolen et al. [84] found an increase in beta-Proteobacteria with thaw, mixed evidence for the disappearance of Actinobacteria, and mixed evidence for an increase in Firmicutes within deeper permafrost layers. Thaw increased the relative abundance of fungi from classes Leotiomycetes, Saccharomycetes, Malasseziomycetes, and Eurotopmycetes [120].

In field experiments, the effects of permafrost thaw on microbial communities and microbially mediated biogeochemical processes are dependent on site hydrology and subsequent effects on soil moisture, oxygen availability, and vegetation community (Figure 3). Simply put, when permafrost thaws, some soils can become wetter, forming thermokarst bogs and fens, whereas others may become dryer, resulting in deeper active layers with decreased water availability (Figure 3). In the "dry scenario," vegetation may shift from tussocks to woody shrubs (i.e., shrubification [127]). This shift may result in the development of a more densely interconnected fungal network due to an increase of mycorrhizal-obligate shrubs [83]. Because soil moisture largely controls redox conditions, it acts as a master variable following thaw, promoting aerobic production of CO_2 in well-drained soils where oxygen is available, and anaerobic production of CO_2, N_2O, and CH_4 in hypoxic, water-logged soils [11, 64, 128, 129]. Soil organic matter decomposition in water-logged soils is substantially slower [130], but because CH_4 and N_2O are more powerful greenhouse gases compared with CO_2 the net effect of soil saturation on the permafrost–climate feedback remains uncertain [131, 132]. Saturated conditions can result in the increased abundance of methanogens and methanotrophs [133]; however, sometimes methanogens may be in very low abundance and take a long time to establish following thaw [12, 134, 135], lowering CH_4 production rates. Multiple studies show that permafrost may contain such low abundance of important microbial functional groups as to limit rates of greenhouse gas production [12, 28, 38, 49, 136]. However, research has also shown that the mixing of the active layer with underlying permafrost can alleviate these functional limitations, thereby enhancing microbial activity and greenhouse gas production [130]. Currently, permafrost regions are experiencing a substantial shift in their carbon and nitrogen dynamics. Many areas are transitioning from functioning as CO_2 sinks to becoming sources of CO_2 due to increased soil organic matter decomposition [13, 137], and the potential for CH_4 and N_2O emissions is anticipated to rise as climate warming progresses and permafrost continues to thaw [11, 138, 139].

Thawing permafrost-affected soils show vigorous nitrogen cycling activity, supported by a rich functional microbial community in both active layer and permafrost soils [68]. This may be due in part to the release of N limitation after thaw [140]. Generally, N_2O is produced during the denitrification processes when nitrate is used as the terminal electron acceptor in

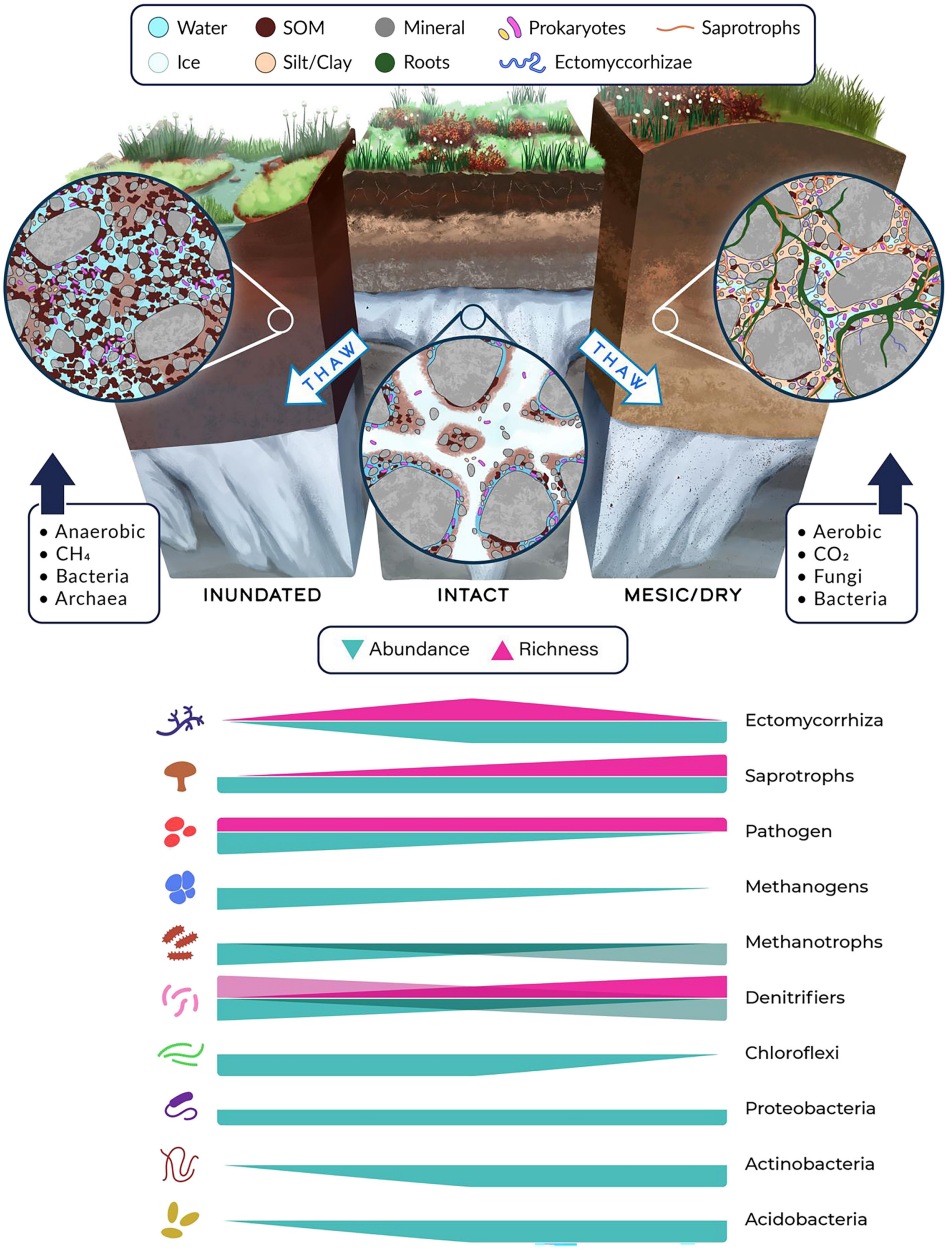

FIGURE 3 | In a simple conceptual model, when permafrost thaws, soils can become saturated (e.g., thermokarst bogs and fens) or drier (e.g., in uplands, water is lost through gravelly terrain), which results in several hypothesized shifts in microbial communities. Changes in the relative abundance and diversity of different microbial groups are based on relationships observed by several investigators [38, 46, 121–126]. Pop outs characterize likely changes in the soil environment at the microbial scale. [Colour figure can be viewed at wileyonlinelibrary.com]

microbial degradation of organic matter. High N_2O production is characterized by a high abundance of a diverse nitrifier and denitrifier community [63, 128]. Ammonia oxidizing Archaea dominate the nitrifier community of Arctic soils [65, 67, 141]; however, N_2O emitting Arctic peat soils have only ammonia oxidizing Archaea, a really narrow diversity of zeta and gamma clades of *Nitrososphaerales* [69]. Truncated denitrification, i.e., denitrification process where microorganisms carry only a fraction of the needed genes, has been shown to be abundant in active layer soils via population genome reconstruction [142]. The completeness of the denitrification pathway and use of the metabolic handoffs among microbial organisms cause soil to be a N_2O sink or a source. Effects of warming on the microbial communities involved in N_2O production and consumption processes have rarely been studied, but a handful of studies have shown either surprisingly few changes [67] or significant changes in abundance of genes involved in the nitrogen cycle [8, 143] and their transcripts [66]. In addition to denitrification, microbes can produce N_2O via fermentative dissimilatory nitrate/nitrite reduction to ammonium (DNRA); however, knowledge of the abundance and activity of these microbes in thawing permafrost soils is still lacking. With anoxic thermokarst formation, the fate of nitrogen from permafrost and overlying soils can change and have a major role in the global N budget [11, 144]. The N cycling microbial community will change with permafrost thaw as mixing with surface soil seems to play a critical role in mitigating functional limitations, e.g., nitrification (e.g., [136]). During this process, microbes from the active layer

may disperse and colonize the permafrost layers, upending any functional limitation, but these are scientific questions to be studied [145].

4 | Enhancing our Understanding of Microbially Mediated Processes in Permafrost Soils

Over the past several decades, we have improved our understanding of permafrost microbial communities and their response to thaw, but we are still far from incorporating this understanding into biogeochemical models of permafrost ecosystems and earth system models [146]. Although some attempts have shown promise [147], these efforts remain limited. Below, we describe some of the factors that limit our understanding and therefore predictive ability of microbially mediated processes in permafrost soils.

Microbially mediated biogeochemical processes occur at multiple scales, from cells to soil pores, pods to plots, and ecosystems to landscapes. Although microbial metabolic processes ultimately control many biogeochemical processes, the different temporal dynamics of metabolic processes, microbial community reorganization, and interactions with soil edaphic properties means that microbial processes measured at one scale may not necessarily link to biogeochemical processes at another scale. For example, at the scale of the cell, DNA transcription to RNA and RNA translation into proteins are important processes controlling cellular metabolism. These processes are highly dynamic and change from second to minute and minute to hour. Transcription informs us about the immediate response of microbial communities to thaw [7, 8, 148], but are less informative about the annual or decadal rates of change until more is known about diurnal and seasonal changes in functional response. Furthermore, permafrost thaw occurs heterogeneously across the landscape because ice content of permafrost varies spatially [149]. The natural heterogeneity of processes such as heat transfer and permafrost degradation are poorly reflected in microcosm or mesocosm laboratory incubations and require landscape-scale measurements of microbial processes in the field to provide insight into the natural variance of the system during thaw [26, 38, 129, 150].

Timescales at which changes in microbial community composition occur differs between permafrost soils and thawing permafrost soils. In intact permafrost, microbial communities are likely fairly static at annual scales, but at scales of centuries and millennia microbial communities may change their resource acquisition strategies [113] while compositionally they can continue to reflect their ecological legacy [26]. Once thawed, changes in community composition will likely occur as changes in energetics and resources is rapid [8], and changes in community composition will likely be tied to changes in energy and resources at annual to decadal timescales. Post-thaw, seasonal changes in deep (> 2 m) permafrost microbial communities are relatively unexplored but might be minimal due to relatively low-temperature fluctuations in deep soils. One interesting area of research could be examining the immigration of new microbial communities into newly thawed permafrost as permafrost contains relatively fewer cells than overlying active layer soils. For instance, the mixing of rhizosphere and native permafrost communities could lead to distinctly new microbial communities and functions. Additionally, in high ice permafrost, thaw could result in rapid mixing of the active layer with permafrost silts below, resulting in rapid immigration and community coalescence. Whereas in low ice upland permafrost soils, there may be minimal mixing of the surface and deeper soils, resulting in slower community change.

At ecosystem to landscape scales, spatial variability is likely to be at least as important as understanding temporal variability in the microbial community response to thaw. Total and active soil microbial communities are driven by soil type [151], and permafrost should be no exception. Permafrost landscapes are derived from geologic processes resulting in a wide variety of ecosystems including fens, bogs, forests, and shrublands each with a unique disturbance history [152] resulting in diverse permafrost characteristics (Figure 2). It should be expected that unique microbial communities will be found among different permafrost soils [26], but the bigger question is whether this unique character is necessarily tied to the current ecosystem, or is it reflective of the multitudinous events of its past or paleoecology, which defines its vegetation, disturbance, and geological history. Because permafrost formation entrains microbial communities in soil, microbial communities in permafrost are likely to be at least somewhat reflective of the environment in which they were deposited and frozen into years ago. Thus, it can become important to not only understand the current ecosystem state that is undergoing thaw, but the paleoecology of the site may hold important information about the types of microorganisms found there. Moreover, one question to examine then is whether the paleoecology of a site might dictate the ecosystem response to thaw due to the unique microorganisms assembled there. The degree to which microorganisms present in intact permafrost may impact soil dynamics post-thaw is not well understood, but likely will depend on an improved understanding of the spatial distribution of microbial communities, their functional potential, and their response to various thaw conditions.

Affiliations

[1]Geology, Minerals, Energy, and Geophysics Science Center, U.S. Geological Survey, California, USA | [2]Natural Resources and the Environment, Center of Soil Biogeochemistry and Microbial Ecology, University of New Hampshire, Durham, New Hampshire, USA | [3]Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA | [4]Natural Resources and Earth Systems Science, University of New Hampshire, Durham, New Hampshire, USA | [5]Department of Biology, California State University Northridge, Northridge, California, USA | [6]Centre for Polar Ecology, University of South Bohemia, České Budějovice, Czech Republic | [7]Department of Biology, Indiana University Bloomington, Bloomington, Indiana, USA | [8]Department of Food Safety, German Federal Institute for Risk Assessment, Berlin, Germany | [9]Biogeochemical Sciences Branch, Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire, USA | [10]Vrije Universiteit, Amsterdam, The Netherlands | [11]Agriculture and Agri-Food Canada, Quebec City, QC, Canada | [12]Department of Environmental Studies, Amherst College, Amherst, Massachusetts, USA | [13]Natural Resources Institute Finland, Helsinki, Finland | [14]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Falmouth, Massachusetts, USA | [15]Department of Ecology, University of Innsbruck, Innsbruck, Austria | [16]Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio,

Finland | [17]Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO, Marseille, France | [18]School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK | [19]GFZ Helmholtz Centre for Geosciences, Section Geomicrobiology, Potsdam, Germany | [20]Environmental Science Division, Argonne National Laboratory, Lemont, Illinois, USA | [21]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska, USA | [22]University of Alaska Fairbanks, Fairbanks, Alaska, USA | [23]University of New Hampshire, Durham, New Hampshire, USA | [24]Institute of Soil Science, University of Hamburg, Hamburg, Germany | [25]Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA | [26]Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland | [27]Laboratory of Soil Cryology, Institute of Physicochemical and Biological Problems in Soil Science, Moscow, Russia | [28]Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic | [29]Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA | [30]State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, China | [31]Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, California, USA | [32]Department of Biology, Indiana University Bloomington, Bloomington, Indiana, USA | [33]Princeton University, Princeton, New Jersey, USA

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

1. M. T. Jorgenson, K. Yoshikawa, M. Kanevskiy, et al., "Permafrost Characteristics of Alaska," in *Proceedings of the Ninth International Conference on Permafrost* (Fairbanks, Alaska: Institute of Northern Engineering, University of Alaska Fairbanks, 2008): 121–122.

2. R. O. Van Everdingen, *Multi-Language Glossary of Permafrost and Related Ground-Ice Terms* (Calgary, Alberta, CA: International Permafrost Association, 2005), https://globalcryospherewatch.org/glossary/.

3. J. Heginbottom, J. Brown, O. Ferrians, and E. S. Melnikov, "Circum-Arctic Map of Permafrost and Ground-Ice Conditions, Version 2," (2002), https://doi.org/10.7265/SKBG-KF16.

4. S. L. Smith, H. B. O'Neill, K. Isaksen, J. Noetzli, and V. E. Romanovsky, "The Changing Thermal State of Permafrost," *Nature Reviews Earth and Environment* 3 (2022): 10–23, https://doi.org/10.1038/s43017-021-00240-1.

5. D. Gilichinsky and S. Wagener, "Microbial Life in Permafrost: A Historical Review," *Permafrost and Periglacial Processes.* 6, no. 243 (1995): 250.

6. M. Rantanen, A. Y. Karpechko, A. Lipponen, et al., "The Arctic Has Warmed Nearly Four Times Faster Than the Globe Since 1979," *Communications Earth & Environment* 3, no. 1–10 (2022): 3.

7. M. J. L. Coolen and W. D. Orsi, "The Transcriptional Response of Microbial Communities in Thawing Alaskan Permafrost Soils," *Frontiers in Microbiology* 6 (2015): 545.

8. R. Mackelprang, M. P. Waldrop, K. M. DeAngelis, et al., "Metagenomic Analysis of a Permafrost Microbial Community Reveals a Rapid Response to Thaw," *Nature* 480 (2011): 368–371.

9. A. T. Tveit, T. Urich, P. Frenzel, and M. M. Svenning, "Metabolic and Trophic Interactions Modulate Methane Production by Arctic Peat Microbiota in Response to Warming," *Proceedings of the National Academy of Sciences* 112 (2015): E2507–E2516.

10. E. A. G. Schuur, B. W. Abbott, R. Commane, et al., "Permafrost and Climate Change: Carbon Cycle Feedbacks From the Warming Arctic," *Annual Review of Environment and Resources* 47 (2022): 343–371, https://doi.org/10.1146/annurev-environ-012220-011847.

11. C. Voigt, M. E. Marushchak, R. E. Lamprecht, et al., "Increased Nitrous Oxide Emissions From Arctic Peatlands After Permafrost Thaw," *Proceedings of the National Academy of Sciences of the United States of America* 114 (2017): 6238–6243.

12. J. G. Ernakovich, L. M. Lynch, P. E. Brewer, F. J. Calderon, and M. D. Wallenstein, "Redox and Temperature-Sensitive Changes in Microbial Communities and Soil Chemistry Dictate Greenhouse Gas Loss From Thawed Permafrost," *Biogeochemistry* 134 (2017): 183–200.

13. J. Ramage, M. Kuhn, A.-M. Virkkala, et al., "The Net GHG Balance and Budget of the Permafrost Region (2000–2020) From Ecosystem Flux Upscaling," *Global Biogeochemical Cycles* 38 (2024): e2023GB007953.

14. C. Bakermans, A. I. Tsapin, V. Souza-Egipsy, D. A. Gilichinsky, and K. H. Nealson, "Reproduction and Metabolism at −10°C of Bacteria Isolated From Siberian Permafrost," *Environmental Microbiology* 5 (2003): 321–326.

15. S. Kobabe, D. Wagner, and E.-M. Pfeiffer, "Characterisation of Microbial Community Composition of a Siberian Tundra Soil by Fluorescence in Situ Hybridisation," *FEMS Microbiology Ecology* 50 (2004): 13–23.

16. E. Rivkina, D. Gilichinsky, S. Wagener, J. Tiedje, and J. McGrath, "Biogeochemical Activity of Anaerobic Microorganisms From Buried Permafrost Sediments," *Geomicrobiology Journal* 15 (1998): 187–193.

17. E. M. Rivkina, E. I. Friedmann, C. P. McKay, and D. A. Gilichinsky, "Metabolic Activity of Permafrost Bacteria Below the Freezing Point," *Applied and Environmental Microbiology* 66 (2000): 3230–3233.

18. A. A. Hansen, R. A. Herbert, K. Mikkelsen, et al., "Viability, Diversity and Composition of the Bacterial Community in a High Arctic Permafrost Soil From Spitsbergen, Northern Norway," *Environmental Microbiology* 9 (2007): 2870–2884.

19. B. Steven, G. Briggs, C. P. McKay, W. H. Pollard, C. W. Greer, and L. G. Whyte, "Characterization of the Microbial Diversity in a Permafrost Sample From the Canadian High Arctic Using Culture-Dependent and Culture-Independent Methods," *FEMS Microbiology Ecology* 59 (2007): 513–523.

20. B. Steven, W. H. Pollard, C. W. Greer, and L. G. Whyte, "Microbial Diversity and Activity Through a Permafrost / Ground Ice Core Profile From the Canadian High," *Arctic* 10 (2008): 3388–3403.

21. D. Gilichinsky, E. Rivkina, V. Shcherbakova, K. Laurinavichuis, and J. Tiedje, "Supercooled Water Brines Within Permafrost-An Unknown Ecological Niche for Microorganisms: A Model for Astrobiology," *Astrobiology* 3 (2003): 331–341.

22. D. Wagner, A. Gattinger, A. Embacher, E.-M. Pfeiffer, M. Schloter, and A. Lipski, "Methanogenic Activity and Biomass in Holocene Permafrost Deposits of the Lena Delta, Siberian Arctic and Its Implication for the Global Methane Budget," *Global Change Biology* 13 (2007): 1089–1099.

23. A. Gittel, J. Bárta, I. Kohoutová, et al., "Distinct Microbial Communities Associated With Buried Soils in the Siberian Tundra," *ISME Journal* 8 (2014): 841–853.

24. M. Varsadiya, T. Urich, G. Hugelius, and J. Bárta, "Microbiome Structure and Functional Potential in Permafrost Soils of the Western Canadian Arctic," *FEMS Microbiology Ecology* 97 (2021): 97, https://doi.org/10.1093/FEMSEC/FIAB008.

25. E. Yergeau, H. Hogues, L. G. Whyte, and C. W. Greer, "The Functional Potential of High Arctic Permafrost Revealed by Metagenomic Sequencing, qPCR and Microarray Analyses," *ISME Journal* 4 (2010): 1206–1214.

26. M. P. Waldrop, C. L. Chabot, S. Liebner, et al., "Permafrost Microbial Communities and Functional Genes Are Structured by Latitudinal and Soil Geochemical Gradients," *ISME Journal* 17 (2023): 1224–1235.

27. T. A. Vishnivetskaya, A. C. Layton, M. C. Y. Lau, et al., "Commercial DNA Extraction Kits Impact Observed Microbial Community Composition in Permafrost Samples," *FEMS Microbiology Ecology* 87 (2014): 217–230.

28. M. P. Waldrop, K. P. Wickland, R. White, A. A. Iii Berhe, J. W. Harden, and V. E. Romanovsky, "Molecular Investigations Into a Globally Important Carbon Pool: Permafrost-Protected Carbon in Alaskan Soils," *Global Change Biology* 16 (2010): 2543–2554.

29. J. K. Jansson and N. Taş, "The Microbial Ecology of Permafrost," *Nature Reviews. Microbiology* 12 (2014): 414–425.

30. R. Mondav, C. K. McCalley, S. B. Hodgkins, et al., "Microbial Network, Phylogenetic Diversity and Community Membership in the Active Layer Across a Permafrost Thaw Gradient," *Environmental Microbiology* 19 (2017): 3201–3218.

31. R. C. Wilhelm, T. D. Niederberger, C. Greer, and L. G. Whyte, "Microbial Diversity of Active Layer and Permafrost in an Acidic Wetland From the Canadian High Arctic," *Canadian Journal of Microbiology* 57 (2011): 303–315.

32. J. Bischoff, K. Mangelsdorf, A. Gattinger, et al., "Response of Methanogenic Archaea to Late Pleistocene and Holocene Climate Changes in the Siberian Arctic," *Global Biogeochemical Cycles* 27 (2013): 305–317, https://doi.org/10.1029/2011GB004238.

33. K. V. Krivushin, V. A. Shcherbakova, L. E. Petrovskaya, and E. M. Rivkina, "Methanobacterium Veterum sp. nov., From Ancient Siberian Permafrost," *International Journal of Systematic and Evolutionary Microbiology* 60 (2010): 455–459.

34. E. Rivkina, V. Shcherbakova, K. Laurinavichius, et al., "Biogeochemistry of Methane and Methanogenic Archaea in Permafrost," *FEMS Microbiology Ecology* 61 (2007): 1–15.

35. V. Shcherbakova, Y. Yoshimura, Y. Ryzhmanova, et al., "Archaeal Communities of Arctic Methane-Containing Permafrost," *FEMS Microbiology Ecology* 92 (2016): fiw135.

36. J. Allan, J. Ronholm, N. C. S. Mykytczuk, C. W. Greer, T. C. Onstott, and L. G. Whyte, "Methanogen Community Composition and Rates of Methane Consumption in Canadian High Arctic Permafrost Soils," *Environmental Microbiology Reports* 6 (2014): 136–144.

37. D. A. Lipson, J. M. Haggerty, A. Srinivas, T. K. Raab, S. Sathe, and E. A. Dinsdale, "Metagenomic Insights Into Anaerobic Metabolism Along an Arctic Peat Soil Profile," *PLoS ONE* 8 (2013): e64659.

38. C. K. McCalley, B. J. Woodcroft, S. B. Hodgkins, et al., "Methane Dynamics Regulated by Microbial Community Response to Permafrost Thaw," *Nature* 514 (2015): 478–481.

39. R. Mondav, B. J. Woodcroft, E.-H. Kim, et al., "Discovery of a Novel Methanogen Prevalent in Thawing Permafrost," *Nature Communications* 5 (2014): 3212.

40. N. Taş, E. Prestat, S. Wang, et al., "Landscape Topography Structures the Soil Microbiome in Arctic Polygonal Tundra," *Nature Communications* 9 (2018): 777.

41. M. E. Holmes, P. M. Crill, W. C. Burnett, et al., "Carbon Accumulation, Flux, and Fate in Stordalen Mire, a Permafrost Peatland in Transition," *Global Biogeochemical Cycles* 36 (2022): e2021GB007113.

42. R. S. Hanson and T. E. Hanson, "Methanotrophic Bacteria," *Microbiology and Molecular Biology Reviews: MMBR* 60 (1996): 439–471.

43. S. Liebner and D. Wagner, "Abundance, Distribution and Potential Activity of Methane Oxidizing Bacteria in Permafrost Soils From the Lena Delta, Siberia," *Environmental Microbiology* 9 (2007): 107–117.

44. M. S. Vecherskaya, V. F. Galchenko, E. N. Sokolova, and V. A. Samarkin, "Activity and Species Composition of Aerobic Methanotrophic Communities in Tundra Soils," *Current Microbiology* 27 (1993): 181–184.

45. V. N. Khmelenina, V. A. Makutina, M. G. Kalyuzhnaya, E. M. Rivkina, D. A. Gilichinsky, and Y. A. Trotsenko, "Discovery of Viable Methanotrophic Bacteria in Permafrost Sediments of Northeast Siberia," *Doklady Biological Sciences: Proceedings of the Academy of Sciences of the USSR, Biological Sciences Sections/Translated From Russian* 384 (2002): 235–237.

46. C. R. Perryman, C. K. McCalley, A. Malhotra, et al., "Thaw Transitions and Redox Conditions Drive Methane Oxidation in a Permafrost Peatland," *Journal of Geophysical Research Biogeosciences* 125 (2020): e2019JG005526.

47. C. Martineau, L. G. Whyte, and C. W. Greer, "Stable Isotope Probing Analysis of the Diversity and Activity of Methanotrophic Bacteria in Soils From the Canadian High Arctic," *Applied and Environmental Microbiology* 76 (2010): 5773–5784.

48. R. Margesin and T. Collins, "Microbial Ecology of the Cryosphere (Glacial and Permafrost Habitats): Current Knowledge," *Applied Microbiology and Biotechnology* 103, no. 6 (2019): 2537–2549, https://doi.org/10.1007/s00253-019-09631-3.

49. S. Liebner, J. Zeyer, D. Wagner, C. Schubert, E.-M. Pfeiffer, and C. Knoblauch, "Methane Oxidation Associated With Submerged Brown Mosses Reduces Methane Emissions From Siberian Polygonal Tundra," *Journal of Ecology* 99 (2011): 914–922.

50. S. Crevecoeur, W. F. Vincent, J. Comte, A. Matveev, and C. Lovejoy, "Diversity and Potential Activity of Methanotrophs in High Methane-Emitting Permafrost Thaw Ponds," *PLoS ONE* 12 (2017): e0188223.

51. C. Knief, "Diversity and Habitat Preferences of Cultivated and Uncultivated Aerobic Methanotrophic Bacteria Evaluated Based on *pmo*A as Molecular Marker," *Frontiers in Microbiology* 6 (2015): 1346.

52. M. C. Y. Lau, B. T. Stackhouse, A. C. Layton, et al., "An Active Atmospheric Methane Sink in High Arctic Mineral Cryosols," *ISME Journal* 9 (2015): 1880–1891.

53. B. T. Stackhouse, T. A. Vishnivetskaya, A. Layton, et al., "Effects of Simulated Spring Thaw of Permafrost From Mineral Cryosol on CO_2 Emissions and Atmospheric CH_4 Uptake," *Journal of Geophysical Research: Biogeosciences* 120 (2015): 1764–1784.

54. L. D'Imperio, C. S. Nielsen, A. Westergaard-Nielsen, A. Michelsen, and B. Elberling, "Methane Oxidation in Contrasting Soil Types: Responses to Experimental Warming With Implication for Landscape-Integrated CH_4 Budget," *Global Change Biology* 23 (2017): 966–976.

55. C. Juncher Jørgensen, K. M. Lund Johansen, A. Westergaard-Nielsen, and B. Elberling, "Net Regional Methane Sink in High Arctic Soils of Northeast Greenland," *Nature Geoscience* 8 (2015): 20–23.

56. C. Voigt, A.-M. Virkkala, G. Hould Gosselin, et al., "Arctic Soil Methane Sink Increases With Drier Conditions and Higher Ecosystem Respiration," *Nature Climate Change* 13 (2023): 1095–1104.

57. C. Knoblauch, U. Zimmermann, M. Blumenberg, W. Michaelis, and E.-M. Pfeiffer, "Methane Turnover and Temperature Response of Methane-Oxidizing bacteria in Permafrost-Affected Soils of Northeast Siberia," *Soil Biology & Biochemistry* 40 (2008): 3004–3013.

58. M. Winkel, J. Mitzscherling, P. P. Overduin, et al., "Anaerobic Methanotrophic Communities Thrive in Deep Submarine Permafrost," *Scientific Reports* 8, no. 1291 (2018): 8.

59. M. Cui, A. Ma, H. Qi, X. Zhuang, and G. Zhuang, "Anaerobic Oxidation of Methane: An "Active" Microbial Process," *Microbiology Open* 4 (2015): 1–11.

60. J. Kao-Kniffin, B. J. Woodcroft, S. M. Carver, et al., "Archaeal and Bacterial Communities Across a Chronosequence of Drained Lake Basins in Arctic Alaska," *Scientific Reports* 5 (2015): 18165.

61. S. Yang, S. E. Anthony, M. Jenrich, et al., "Microbial Methane Cycling in Sediments of Arctic Thermokarst Lagoons," *Global Change Biology* 29 (2023): 2714–2731.

62. J. Hashemi, D. A. Lipson, K. A. Arndt, et al., "Thermokarst Landscape Exhibits Large Nitrous Oxide Emissions in Alaska's Coastal Polygonal Tundra," *Communications Earth & Environment* 5 (2024): 473.

63. M. E. Marushchak, J. Kerttula, K. Diáková, et al., "Thawing Yedoma Permafrost Is a Neglected Nitrous Oxide Source," *Nature Communications* 12 (2021): 7107.

64. M. Marushchak, A. Pitkämäki, H. Koponen, C. Biasi, M. Seppala, and P. Martikainen, "Hot Spot for Nitrous Oxide Emissions Found in Different Types of Permafrost Peatlands," *Global Change Biology* 17 (2011): 2601–2614.

65. R. J. E. Alves, W. Wanek, A. Zappe, et al., "Nitrification Rates in Arctic Soils Are Associated With Functionally Distinct Populations of Ammonia-Oxidizing archaea," *ISME Journal* 7 (2013): 1620–1631.

66. J. Hultman, M. P. Waldrop, R. Mackelprang, et al., "Multi-Omics of Permafrost, Active Layer and Thermokarst Bog Soil Microbiomes," *Nature* 521 (2015): 208–212.

67. E. Lamb, S. Han, B. Lanoil, et al., "A High Arctic Soil Ecosystem Resists Long-Term Environmental Manipulations," *Global Change Biology* 17 (2011): 3187–3194.

68. E. Ramm, C. Liu, P. Ambus, et al., "A Review of the Importance of Mineral Nitrogen Cycling in the Plant-Soil-Microbe System of Permafrost-Affected Soils—Changing the Paradigm," *Environmental research letters: ERL [Web site]* 17 (2022): 013004.

69. H. M. P. Siljanen, R. J. E. Alves, J. G. Ronkainen, et al., "Archaeal Nitrification Is a Key Driver of High Nitrous Oxide Emissions From Arctic Peatlands," *Soil Biology & Biochemistry* 137 (2019): 107539.

70. S. A. Hetz, A. Poehlein, and M. A. Horn, "Whole-Genome Sequences of Two New Caballeronia Strains Isolated From Cryoturbated Peat Circles of the Permafrost-Affected Eastern European Tundra," *Microbiology Resource Announcements* 9 (2020), https://doi.org/10.1128/MRA.00731-20.

71. E. Bellemain, M. L. Davey, H. Kauserud, et al., "Fungal Palaeodiversity Revealed Using High-Throughput Metabarcoding of Ancient DNA From Arctic Permafrost," *Environmental Microbiology* 15 (2013): 1176–1189.

72. G. Kochkina, N. Ivanushkina, S. Ozerskaya, et al., "Ancient Fungi in Antarctic Permafrost Environments," *FEMS Microbiology Ecology* 82 (2012): 501–509.

73. S. Ozerskaya, G. Kochkina, N. Ivanushkina, and D. A. Gilichinsky, "Fungi in Permafrost," in *Permafrost Soils*, ed. R. Margesin (Berlin, Heidelberg: Springer Berlin Heidelberg, 2009): 85–95.

74. M. C. Lydolph, J. J. Jonas, A. P. Peter, et al., "Beringian Paleoecology Inferred From Permafrost-Preserved Fungal DNA," *Applied and Environmental Microbiology* 71 (2005): 1012–1017.

75. T. H. da Silva, E. C. Queres Gomes, V. N. Gonçalves, et al., "Does Maritime Antarctic Permafrost Harbor Environmental Fungi With Pathogenic Potential?," *Fungal Biology* 126 (2022): 488–497.

76. B. Frey, T. Rime, M. Phillips, et al., "Microbial Diversity in European Alpine Permafrost and Active Layers," *FEMS Microbiology Ecology* 92 (2016): fiw018.

77. Y.-L. Chen, Y. Deng, J.-Z. Ding, et al., "Distinct Microbial Communities in the Active and Permafrost Layers on the Tibetan Plateau," *Molecular Ecology* 26 (2017): 6608–6620.

78. Y. Jiang, L. Yang, S. Wu, and T. Zhou, "Warming Changes the Composition and Diversity of Fungal Communities in Permafrost," *Annals of Microbiology* 73 (2023): 1–12.

79. S. K. Schmidt, L. Vimercati, J. L. Darcy, et al., "A Naganishia in High Places: Functioning Populations or Dormant Cells From the Atmosphere?," *Mycology* 8 (2017): 153–163.

80. F. Wirth and L. Z. Goldani, "Epidemiology of Rhodotorula: An Emerging Pathogen," *Interdisciplinary Perspectives on Infectious Diseases* 2012 (2012): 465717.

81. W. Hu, Q. Zhang, D. Li, et al., "Diversity and Community Structure of Fungi Through a Permafrost Core Profile From the Qinghai-Tibet Plateau of China," *Journal of Basic Microbiology* 54 (2014): 1331–1341.

82. C. S. Delavaux, R. J. Ramos, S. L. Sturmer, and J. D. Bever, "Environmental Identification of Arbuscular Mycorrhizal Fungi Using the LSU rDNA Gene Region: An Expanded Database and Improved Pipeline," *Mycorrhiza* 32 (2022): 145–153.

83. U. M. E. Schütte, J. A. Henning, Y. Ye, et al., "Effect of Permafrost Thaw on Plant and Soil Fungal Community in a Boreal Forest: Does Fungal Community Change Mediate Plant Productivity Response?," *Journal of cology* 107 (2019): 1737–1752.

84. M. J. L. Coolen, J. van de Giessen, E. Y. Zhu, and C. Wuchter, "Bioavailability of Soil Organic Matter and Microbial Community Dynamics Upon Permafrost Thaw," *Environmental Microbiology* 13 (2011): 2299–2314.

85. J.-M. Alempic, A. Lartigue, A. E. Goncharov, et al., "An Update on Eukaryotic Viruses Revived From Ancient Permafrost," *Viruses* 15 (2023), 564.

86. R. Wu, E. M. Bottos, V. G. Danna, J. C. Stegen, J. K. Jansson, and M. R. Davison, "RNA Viruses Linked to Eukaryotic Hosts in Thawed Permafrost," *mSystems* 7 (2022): e0058222.

87. R. McKie, *Arctic Zombie Viruses in Siberia Could Spark Terrifying new Pandemic, Scientists Warn* (New York, NY: Guardian, 2024).

88. D. W. Griffin, E. E. Silvestri, C. Y. Bowling, T. Boe, D. B. Smith, and T. L. Nichols, "Anthrax and the Geochemistry of Soils in the Contiguous United States," *Geosciences (Switzerland)* 4 (2014): 114–127.

89. R. Mackelprang, R. A. Barbato, A. M. Ramey, U. M. E. Schütte, and M. P. Waldrop. "Cooling Perspectives on the Risk of Pathogenic Viruses From Thawing Permafrost," *mSystems* (2025): e00042-24, https://doi.org/10.1128/msystems.00042-24.

90. A. Waits, A. Emelyanova, A. Oksanen, K. Abass, and A. Rautio, "Human Infectious Diseases and the Changing Climate in the Arctic," *Environmental International* 121 (2018): 703–713.

91. A. J. Parkinson, "Arctic Human Health Initiative," *International Journal of Circumpolar Health* 69 (2010): 1–44.

92. P. Biagini, C. Thèves, P. Balaresque, et al., "Variola Virus in a 300-Year-Old Siberian Mummy," *New England Journal of Medicine* 367 (2012): 2057–2059.

93. R. Stone, "Public Health. Is Live Smallpox Lurking in the Arctic?," *Science* 295 (2002): 2002.

94. J. B. Emerson, S. Roux, J. R. Brum, et al., "Host-Linked Soil Viral Ecology Along a Permafrost Thaw Gradient," *Nature Microbiology* 3 (2018): 870–880, https://doi.org/10.1038/s41564-018-0190-y.

95. G. Trubl, J. A. Kimbrel, J. Liquet-Gonzalez, et al., "Active Virus-Host Interactions at Sub-Freezing Temperatures in Arctic Peat Soil," *Microbiome* 9, no. 1 (2021): 208.

96. M. Legendre, A. Lartigue, L. Bertaux, et al., "In-Depth Study of Mollivirus Sibericum, a New 30,000-Y-Old Giant Virus Infecting Acanthamoeba," *Proceedings of the National Academy of Sciences* 112 (2015): E5327–E5335.

97. J. R. Schrad, J. S. Abrahão, J. R. Cortines, and K. N. Parent, "Structural and Proteomic Characterization of the Initiation of Giant Virus Infection," *Cell* 181 (2020): 1046–1061.e6.

98. A. Aksozek, K. McClellan, K. Howard, J. Y. Niederkorn, and H. Alizadeh, "Resistance of *Acanthamoeba castellanii* Cysts to Physical, Chemical, and Radiological Conditions," *Journal of Parasitology* 88 (2002): 621–623.

99. R. Sriram, M. Shoff, G. Booton, P. Fuerst, and G. S. Visvesvara, "Survival of Acanthamoeba Cysts After Desiccation for More Than 20 Years," *Journal of Clinical Microbiology* 46 (2008): 4045–4048.

100. B. Longdon, M. A. Brockhurst, C. A. Russell, J. J. Welch, and F. M. Jiggins, "The Evolution and Genetics of Virus Host Shifts," *PLoS Pathogens* 10 (2014): e1004395.

101. J. H. Ellwanger and J. A. B. Chies, "Zoonotic Spillover: Understanding Basic Aspects for Better Prevention," *Genetics and Molecular Biology* 44 (2021): e20200355.

102. K. Hueffer, D. Drown, V. Romanovsky, and T. Hennessy, "Factors Contributing to Anthrax Outbreaks in the Circumpolar North," *EcoHealth* 17 (2020): 174–180.

103. T. J. Seitz, U. M. E. Schütte, and D. M. Drown, "Soil Disturbance Affects Plant Productivity via Soil Microbial Community Shifts," *Frontiers in Microbiology* 12 (2021): 619711.

104. L. Ghelardini, N. Luchi, F. Pecori, et al., "Ecology of Invasive Forest Pathogens," *Biological Invasions* 19 (2017): 3183–3200.

105. R. Seidl, G. Klonner, W. Rammer, et al., "Invasive Alien Pests Threaten the Carbon Stored in Europe's Forests," *Nature Communications* 9 (2018): 1626.

106. J. D. Bever, S. A. Mangan, and H. M. Alexander, "Maintenance of Plant Species Diversity by Pathogens," *Annual Review of Ecology, Evolution, and Systematics* 46 (2015): 305–325.

107. M. B. Eppinga, M. Baudena, D. J. Johnson, et al., "Frequency-Dependent Feedback Constrains Plant Community Coexistence," *Nature Ecology & Evolution* 2 (2018): 1403–1407.

108. G. Wang, H. M. Burrill, L. Y. Podzikowski, et al., "Dilution of Specialist Pathogens Drives Productivity Benefits From Diversity in Plant Mixtures," *Nature Communications* 14 (2023): 8417.

109. A. Burkert, T. A. Douglas, M. P. Waldrop, and R. Mackelprang, "Changes in the Active, Dead, and Dormant Microbial Community Structure Across a Pleistocene Permafrost Chronosequence," *Applied and Environmental Microbiology* 85, no. 7 (2019): e02646-18.

110. N. S. Panikov and M. V. Sizova, "Growth Kinetics of Microorganisms Isolated From Alaskan Soil and Permafrost in Solid Media Frozen Down to −35°C," *FEMS Microbiology Ecology* 59 (2007): 500–512.

111. S. J. Tuorto, P. Darias, L. R. McGuinness, et al., "Bacterial Genome Replication at Subzero Temperatures in Permafrost," *ISME Journal* 8 (2014): 139–149.

112. S. R. James, B. J. Minsley, J. W. McFarland, E. S. Euskirchen, C. W. Edgar, and M. P. Waldrop, "The Biophysical Role of Water and Ice Within Permafrost Nearing Collapse: Insights From Novel Geophysical Observations," *Journal of Geophysical Research: Earth Surface* 126 (2021): e2021JF006104.

113. M.-C. Leewis, R. Berlemont, D. C. Podgorski, et al., "Life at the Frozen Limit: Microbial Carbon Metabolism Across a Late Pleistocene Permafrost Chronosequence," *Frontiers in Microbiology* 11 (2020): 1–15.

114. H. L. Ayala-del-Rio, P. S. Chain, J. J. Grzymski, et al., "The Genome Sequence of Psychrobacter Arcticus 273-4, a Psychroactive Siberian Permafrost Bacterium, Reveals Mechanisms for Adaptation to Low-Temperature Growth," *Applied and Environmental Microbiology* 76 (2010): 2304–2312.

115. I. Raymond-Bouchard, J. Goordial, Y. Zolotarov, et al., "Conserved Genomic and Amino Acid Traits of Cold Adaptation in Subzero-Growing Arctic Permafrost Bacteria," *FEMS Microbiology Ecology* 94 (2018): fiy023.

116. J. Goordial, I. Raymond-Bouchard, Y. Zolotarov, et al., "Cold Adaptive Traits Revealed by Comparative Genomic Analysis of the *Eurypsychrophile rhodococcus* sp. JG3 Isolated From High Elevation McMurdo Dry Valley Permafrost, Antarctica," *FEMS Microbiology Ecology* 92 (2016): fiv154.

117. B. Steven, R. Léveillé, W. H. Pollard, and L. G. Whyte, "Microbial Ecology and Biodiversity in Permafrost," *Extremophiles: Life Under Extreme Conditions* 10 (2006): 259–267.

118. N. C. S. Mykytczuk, S. J. Foote, C. R. Omelon, G. Southam, C. W. Greer, and L. G. Whyte, "Bacterial Growth at −15°C; Molecular Insights From the Permafrost Bacterium Planococcus Halocryophilus Or1," *ISME Journal* 7 (2013): 1211–1226.

119. R. Mackelprang, A. Burkert, M. Haw, et al., "Microbial Survival Strategies in Ancient Permafrost: Insights From Metagenomics," *ISME Journal* 11 (2017): 2305–2318.

120. R. A. Barbato, R. M. Jones, T. A. Douglas, et al., "Not All Permafrost Microbiomes Are Created Equal: Influence of Permafrost Thaw on the Soil Microbiome in a Laboratory Incubation Study," *Soil Biology & Biochemistry* 167 (2022): 108605.

121. J. R. Deslippe, M. Hartmann, S. W. Simard, and W. W. Mohn, "Long-Term Warming Alters the Composition of Arctic Soil Microbial Communities," *FEMS Microbiology Ecology* 82 (2012): 303–315.

122. J. Geml, L. N. Morgado, T. A. Semenova, J. M. Welker, M. D. Walker, and E. Smets, "Long-Term Warming Alters Richness and Composition of Taxonomic and Functional Groups of Arctic fungi," *FEMS Microbiology Ecology* 91 (2015): fiv095.

123. J. Geml, T. A. Semenova, L. N. Morgado, and J. M. Welker, "Changes in Composition and Abundance of Functional Groups of Arctic Fungi in Response to Long-Term Summer Warming," *Biology Letters* 12 (2016): 20160503.

124. S. Mundra, R. Halvorsen, H. Kauserud, et al., "Ectomycorrhizal and Saprotrophic fungi Respond Differently to Long-Term Experimentally Increased Snow Depth in the High Arctic," *MicrobiologyOpen* 5 (2016): 856–869.

125. T. A. Semenova, L. N. Morgado, J. M. Welker, M. D. Walker, E. Smets, and J. Geml, "Compositional and Functional Shifts in Arctic Fungal Communities in Response to Experimentally Increased Snow Depth," *Soil Biology & Biochemistry* 100 (2016): 201–209.

126. T. A. Semenova, L. N. Morgado, J. M. Welker, M. D. Walker, E. Smets, and J. Geml, "Long-Term Experimental Warming Alters Community Composition of Ascomycetes in Alaskan Moist and dry Arctic Tundra," *Molecular Ecology* 24 (2015): 424–437.

127. M. Sturm, C. Racine, and K. Tape, "Increasing Shrub Abundance in the Arctic," *Nature* 411 (2001): 546–547.

128. K. Palmer, C. Biasi, and M. A. Horn, "Contrasting Denitrifier Communities Relate to Contrasting N_2O Emission Patterns From Acidic Peat Soils in Arctic Tundra," *ISME Journal* 6 (2012): 1058–1077.

129. M. P. Waldrop, J. W. McFarland, K. L. Manies, et al., "Carbon Fluxes and Microbial Activities From Boreal Peatlands Experiencing Permafrost Thaw," *Journal of Geophysical Research: Biogeosciences* 126 (2021): 1–19.

130. C. Schädel, M. K. F. Bader, E. A. G. Schuur, et al., "Potential Carbon Emissions Dominated by Carbon Dioxide From Thawed Permafrost Soils," *Nature Climate Change* 6 (2016): 950–953.

131. B. W. Abbott and J. B. Jones, "Permafrost Collapse Alters Soil Carbon Stocks, Respiration, CH_4, and N_2O in Upland Tundra," *Global Change Biology* 21 (2015): 4570–4587.

132. C. Knoblauch, C. Beer, S. Liebner, M. N. Grigoriev, and E.-M. Pfeiffer, "Methane Production as Key to the Greenhouse Gas Budget of Thawing Permafrost," *Nature Climate Change* 8 (2018): 309–312.

133. S. Liebner, L. Ganzert, A. Kiss, S. Yang, D. Wagner, and M. M. Svenning, "Shifts in Methanogenic Community Composition and Methane Fluxes Along the Degradation of Discontinuous Permafrost," *Frontiers in Microbiology* 6 (2015): 356.

134. S. Holm, J. Walz, F. Horn, et al., "Methanogenic Response to Long-Term Permafrost Thaw Is Determined by Paleoenvironment," *FEMS Microbiology Ecology* 96 (2020): 1–13.

135. C. Knoblauch, C. Beer, A. Schuett, et al., "Carbon Dioxide and Methane Release Following Abrupt Thaw of Pleistocene Permafrost Deposits in Arctic Siberia," *Journal of Geophysical Research. Biogeosciences* 126 (2021), e2021JG006543.

136. S. Monteux, F. Keuper, S. Fontaine, et al., "Carbon and Nitrogen Cycling in Yedoma Permafrost Controlled by Microbial Functional Limitations," *Nature Geoscience* 13 (2020): 794–798.

137. C. R. See, A.-M. Virkkala, S. M. Natali, et al., "Decadal Increases in Carbon Uptake Offset by Respiratory Losses Across Northern Permafrost Ecosystems," *Nature Climate Change* 14 (2024): 853–862.

138. G. Hugelius, J. Loisel, S. Chadburn, et al., "Large Stocks of Peatland Carbon and Nitrogen Are Vulnerable to Permafrost Thaw," *Proceedings of the National Academy of Sciences* 117 (2020): 20438–20446.

139. M. J. Lara, D. H. Lin, C. Andresen, V. L. Lougheed, and C. E. Tweedie, "Nutrient Release From Permafrost Thaw Enhances CH_4 Emissions From Arctic Tundra Wetlands," *Journal of Geophysical Research. Biogeosciences* 124 (2019): 1560–1573.

140. R. Wegner, C. Fiencke, C. Knoblauch, L. Sauerland, and C. Beer, "Rapid Permafrost Thaw Removes Nitrogen Limitation and Rises the Potential for N_2O Emissions," *Nitrogen* 3 (2022): 608–627.

141. S. Leininger, T. Urich, M. Schloter, et al., "Archaea Predominate Among Ammonia-Oxidizing Prokaryotes in Soils," *Nature* 442 (2006): 806–809.

142. I. S. Pessi, S. Viitamäki, A.-M. Virkkala, et al., "In-Depth Characterization of Denitrifier Communities Across Different Soil Ecosystems in the Tundra," *Environmental Microbiomes* 17 (2022): 30.

143. C. R. Penton, C. Yang, L. Wu, et al., "NifH-Harboring Bacterial Community Composition Across an Alaskan Permafrost Thaw Gradient," *Frontiers in Microbiology* 7 (2016): 1894.

144. R. A. Finger, M. R. Turetsky, K. Kielland, R. W. Ruess, M. C. Mack, and E. S. Euskirchen, "Effects of Permafrost Thaw on Nitrogen Availability and Plant–Soil Interactions in a Boreal Alaskan Lowland," *Journal of Ecology* 104, no. 6 (2016): 1542–1554.

145. J. G. Ernakovich, R. A. Barbato, V. I. Rich, et al., "Microbiome Assembly in Thawing Permafrost and Its Feedbacks to Climate," *Global Change Biology* 28 (2022): 5007–5026.

146. C. Schädel, B. M. Rogers, D. M. Lawrence, et al., "Earth System Models Must Include Permafrost Carbon Processes," *Nature Climate Change* 14 (2024): 114–116.

147. Y. Oh, Q. Zhuang, L. Liu, et al., "Reduced Net Methane Emissions Due to Microbial Methane Oxidation in a Warmer Arctic," *Nature Climate Change* 10 (2020): 317–321.

148. K. S. Messan, R. M. Jones, S. J. Doherty, K. Foley, T. A. Douglas, and R. A. Barbato, "The Role of Changing Temperature in Microbial Metabolic Processes During Permafrost Thaw," *PLoS ONE* 15 (2020), e0232169.

149. E. F. Belshe, E. A. G. Schuur, B. M. Bolker, and R. Bracho, "Incorporating Spatial Heterogeneity Created by Permafrost Thaw Into a Landscape Carbon Estimate," *Journal of Geophysical Research: Biogeosciences* 117 (2012): G01026.

150. J. Comte, A. Monier, S. Crevecoeur, C. Lovejoy, and W. F. Vincent, "Microbial Biogeography of Permafrost Thaw Ponds Across the Changing Northern Landscape," *Ecography* 39 (2016): 609–618.

151. M. S. Girvan, J. Bullimore, J. N. Pretty, A. M. Osborn, and A. S. Ball, "Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils," *Applied and Environmental Microbiology* 69 (2003): 1800–1809.

152. M. T. Jorgenson, H. Jennifer, K. Mikhail, et al., "Reorganization of Vegetation, Hydrology and Soil Carbon After Permafrost Degradation Across Heterogeneous Boreal Landscapes," *Environmental Research Letters: ERL [Web Site]* 8 (2013): 035017.

Appendix 1

List of References That Support the Dates of Milestones in Permafrost Microbiology Research in Figure 1

151.Abyzov, S.S., Lipenkov, V.Y., Bobin, N.E., and Koudryashov, B.B. (1982) The Microflora of the Central Antarctic Glacier and the Control Methods of the Sterile Isolation of the Ice Core for Microbiological Analysis. Izvestiya Akademii Nauk Sssr Seriya Biologicheskaya: 537–548.

152.Boyd, W.B., JW Boyd (1962) Presence of Azotobacter Species in Polar Regions. Journal of Bacteriology 83: 429–430.

153.Boyd, W.B., JW Boyd (1964) The Presence of Bacteria in Permafrost of the Alaskan Arctic. Canadian Journal of Microbiology 10: 917–919.

154.Buongiorno, J., Bird, J., Krivushin, K. et al. (2016) Draft Genome Sequence of Antarctic Methanogen Enriched From Dry Valley Permafrost. Genome Announcements 4: e01362–e01316.

155.Cameron, R.E., and Morelli, F.A. (1974) Viable Microorganisms From Ancient Ross Island and Taylor Valley Drill Core. Antarctic Journal of the United States 9: 113–116.

156.Chauhan, A., Layton, A.C., Vishnivetskaya, T.A., Williams, D., Pfiffner, S.M., Rekepalli, B., Stackhouse B., Lau M.C.Y., Phelps T.J., Mykytczuk N., Ronholm J., Whyte L., Onstott T.C., Sayler G.S. (2014) Metagenomes From Thawing Low-Soil-Organic-Carbon Mineral Cryosols and Permafrost of the Canadian High Arctic. Genome Announcements 2: e01217–e01214.

157.Gilichinsky, D., Khlebnikova, G., Zvyagintsev, D., Fyodorov-Davydov, D., and Kudryavtseva, N. (1989) Microbiology of Sedimentary Materials in the Permafrost Zone. International Geological Review 31: 847–858.

158.Gilichinsky, D.A., Soina, V.S., and Petrova, M.A. (1993) Cryoprotective Properties of Water in the Earth Cryollitosphere and Its Role in Exobiology. Origins of Life and Evolution of the Biosphere 23: 65–75.

159.James, N., and Sutherland, M. (1942) Are There Living Bacteria in Permanently Frozen Subsoil? Can J Res Sect C Bot Sci 20. Canadian Journal of Research 20C: 228–235.

160.Khlebnikova, G.M., Gilichinskyii, D.A., Fedorov-Davydov, D.C., and Vorob'eva, E.A. (1990) Quantitative Evaluation of Microorganisms in Permafrost Deposits and Buried Soils. Microbiology 59: 106–112.

161.Legendre, M., Bartoli, J., Shmakova, L., Jeudy, S., Labadie, K., Adrait, A., Lescot M., Poirot O., Bertaux L., Bruley C., Couté Y., Rivkina E., Abergel C., Claverie J.M. (2014) Thirty-Thousand-Year-Old Distant Relative of Giant Icosahedral DNA Viruses With a Pandoravirus Morphology. Proceedings of the National Academy of Sciences of the United States of America 111: 4274–4279.

162.Omelyansky, V. (1911) Bakteriologicheskoe Issledovanie Sanga Mamonta Prilegayushchei Pochvy (Bacteriological Investigation of the Sanga Mammoth and Surrounding Soil). Archive of Biological Sciences (In Russian) 16: 335–340.

163.Petrova, M.A., Midlin, S.Z., Soina, V.S., Gorlenko, Z.M., and Nikiforov, V.G. (1992) Isolation of Mercury Resistant Bacteria From Permafrost Samples of Kolyma Lowland. In 1st International Conference on Cryopedology. Pushchino, Russia, pp. 247–251.

164.Rivkina, E.M., Samarkin, V.A., and Gilichinskii, D.A. (1992) Methane in Permafrost Sediments on the Kolyma-Indigirka Lowland. Doklady Akademii Nauk 323: 559–562.

165.Shatilovich, A.V., Tchesunov, A.V., Neretina, T.V., Grabarnik, I.P., Gubin, S.V., Vishnivetskaya, T.A., Onstott T.C., Rivkina E.M. (2018) Viable Nematodes From Late Pleistocene Permafrost of the Kolyma River Lowland. Doklady Biological Sciences 480: 100–102.

166.Shi, T., Reeves, R.H., Gilichinsky, D.A., and Friedmann, E.I. (1997) Characterization of Viable Bacteria From Siberian Permafrost by 16S rDNA Sequencing. Microbial Ecology 33: 169–179.

167.Shmakova, L., Malavin, S., Iakovenko, N., Vishnivetskaya, T., Shain, D., Plewka, M., and Rivkina, E. (2021) A Living Bdelloid Rotifer From 24,000-Year-Old Arctic Permafrost. Current Biology 31: R712–r713.

168.Simon, C., Wiezer, A., Strittmatter, A.W., and Daniel, R. (2009) Phylogenetic Diversity and Metabolic Potential Revealed in a Glacier Ice Metagenome. Applied and Environmental Microbiology 75: 7519–7526.

169.Sipes, K., Vishnivetskaya, T.A., Eddie, A., Spirina, E.V., Onstott, T.C., Rivkina, E.M., and Lloyd, K.G. (2019) Metagenome-Assembled Genomes (MAGs) and Single-Cell Assembled Genomes (SAGs) Reveal a Marine Influenced Microbial Presence in 1 Ma old Non-Marine Permafrost of the Eastern Siberia Kolyma Region. In 8th International Conference on Polar and Alpine Microbiology. Hamilton, New Zealand, p. 40.
170.Soina, V., and Vorobyova, E. (1994) Preservation of Microbial Cell Structures in the Permafrost. In Viable Microorganisms in PermafrostGilichinsky, D. (ed). Pushchino: Pushchino Research Centre, Russian Academy of Sciences, pp. 37–48.
171.Soina, V.S., Lebedeva, E.V., Golyshina, O.B., Fedorov-Davydov, D.G., and Gilichinsky, D.A. (1991) Nitrifying Bacteria in the Permafrost of Kolyma Lowland. Microbiology 60: 187–190.
172.Tiedje, J.M., Smith, G., Simkins, S., Holben, W., Finney, C., and Gilichinsky, D.A. (1994) Recovery of DNA, Denitrifiers and Patterns of Antibiotic Sensitivity in Microorganisms From Ancient Permafrost Soil of Eastern Siberia. In Viable Microorganisms in PermafrostGilichinsky, D. (ed). Pushchino: Pushchino Research Centre, Russian Academy of Sciences, pp. 83–98.
173.Vainshtein, M., Gogotova, G., and Hippe, H. (1994) A Sulfate-Reducing Bacterium From the Permafrost. In Viable Microorganisms in PermafrostGilichinsky, D. (ed). Pushchino: Pushchino Research Centre, Russian Academy of Sciences, pp. 68–74.
174.Vishnivetskaya, T., Kathariou, S., McGrath, J., Gilichinsky, D., and Tiedje, J.M. (2000) Low-Temperature Recovery Strategies for the Isolation of Bacteria From Ancient Permafrost Sediments. Extremophiles 4: 165–173.
175.Vishnivetskaya, T.A., Erokhina, L.G., Gilichinsky, D.A., and Vorobyova, E.A. (1997) Blue-Green and Green Algae From Arctic Permafrost. Earth Cryosphere 1: 71–76.
176.Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky D., Rivkina E., Vishnivetskaya T. (1997) The Deep Cold Biosphere: Facts and Hypothesis. FEMS Microbiology Reviews 20: 277–290.
177.Woodcroft, B.J., Singleton, C.M., Boyd, J.A., Evans, P.N., Emerson, J.B., Zayed, A.A.F., Hoelzle R.D., Lamberton T.O., McCalley C.K., Hodgkins S.B., Wilson R.M., Purvine S.O., Nicora C.D., Li C., Frolking S., Chanton J.P., Crill P.M., Saleska S.R., Rich V.I., Tyson G.W. (2018) Genome-Centric View of Carbon Processing in Thawing Permafrost. Nature 560: 49–54.
178.Yashina, S., Gubin, S., Maksimovich, S., Yashina, A., Gakhova, E., and Gilichinsky, D. (2012) Regeneration of Whole Fertile Plants From 30,000-Y-Old Fruit Tissue Buried in Siberian Permafrost. Proceedings of the National Academy of Sciences of the United States of America 109: 4008–4013.
179.Zhou, J.Z., Davey, M.E., Figueras, J.B., Rivkina, E., Gilichinsky, D., and Tiedje, J.M. (1997) Phylogenetic Diversity of a Bacterial Community Determined From Siberian Tundra Soil DNA. Microbiology-UK 143: 3913–3919.
180.Zvyagintsev, D., Gilichinskii, D., Blagodatsky, S., Vorob'eva, E., Khlebnikova, G., Arkhangelov, A., and Kudryavtseva, N. (1985) Survival Time of Microorganisms in Permanently Frozen Sedimentary Rocks and Buried Soils. Microbiology 54: 131–136.