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Title
Permafrost as a potential pathogen reservoir

Permalink
https://escholarship.org/uc/item/50s30845

Journal
One Earth, 5(4)

ISSN
2590-3330

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Publication Date
2022-04-01

DOI
10.1016/j.oneear.2022.03.010

Peer reviewed

1   **One Earth Review**: Permafrost as a Potential Pathogen Reservoir

2

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10

11   **Summary**

12   The Arctic is currently warming at unprecedented rates due to global climate change, resulting in

13   thaw of large tracts of permafrost soil. A grand challenge is to understand the implications of permafrost

14   thaw on human health and the environment. Permafrost is a reservoir of mostly uncharacterized

15   microorganisms and viruses, many of which could be viable. Given our limited knowledge of resident

16   permafrost microbes, we also lack the basis to judge if they pose risks to humans, animals and plants. Here,

17   we delve into features of permafrost as a microbial habitat, and discuss what is known about the potential

18   for microbial pathogens to emerge in a warming climate as permafrost thaws. This review has broader

19   implications for human health and ecosystem sustainability in the new arctic environment that will emerge

20   in a thawed permafrost landscape.

21

22   **Keywords: p**ermafrost, climate change, emerging pathogen, soil microbiome, soil virus

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24

25   **Introduction**

26   Current predictions of permafrost land coverage range between 15–24% of the Northern

27   hemisphere[1,2]. Permafrost soils are among the largest terrestrial carbon reservoirs on the planet and in the

28   Northern hemisphere permafrost soils are estimated to contain 1014 petagrams carbon to a depth of 3

29   meters[3]. By definition, soils that have remained frozen for at least two consecutive years are accepted as

30   permafrost, but most formations are much older than that and originate from prior glaciation periods. Frozen

31   permafrost soils are overlain by an active layer which undergoes seasonal freeze-thaw cycles. Depending

32   on when the soil froze, permafrost can contain organic depositions from prior periods of Earth's

paleoclimate, including periods with a warmer and more carbon dioxide (CO_2) rich atmosphere. Active layer carbon stocks are newer deposits in comparison to permafrost[3].

Permafrost is thawing due to anthropogenic climate change, and we can already observe large scale changes in permafrost regions. Soil temperatures have increased by up to 2°C over the last decade and the active layer has deepened significantly[4]. Global warming has disproportionately warmed both high-latitude and high-altitude permafrost (i.e., Tibetan plateau) areas, and these regions are projected to continue to experience greater temperature increases compared to other regions of the world[5]. As a result, on a global scale, 22–64% of permafrost is expected to thaw by 2100[6]. Thawing permafrost creates a positive feedback loop by releasing nutrients such as water, allowing plants and microorganisms to grow. In low drainage areas containing ice-rich permafrost or massive ground ice, gradual thaw has resulted in ground subsidence and inundation where formation of bogs, fens, and thermokarst (abrupt thaw) lakes mainly support microbial anaerobic fermentation and methane (CH_4) production[7,8]. Thawed permafrost carbon stocks are rapidly turned to CH_4 which further contributes to greenhouse gas accumulation[9,10]. However, these observations do not reflect the entirety of the complex changes that Arctic landscapes are currently experiencing. Increasingly warmer and longer summer seasons rapidly melt ground ice where abrupt thaw intensifies subsidence and deepens the active layer[11]. Abrupt thaw in upland areas results in active layer detachments or large scale landslides, and forms gullies and thaw slumps[12]. Model estimations predict abrupt thaw to cause carbon losses that are equivalent to 40% of those expected from the gradual thaw[13]. Currently we have limited understanding of how microbes respond to such sudden changes and shifting quantities in ice, carbon, and nutrient deposits[4].

Here, we delve into features of permafrost that could make it a reservoir for microbes, including pathogens, and discuss what is known about the potential for microbial pathogens to emerge in a warming climate as permafrost thaws. In most cases we find that the threats to public health are minor. We show that although there is potential for bacterial human pathogens, such as anthrax, to be problematic as permafrost thaws, there is less potential for other human pathogens. Most of the microorganisms and viruses that have been identified in permafrost to date target non-human hosts, such as plants and microorganisms. Plant pathogens could, however, pose a risk to native and introduced crops. Although fragments of human viruses have been recovered from remains preserved by permafrost, none have been shown to be infectious to humans. However, many of the microorganisms and viruses residing in permafrost have not yet been isolated or characterized. Therefore, their potential to pose future risks to humans, animals and plants warrants consideration. These risks may increase with climate change as the landscape and vegetation changes and the human population becomes increasingly exposed to thawed permafrost lands.

MICROBES IN PERMAFROST

67   Permafrost soils have been shown to be microbially diverse terrestrial ecosystems[7]. When frozen,
68   many of the resident microorganisms in permafrost, unlike macroorganisms — 'think wooly mammoths'
69   — were able to adapt and survive in subzero temperatures[7,14]. These psychrotolerant microorganisms[15] also
70   have to endure other stressful conditions, including high salinity, and low water and nutrient availability[16-
71   18]; as well as anoxia and low pH in frozen bogs and fens[19,20]. Permafrost "microbes" include bacteria,
72   archaea, fungi, algae, protists and viruses. Protozoa have also been isolated from permafrost[21]. The types
73   of microbes and their ability to survive depend on a variety of factors including the permafrost age and
74   chemical composition. Whether any of these microbes are potentially pathogenic to humans, other animals,
75   or plants is a current concern and the topic of considerable debate[22].

76   Previous reviews have summarized the potential for pathogen emergences from other cryosphere
77   environments, including glaciers and ice patches[23-25]. For example, high frequencies of influenza A viral
78   RNAs were detected in Siberian Lake ice that can potentially be transmitted via migratory birds[26]. In another
79   study, one arthropod RNA virus and one plant DNA virus were recovered from ice patches in the Canadian
80   Arctic[27]. Although outside the scope of this review that is focused on permafrost, these reports underscore
81   the potential for the cryosphere to serve as a reservoir of emerging pathogens.

82

83   **Bacteria and archaea in permafrost**

84   It is known that the microbial communities that inhabit active layer and permafrost soil layers are
85   quite different in composition[7]. Active layer and permafrost microbes also have different vulnerabilities to
86   increases in global temperatures. As permafrost thaws, the depth of the active layer increases. As a result,
87   there is usually an increase in microbial activity in the newly formed active layer which in return intensifies
88   greenhouse gas emissions[7] and a transition in the composition of the microbial community[28]. Soil moisture
89   content and overlying vegetation have been shown to be key drivers of the microbial response to permafrost
90   thaw[20,29,30]. In addition, differences in soil conditions, e.g., temperature, redox chemistry, moisture content,
91   are clearly of importance in determining the thaw response[28].

92   Permafrost soils are shown to contain a wide range of soil bacteria, predominantly from the
93   Terrabacteria supergroup, including *Actinobacteria*, *Firmicutes*, *Cyanobacteria* and *Chloroflexi*, as well as
94   *Bacteroidetes* and *Proteobacteria*[7]. Some representatives of less well known phyla have also been
95   discovered in permafrost metagenomes, including metagenome-assembled genomes corresponding to
96   Candidate phylum *Eremiobacteraeota* and Candidate phylum *Dormibacteraeota* in intact and thawing
97   permafrost[31]. Similar to bacteria, many different species of archaea have been found in permafrost,
98   including representatives of *Euryarchaeota*, *Crenarchaeota* and *Thaumarchaeota*. To date, the most
99   commonly observed archaeal species in thawing permafrost are methanogenic *Euryarchaeota* that can use
100   fermentation byproducts to produce CH_4[32].

101   Conditions in permafrost can preserve both nucleic acids and live cells that have mechanisms for

102 survival under long-term freezing conditions. Potential resources in permafrost, including organic carbon

103 nutrients and water are key to ensuring long-term survival. Because living organisms require water for

104 survival, permafrost poses a unique challenge due to low water availability. Even a thin layer of salt brine

105 can serve as a water reservoir because the salt increases the freezing temperature of water. Liquid water has

106 been detected in permafrost at $-10°C$[33] and bacteria have been shown to be metabolically active at that

107 temperature[34].

108   Increasing permafrost age also increases the necessity for microbial cells to cope with extended

109 periods of stressful conditions[28,35]. Mackelprang et al., (2017)[28] used 16S rRNA amplicon and metagenomic

110 sequencing to profile the permafrost microbial communities existing in a permafrost chronosequence that

111 ranged from 19000 to 33000 years ago (Pleistocene Era)[28]. They found that the age of the permafrost

112 influenced the types of microbes present. For example, representatives of *Firmicutes* that can form resistant

113 spores increased in relative abundance with age of the permafrost. Functional analysis of metagenomes

114 revealed an enrichment in pathways for degradation of recalcitrant plant material and stress-related genes

115 in older permafrost[36].

116   Most research has focused on understanding how interactions among post-thaw permafrost

117 microbiomes are deterministic of observed carbon degradation rates and pathways. For example, recent

118 evidence suggests that conditions mediated by thaw could give rise to acetoclastic methanogenesis where

119 hydrogenotrophic methanogenesis was predicted to be dominant in intact permafrost[37]. Drainage driven dry

120 soil conditions, on the other hand, can enhance aerobic processes and fuel CO_2 production especially in

121 uplands[10]. Under these conditions easily decomposable sugars, simple amino acids and lipids are depleted

122 due to oxidative decomposition, where hydrophobic carbon compounds are preserved in deep soils. In

123 addition, multi-omics analyses of new thermokarst bog and fen formations showed high abundances of

124 methanogenesis-related genes, transcripts and proteins, suggesting that CH_4 generation will increase in

125 these systems once permafrost thaws[16,32]. This has important implications for greenhouse gas emissions

126 because CH_4 is a more potent greenhouse gas than CO_2. By contrast, little is known about the risk of

127 pathogen exposure in thawed permafrost.

128

129 **Viruses in permafrost**

130   Viruses are the most numerically abundant biological entities on Earth[38] and we have just begun to

131 investigate the full extent of viral diversity in thawing permafrost soils. Recent surveys in the active layer

132 of permafrost soils have identified that the vast majority of viruses are double-stranded (ds) DNA

133 bacteriophages (phage; viruses that only infect bacteria, 'Bacteriophage' in **Figure 1**). These phages are

134 largely unrelated to other known phages and a large portion of the community is active[39]. The phage

community composition changes with permafrost thaw from soil-virus-like assemblages in the palsas with intact permafrost to a mixture of soil-like and aquatic-like in partially thawed bog to aquatic-virus-like assemblages in the fully thawed inundated fen habitats[40,41]. Phage genomes are attuned to their environmental conditions by carrying auxiliary metabolic genes (AMGs) that can help their hosts overcome metabolic bottlenecks such as resource limitations. For example, phages detected in thawing permafrost peatlands (soils with a large quantity of organic matter due to incomplete decomposition) were found to carrying an array of glycoside hydrolases that breakdown complex carbohydrates into labile carbohydrates which in turn sustains a wide diversity of microorganisms[40,41].

Phages are the most intensely studied type of viruses in environmental systems, and in contrast our knowledge on ssDNA and RNA viruses is limited, especially in permafrost ('Virus' in **Figure 1**). This has been largely due to (1) dsDNA phages being ubiquitous and abundant, (2) common methods to capture ssDNA or RNA viruses preclude quantitative ecological inferences[42] or exclude them (e.g., metagenomes don't capture RNA viruses), and (3) most ssDNA and RNA viruses appear to infect eukaryotes[43,44]. This suggests that we are missing an unknown quantity of environmental viruses that could be emerging pathogens.

Recently, a protocol was published to increase our detection and characterization of ssDNA viruses in soils underlain with permafrost[45]. While ssDNA viruses were detected, they represented a small fraction of the viral diversity and abundance, and the potential hosts were bacteria or micro-eukaryotes (e.g., fungi and amoeba)[45]. Other studies of ssDNA[46] and RNA viruses[29,47] in non-permafrost soils showed that they are abundant and diverse groups of viruses with clear differences in bulk-soil versus rhizosphere communities, and also identified bacteria or micro-eukaryotes as potential hosts. However, we have yet to fully understand the full extent of global viral diversity, including in permafrost soils [29,46,47].

Microbial activity in permafrost

Permafrost environments are subzero and often anoxic, which has led many to believe that resident microbes are not active. Over the last decade there has been mounting research revealing diverse assemblages of active bacteria and viruses from permafrost environments[39,48,49]. A multi-omics approach (i.e., metagenomics, metatranscriptomics and metaproteomics) was used to determine which microbial populations were active along a permafrost thaw gradient[16]. The proteome data revealed several proteins that were expressed by permafrost microorganisms to equip them to survive the harsh conditions in permafrost, including cold shock proteins. The metatranscriptome data also revealed actively transcribing bacterial populations in permafrost, including representatives of *Firmicutes*, *Bacteroidetes*, *Acidobacteria* and *Proteobacteria* phlya[16]. It is noteworthy that the active microbes detected in permafrost in the study[16] by Hultman and colleagues were representatives of 'typical' environmental bacteria, not pathogens.

169 With respect to viruses, there is a prevailing reductionist point of view that claims viruses are not
170 truly active, but rather they are like enzymes where random collisions lead to a series of chemical reactions.
171 This is because without cells, viruses cannot multiply, and they do not have the ability to generate ATP.
172 Therefore, to determine if a virus is active, evidence is needed that the virus is able to infect a host and
173 cause a change in the host's ecology. In previous work, viruses have been co-cultured with putative hosts
174 to determine activity but determining the host and culturing a virus can be difficult and time consuming[50].
175 Several advances have recently been developed to determine activity of viruses in environmental samples.
176 A few examples include: (1) bioorthogonal noncanonical amino acid tagging (BONCAT) which is a
177 technique that adds a synthetic amino acid to a sample that allows the tracking and quantification of newly
178 synthesized proteins [51]; (2) Stable Isotope probing (SIP) which uses an isotope with more mass to label
179 newly synthesized DNA[39]; (3) and multi-omics (i.e., metagenomics, metatranscriptomics, and
180 metaproteomics[29]), such as described above[16], that can reveal viral presence, gene expression, and protein
181 production in a sample. Unfortunately, viruses can have several infection styles, each having variable
182 effects[42] (warranting further investigation). While these techniques have been applied to an array of habitats,
183 few have been applied to determine viral activity in permafrost. Recently, SIP-metagenomics was used to
184 determine viral activity in bog samples[39] that were incubated for six to twelve months under conditions
185 simulating the freezing winter temperatures in the presence of $H_2^{18}O$. Active populations incorporated ^{18}O
186 into their DNA when replicating and could thus be distinguished from inactive populations. This study
187 revealed that only a subset of bacteria were active under the simulated winter conditions and suggested that
188 they survived by fermentation and carbon scavenging processes. However, the majority of the detected
189 phages were active and linked to the active bacterial populations, highlighting their importance in
190 structuring of the microbial community. Note that this study focused on active phages that have prokaryotic
191 hosts. Thus, the activity of potential plant, animal or human viruses was not determined.
192

193 **PATHOGENS IN PERMAFROST**
194 An estimated 5 million people currently live in Arctic regions that have underlying permafrost
195 lands[52]. This number is even higher when including tourism and trade in sub-Arctic permafrost areas[53,54].
196 As permafrost thaws there is thus a potential for emerging pathogens to infect humans. Building on current
197 knowledge about microbial life and activity in permafrost in general, we will focus on what has been
198 reported about potential pathogens and exposures from thawed permafrost. We will highlight examples of
199 bacterial, fungal and viral pathogens in permafrost that can pose future risks to humans, other animals and
200 plants (details of each in **Table 1**). Our survey of the literature will conclude that we still have much to
201 learn about potential pathogen risks from thawing permafrost.
202

Potential bacterial pathogens in permafrost

Bacterial pathogens may be preserved in a quiescent state in permafrost and become activated when the permafrost thaws and conditions are favorable for growth ('Bacterial Pathogen' in **Figure 1**). One of the best-known examples is that of *Bacillus anthracis*, the agent of anthrax disease that is globally distributed with potential risks to humans, livestock and wildlife[55]. Spores, such as those from *Bacillus anthracis*, are notoriously robust during long-term storage in freezing conditions ('Anthrax Spores' in **Figure 1**). In 2016, an outbreak of anthrax was reported in Siberia, due to exposure to infected carcasses that had previously been frozen in permafrost. As the permafrost thawed the carcasses became exposed and anthrax spores were released. As a result, one person and over 2000 reindeer died after ingestion of the spores[56,57]. Anthrax outbreaks have also occurred when infected cattle that were buried were re-exposed after permafrost thaw[58].

Stella et al. (2020)[59] modeled the risk of future anthrax exposure and found that the annual risk of anthrax infection is strongly correlated with active layer depth. The model distinguished between freshly released spores from thawed carcasses to those that become reactivated as a result of permafrost thaw. Seasonal forcing by thawing and herding was found to favor endemic disease transmission. Several knowledge gaps exist that are needed to improve the model, including how spores are vertically distributed in permafrost soils. Their results suggest that seasonal thawing of permafrost, together with longer periods of warmer temperatures, could result in an increased prevalence of re-emergence of Anthrax spores and increased risk of associated disease in the Arctic[59]. A compounding reason for anthrax outbreaks is an increase in reindeer with a lack of immunity[60] that could contribute to the spread and infectious capacity of the pathogens. Therefore, one potential mitigation strategy to help control future outbreaks would be to increase reindeer vaccinations.

There are several reports about prevalence of antibiotic resistance genes in permafrost bacteria[61-63] that could make known pathogens more problematic. Antibiotic resistance poses a threat to human health by reducing the efficacy of antibiotic treatments. Antibiotic resistance in pathogenic bacteria is assumed to be a modern phenomenon. However, targeted metagenomic analyses from 30,000-year-old Alaskan permafrost showed resistance to β-lactam, tetracycline and glycopeptide predating the discovery and use of medicinal antibiotics[64]. This finding suggests that permafrost can serve as a reservoir of antibiotic resistance genes. Prevalence of antibiotic resistance in permafrost bacteria is likely an emergent property for competitive fitness rather than pathogenicity. As permafrost thaws, there is the potential for an increase in bacterial populations that bear plasmids with antibiotic resistance genes that have the potential to spread. However, this potential concern is likely no greater than that posed by non-permafrost soils that also harbor abundant antibiotic resistance genes.

Potential fungal pathogens in permafrost

Sequencing has revealed that several fungi are present in permafrost, including species of *Geomyces, Cladosporium, Aspergillus* and *Penicillium*[65]. Some fungi that have been isolated from permafrost are potential plant pathogens ('Fungi' in **Figure 1**). Ancient permafrost from Siberia (16,000–32,000 years old) was found to contain several fungal OTUs from 3 phyla: Ascomycetes, Basidiomycetes and Mucormycota[66]. This study found that approximately a third of the species detected were representative of plant-associated fungi, including pathogens. Within thermokarst bogs, the plant pathogens *Galerina paludosa* and *Hyaloscypha* increased in relative abundances following permafrost thaw[67]. The fungi were likely trapped together with plant material as the permafrost froze. Most fungal isolates from permafrost form small conidia suggesting that this is a survival mechanism for long-term incubation in the cryosphere. Whether the fungi were still alive or not is not known. However, several of the fungal taxa were designated to be putative psychrotrophic fungi with representatives known to produce dark pigments and spores to help with survival under stress conditions.

Representatives of fungal insect pathogens have also been detected in ancient Siberian permafrost[66,68]. Some taxa were putative colonizers of skin or dung of mammals, potentially reflecting the presence of a range of insect and other animal species that thrived at the time (Pleistocene to early Holocene areas). Lydolph et al (2005)[68] studied ancient DNA from a permafrost tunnel in Siberia that ranged from modern age to 400,000 years ago. They also found ancient DNA with fungal representatives of coprophilic and keratophilic fungi, as well as plant pathogens. The main representatives were from Ascomycetes, Basidiomycetes and Zygomycetes phyla. However, no active fungal pathogens in permafrost that directly infect humans have been reported to date to our knowledge.

Potential viral pathogens in permafrost

It is currently unknown how many viruses are trapped in permafrost, but once thawed, the so-called "sleeping" or "zombie" viruses may become free to infect hosts. Although viruses can be preserved in a frozen environment for long periods of time[69], endurance of environment pressures during thawing without bursting is critical for viruses to remain intact and infectious. One example of a class of viruses that have managed to survive in ancient permafrost are giant viruses. Giant viruses or nucleocytoplasmic large DNA viruses (NCDLV) are known to have durable multiple layers enclosing virion nucleoids making them resistant to the harsh permafrost environment[70] ('Giant viruses' in **Figure 1**). Two members of giant viruses, *Pithovirus sibericum*[50] and *Mollivirus sibericum*[71] were previously isolated and revived from 30,000-year-old Siberian permafrost. Although these viruses were preserved frozen in permafrost for at least ~30,000 years, after thaw they were able to infect a modern-day version of their host[50]. The isolated giant viruses were reported to have thick outer walls of 40–70 nm[71,72] comparable to 20–80 nm for Gram positive

271   bacteria[73]. However, the giant viruses that have been characterized to date do not pose a threat to humans

272   and other animals because their natural hosts are microeukaryotes, such as protists and algae ('Protozoa' in

273   **Figure 1**). Another giant virus, Mimivirus, that was first isolated from water samples for investigating a

274   pneumonia outbreak[74], was however, found to be capable of entering human and mouse myeloid cells

275   resulting in a significant increase in viral DNA and a decrease in macrophage viability[75]. A clinical study

276   also reported a high prevalence of Mimivirus antibodies in pneumonia patients with unknown underlying

277   mechanisms[76]. Although viruses often have a narrow and specific host range, these findings challenge the

278   assumption that microeukaryotes are exclusive hosts for giant viruses, highlighting a major knowledge gap

279   in virus-host relationships. Currently, however, there is no evidence that isolated and revived giant viruses

280   from permafrost can cause human infections.

281   The majority of DNA viruses in permafrost environments are phages and therefore cannot cause

282   disease in humans. In fact, phage are arguably the most well-studied type of virus and the majority of them

283   (and viruses in general) are host specific. This means that they can only infect bacteria that are the same

284   species and often only the same strain[77,78]. Despite phages being largely bacterial host-specific, they appear

285   to be major community-structuring agents that uphold a diverse permafrost microbiome. A diverse

286   microbiome prevents any specific bacterium, e.g., a human pathogen, from growing in abundance. This

287   means that beyond phages killing pathogenic bacteria, thereby protecting us, they can create an ecosystem

288   where pathogenic bacteria are less likely to be of concern to humans[79]. Given current evidence of the wide-

289   spread presence of phages in all soils, including permafrost, they are very unlikely to represent a threat to

290   humans.

291   Other types of DNA viruses, such as Variola virus which causes smallpox, that infect humans may

292   be present in permafrost environments, but they have not been detected in sufficient numbers or good

293   quality to pose a threat[80]. Because DNA viruses co-evolve and replicate in the presence of their hosts, in

294   order for pathogenic human viruses to be present in sufficient quantities to cause infection, human/animal

295   remains would have to be preserved in a condition that allows for viral survival. However, this is highly

296   unlikely. For example, Variola virus DNA was recovered from several archeological sites in northeastern

297   Siberia dating back to the late 17th to 19th century. Smallpox victims mummified in the permafrost were

298   found, but the Variola virus DNA was highly degraded and thus the virus was not infectious[81,82]. Therefore,

299   although human DNA viral pathogens can be detected in permafrost, the risk of infection is negligible and

300   only of concern if the virus is intact and in sufficient quantities to cause disease. It is noteworthy that to

301   date there have been no reported cases of viral infections from thawed permafrost in humans.

302   To our knowledge, there is no genetically informed ecological study of the RNA viral community

303   in permafrost. One reason that RNA viruses are understudied compared to DNA viruses is that RNA is less

304   stable compared to DNA[83] and more prone to degradation during the extraction process. One example of

305 an RNA virus is the H1N1 influenza A virus that caused the 1918 pandemic. This virus killed an estimated
306 50 million people, which overwhelmed the burial of infected humans leading to mass graves and unburied
307 corpses[84]. These mass graves largely went unmarked leaving the potential for humans to interact with these
308 corpses should permafrost thaw. In the 1990s many trials were attempted to reconstruct or revive the 1918
309 pandemic influenza virus from frozen samples, but they were unsuccessful because the RNA was highly
310 degraded. In the early 2000s, advances in technology allowed for the successful reconstruction of the H1N1
311 virus from permafrost, but it was not viable[80]. Complete hemagglutinin (HA) genes, encoding binding
312 proteins to initiate infection, were detected from the reserve-transcribed RNAs extracted from the frozen
313 lung tissues of 1918 influenza victims who were buried in a mass grave in permafrost[85]. Although this last
314 example could be taken as a cause for concern, the viruses were not infectious. Further, ancient RNA
315 extracted from wolf tissues buried in Late Pleistocene permafrost showed no traces of RNA viruses[86]. To
316 date, there is no report of intact and infectious RNA viruses directly isolated from permafrost. Therefore,
317 although RNA viruses can be preserved in permafrost, based on our current knowledge, the risk of these
318 RNA viruses being infectious to humans or other animals is unlikely.

319 Permafrost thaw is causing major changes in plant composition and productivity and will
320 undoubtedly affect plant viral pathogens[87]. Previously, plant viral pathogens such as Ancient Caribou Feces
321 associated Virus (aCFV) and Tomato Mosaic Tobamovirus (ToMV) were recovered from ice[27,88]. aCFV is
322 a DNA virus that can infect *Nicotiana benthamiana* (a close relative of tobacco) and may even infect other
323 plants or fungi (e.g., mosses, lichens), and was recovered from caribou feces dating back 700 yrs BP[27]. To
324 test if aCFV was still infectious, a modern version of *N. benthami*ana was exposed and successfully
325 infected. Although the plant became infected, it had an asymptomatic infection, likely due to changes in
326 the plant species over time. Unlike aCFV, ToMV is one of the most common plant viruses, because it has
327 the ability to infect many different plants such as cucumber, lettuce, beet, and pepper[89]. This RNA virus is
328 very stable, having previously been shown to survive UV radiation and desiccation, and was recovered
329 from ice cores dating back up to 140,000 years old[88]. It is unknown if the recovered ToMV could still be
330 infectious, but it presents a potential problem because it is one of the most destructive diseases, resulting in
331 serious crop losses[90]. The introduction of non-native crops to soils, such as recently thawed permafrost
332 soils, could also increase the likelihood of a viral plant epidemic[87,90].

333

334 **CONCLUSIONS AND PERSPECTIVE**

335 Although we have focused on permafrost as a potential reservoir of pathogens, there are other
336 factors to consider in a warming Arctic climate. As we have summarized in this review, although some of
337 the microorganisms and viruses that are preserved in permafrost can be active after thaw, the risks to human
338 health are generally low (**Figure 2**). In the age of COVID-19, the human population has become painfully

aware of the threat of emerging pathogens from 'wild' spaces. Although speculative, there is still a risk that an unknown pathogen could arise from 'wild' permafrost where it has survived over a long period of time in a dormant or quiescent state. A more immediate concern is expansion of modern infectious diseases via vectors (e.g., ticks and mosquitoes) in areas where permafrost has thawed and the soil becomes wetter, or collapses into thermokarst bogs, as the climate warms[91-93]. Increased temperature and precipitation can further expand the geographical distribution of animal vectors[94]. Many of these vectors have been reported to carry human pathogens such as *Francisella tularensis*[95], West Nile virus[96] and Zika[97]. Additionally, transmission vectors can facilitate the spreading of permafrost bacterial pathogens. For instance, anthrax was reported to be carried by both mosquitos (*Aedes aegypti* and *Aedes taeniorhynchus*) and flies (*Stomoxys calcitrans*)[98]. Another pending concern is that increased temperature is correlated with an increase in food and waterborne diseases[99]. Accordingly, there have been recent reports of several enteric disease outbreaks in the Arctic that were caused by bacterial pathogens, including Campylobacter, E. coli 0157:H7, *Vibrio parahemolyticus* and Salmonella. Other vector-borne diseases (e.g., rodents) that could increase in frequency in a warmer Arctic environment include TBE, *Nephropathia epidemia*, tularemia, borreliosi, malaria, toxoplasmosis, and Sindbis Virus infections[22]. Finally, airborne diseases including viral respiratory tract infections and influenza have been correlated to shifts in temperature and humidity. However, the research underlying increased disease prevalence in the Arctic is still scarce. Therefore, we conclude with a call to the broader research community to increase research efforts in this area, as recently highlighted in a general call to increase research on the impact of climate change on microorganisms in the environment[100].

Author Contributions

J.K.J. coordinated the scope and content of the review. N.T., R.W. and G.T. drafted Figure 1. R.W. drafted Figure 2. All authors contributed equally to the writing of this review.

Competing interest statement

The authors declare no conflict of interest.

Additional information

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Relevant hashtags would be: #Climate, #Soil, #Micorbiome, #Virus, #Permafrost, #Pathogen

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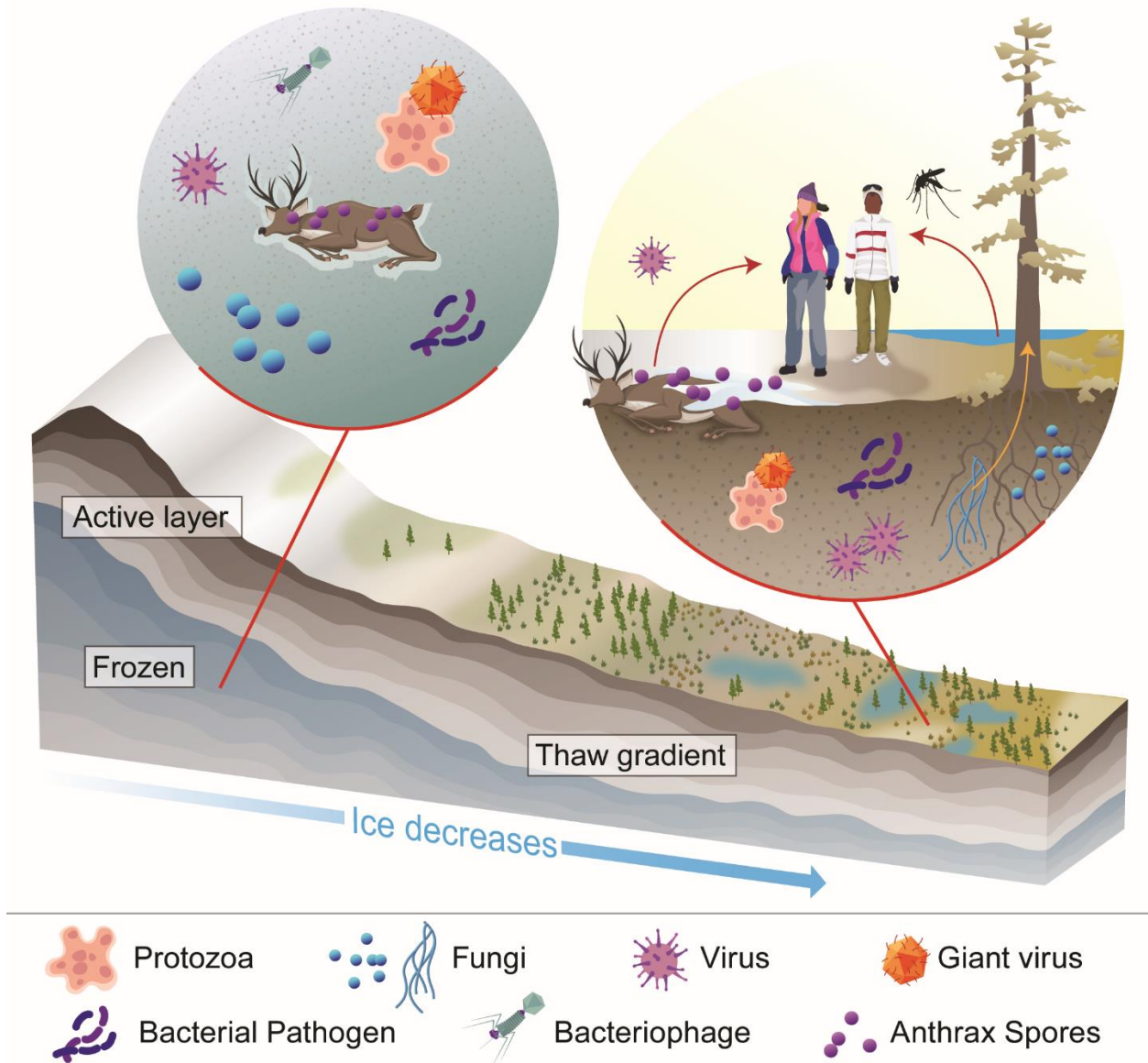
Figure 1. Emergence of potential pathogens along a permafrost thaw gradient. Left inset illustrates examples of microbes in permafrost, including non-pathogenic (e.g., 'Bacteriophage' and 'Giant viruses' with bacteria and protozoa as respective hosts) and potential pathogenic microbes (e.g., spores of 'Fungi', 'Anthrax spores', other 'Virus'). Right inset illustrates examples of pathogen emergence in thawed permafrost, including anthrax spores from animal carcasses and potential disease transmission vectors like mosquitoes.

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Figure 2. Potential Risks of Microbial and viral pathogens in permafrost

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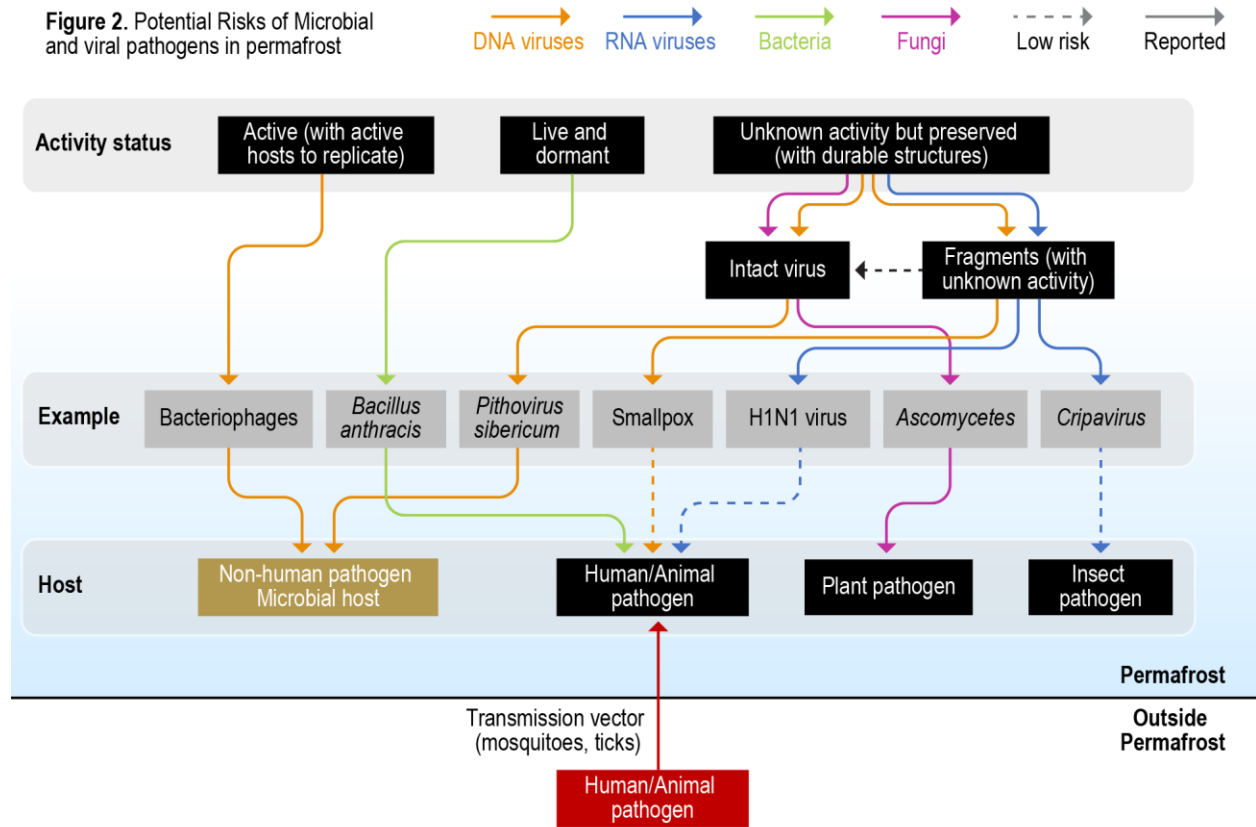
Figure 2. Microbes in permafrost can infect a diverse range of hosts. Microbes in permafrost are categorized into three activity statuses: 'Active', 'Live and dormant' and 'Unknown activity but preserved' in either intact or fragmented forms. Examples of microbes ('DNA viruses', orange lines; 'RNA viruses', blue lines; 'Bacteria', green lines; and 'Fungi', pink lines) are listed that potentially infect bacteria, protozoa, humans/animals, plants and insects. Low potential pathogenicity cases are indicated with dashed lines. Reported pathogenicity cases are indicated with solid lines.

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Table 1. Examples of potential pathogens in permafrost and associated ice patches

*Indicates non-permafrost cryosphere samples

Biological entity	Name	Disease caused	Type of pathogen	Sample detected	Outbreak	Reference
Bacterium	*Bacillus anthracis*	Anthrax	Human or animal pathogen	Siberia permafrost	In 2016, a person and thousands of reindeer died; between 1897 and 1925, 1.5 million deer died	[56-58]
DNA virus	Variola virus	smallpox	Human or animal pathogen	Northeastern Siberia	NA	[81,82]
	Ancient Caribou Feces associated Virus (aCFV)	asymptomatic infection	Plant pathogen Fungal pathogen	*700-y-old caribou feces from a subarctic ice patch	NA	[27]
Fungus	Members of the genera Ascochyta, Entyloma, Epicoccum, Fusarium, Gaeumannomyces, Microdochium, Phaeosphaeria, and Phoma	Leaf spot, defoliation, and a reduction in yield and crop value	Plant pathogen	Ancient permafrost from Siberia (16,000-32,000 years old)	NA	[66]
	Members of Cordyceps, Paecilomyces	Paralysis or mortality	Insect pathogen	Ancient permafrost from Siberia (16,000-32,000 years old)	NA	[66,68]
RNA virus	Cripavirus	Paralysis or mortality	Insect pathogen	700-year-old caribou feces in subarctic	NA	[27]
	H1N1 virus	Influenza	Human or animal pathogen	frozen lung tissues of 1918 influenza victims	NA	[85]
	Tomato Mosaic Tobamovirus (ToMV)	Tomato mosaic disease	Plant pathogen	*140,000-year-old Greenland ice core	NA	[88]