MICROBIAL GENOMICS

REVIEW

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Microbial genomics amidst the Arctic crisis

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Abstract

The Arctic is warming – fast. Microbes in the Arctic play pivotal roles in feedbacks that magnify the impacts of Arctic change. Understanding the genome evolution, diversity and dynamics of Arctic microbes can provide insights relevant for both fundamental microbiology and interdisciplinary Arctic science. Within this synthesis, we highlight four key areas where genomic insights to the microbial dimensions of Arctic change are urgently required: the changing Arctic Ocean, greenhouse gas release from the thawing permafrost, 'biological darkening' of glacial surfaces, and human activities within the Arctic. Furthermore, we identify four principal challenges that provide opportunities for timely innovation in Arctic microbial genomics. These range from insufficient genomic data to develop unifying concepts or model organisms for Arctic microbiology to challenges in gaining authentic insights to the structure and function of low-biomass microbiota and integration of data on the causes and consequences of microbial feedbacks across scales. We contend that our insights to date on the genomics of Arctic microbes are limited in these key areas, and we identify priorities and new ways of working to help ensure microbial genomics is in the vanguard of the scientific response to the Arctic crisis.

INTRODUCTION

The accelerated warming of the Arctic is already resulting in the loss of sea ice, the recession of glaciers and the expansion of wildfires [1, 2], with the consequences of these impacts already reaching far beyond the Arctic region [3–5]. Within the 'business as usual' scenario presented by the Intergovernmental Panel on Climate Change (IPCC) (RCP8.5) [6], it is likely that regions of the Arctic will experience up to 10 °C warming by the end of the century [7]. For a region that can be defined by July monthly mean temperatures of 10 °C or less, it is clear that an additional warming of 10 °C will have extensive impacts [8]. As a result, the Arctic is one of the areas in greatest danger from the current climate crisis [9]. Since microbes inhabit many of the critical interfaces between the Arctic environment and its climate [10, 11], they will experience impacts and prompt feedbacks as a result of the Arctic crisis. However, the climate interactions of Arctic microbes are still somewhat overlooked within contemporary syntheses [12]. Herein, we contend that understanding microbial responses to Arctic warming, and indeed predicting whether Arctic microbes will fuel further feedbacks, requires exploration of Arctic microbial genomic potential and the fusion of genomic insights with those garnered from diverse academic disciplines.

Arctic microbes as first responders

Microbes are the first responders to the Arctic crisis. Small in size but large in number, microbes inhabit diverse niches across the Arctic. Their generation times are typically much shorter than plant or animal inhabitants of the Arctic and often well within seasonal or synoptic timescales [13, 14], allowing rapid changes in Arctic microbial populations in response to climate changes. Specifically, many microbial niches are interposed at the margins between frozen substrates (e.g. brine channels in sea ice, permafrost, glacial weathering crusts, englacial vein boundaries). Here, liquid water is limited, which hinders microbial activity [15–17]. Thus, changes in temperature that switch Arctic environments from frozen solids into melted liquids can radically alter the niches available to microbial populations. Through their growth and nutrient cycling, the collective regional-scale responses of Arctic microbes can influence biogeochemical

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Abbreviations: IPCC, Intergovernmental Panel on Climate Change; MAG, metagenome-assembled genome; tDOM, terrestrially derived organic matter.

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cycles on regional and global scales [18–20]. Consequently, members of Arctic microbiota have been considered both sentinels and amplifiers of global climate change [10]. The key to how Arctic microbes both sense changes in their local environment and amplify the global impacts of these changes is to be found within their genomes.

The microbiology of a crisis

The purpose of this review article is to stimulate the response of microbial genomics as a field to the Arctic crisis. Some of the most innovative and significant conceptual and technical developments within the history of microbiology have been stimulated by crises: from germ theory [21] to real-time genomic epidemiology [22]. Confronting the Arctic crisis presents an imperative to address many gaps in our fundamental knowledge of cold-region microbiology, which potentially constrain climate models and the informing of policymakers. Furthermore, in crisis there is also opportunity, and bioprospecting of the Arctic is recognized as an emerging field [23]. However, beyond potential influence on climatology, policy or economy, the study of Arctic microbial genomics is merited in its own right, for many fundamental gaps remain in our knowledge of Arctic microbes. In any case, the prospect of rapid and radical change in the microbial ecosystems of the Arctic must prompt the systematic investigation of genomic diversity within these ecosystems before they are overridden by the effects of warming, the rate of which is unprecedented in human history [9]. Therefore, this review will offer a primer on key microbial habitats and processes in the Arctic, before considering important challenges and potential opportunities for microbial genomics in confronting the Arctic crisis.

Impact Statement

The Arctic is a vast region of our planet that is warming very quickly as Earth's climate is changing. This means changes in the Arctic can have impacts that matter globally. A diverse range of microbes are well adapted for life in Arctic environments, but many of these microbes are responding to change in the Arctic and some even drive feedbacks that may affect climate change itself. Our understanding of these adaptations, responses and feedbacks can be improved by integrating microbial genomics with other disciplines of Arctic science. Our review identifies four key areas in which microbes are playing crucial roles in the changing lands and seas of the Arctic, and a further four areas in which the innovative use of genomics can address important gaps in our fundamental knowledge of Arctic microbiology and its implications for a changing Arctic and our warming world.

KEY MICROBIAL PROCESSES IN CRITICAL ZONES OF ARCTIC CHANGE

Within this section, we consider the changing microbiology of four ice-cold hot-spots of microbial diversity, activity and feedbacks in the Arctic climate system (Fig. 1). These range from the changing Arctic Ocean and permafrost thaw to glacial ecosystems and human activities in the Arctic. For each of these critical zones, microbe-mediated processes interacting with climate change are highlighted and areas are identified where improved understanding of the genomic foundations of Arctic microbiomes is required.

Sea ice habitat loss in the Arctic Ocean

At its maximum, Arctic sea ice currently extends to around 15 million km^2, blanketing almost all of the Arctic Ocean with a solid frozen cap. In the 40 years of satellite observations, the extent of sea ice in the Arctic Ocean has declined considerably, from a September minimum of 7.7 million km^2 in 1979 to the lowest extent of 3.6 million km^2 in 2012, with the last 13 years representing the thirteen lowest extents in the satellite era (http://www.climate.gov/news-features/understanding-climate/climate-change-minimum-arctic-sea-ice-extent). The loss of sea ice itself prompts changes in sea ice albedo feedback, where the high surface reflectance of sea ice to solar energy is decreased in contrast to the increased absorption of solar energy to the darker surface of open water [24]. These changes have the potential to influence the planetary energy budget [25].

Sea ice is a complex microbial habitat marked by profound gradients in temperature, chemistry and salinity across a vertical profile of a few metres [15]. Within the sea ice column, microbes inhabit highly saline waters within pore spaces and brine channels are created as ice formation excludes dissolved salts [15]. The interface between the base of the sea ice column and underlying seawater is marked by high densities of microbes [15, 26]. Sea ice microbes are perennially active [27, 28]. Long hours of sunlight during the polar day supports a net autotrophic ecosystem [15] driven by eukaryotic phototrophy, primarily from diatoms such as *Fragilariopsis* [29, 30]. The exudation of organic carbon from sea ice diatoms supports a diverse range of bacterial heterotrophs, Archaea, protists and meiofauna associated with the sea ice [15, 31–33]. The export of sea ice organic carbon nourishes the food web of the underlying water column and seabed [34], emphasizing the importance of sea ice habitats in the functioning of the Arctic Ocean ecosystem [35]. The declining extent of sea-ice coverage, therefore, has profound impacts on the broader Arctic Ocean ecosystem, and the loss of thicker, structurally more complex, multi-year sea ice diminishes the range of productive niches available to sea-ice microbes [36, 37]. The loss of multi-year sea ice in a region is associated with long-term taxonomic shifts in the microbial communities of underlying water, for example, communities in the Beaufort Sea [38, 39] showed a decline in the abundance of multiple microbial groups relevant to biogeochemical cycling within the region. These included *Bacteroidetes*, which are typically associated with processing complex organic carbon [40], likely from diatoms, and

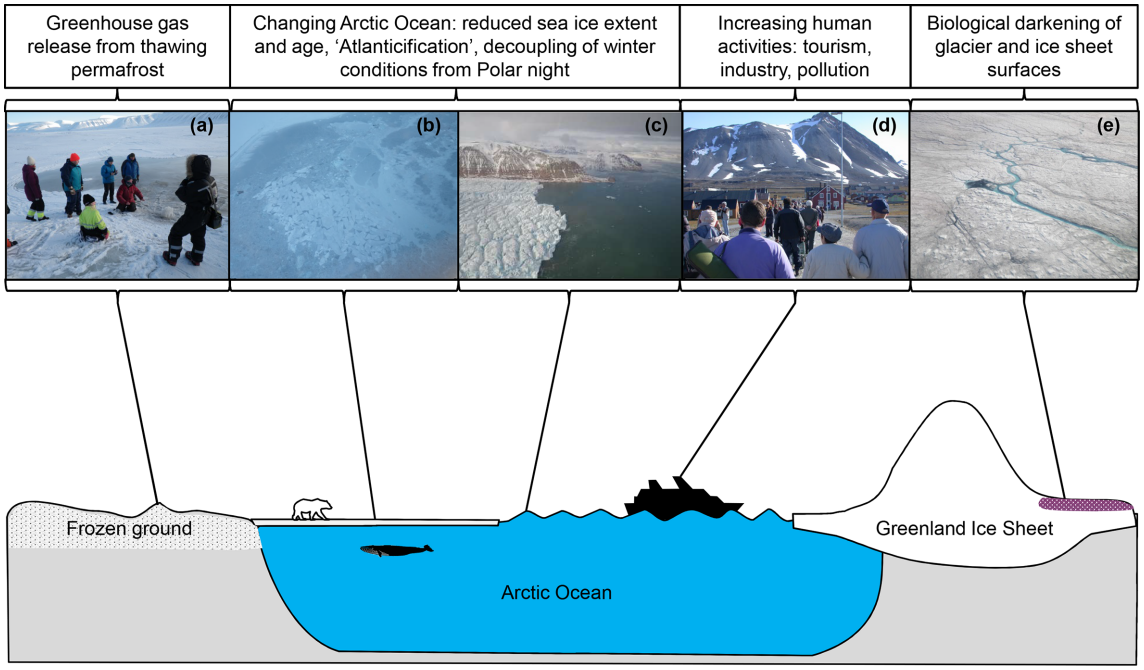

Fig. 1. Ice-cold hot-spots of microbial change within the warming Arctic. The images demonstrate: a controlled release of methane-saturated groundwater from High Arctic permafrost (a); first year sea ice in winter (b); a marine-terminating glacier meeting open water in a High Arctic fjord in winter (c); cruise ship visitors at Ny Ålesund, a Svalbard settlement used for coal mining and scientific research for over a century (d); glacier algae growth and cryoconite accumulation in the Dark Zone of the Greenland Ice Sheet (e). All photographs are from the personal collection of A. Edwards.

marked reduction in the abundance of ammonia-oxidizing Thaumarchaeota, which correlates with a decline in nitrate, the limiting nutrient for productivity in the region

Concomitant with the loss of sea ice, the expansion of open water as a habitat in the Arctic Ocean is prompting the immigration of microbial groups once thought limited to lower latitudes, with potential effects on bacterial lineages that may be endemic in the Arctic Ocean [41, 42]. Most notably, coccoid picocyanobacteria (*Synechococcus* sp.), major marine primary producers in lower-latitude oceans, were once considered essentially absent in the colder waters of the Arctic Ocean [43]; however, they are now found as far north as 82.5°N, with abundant populations in Atlantic water reaching north-west of Svalbard (79°N) [44]. Picocyanobacteria such as *Synechococcus* and *Prochlorococcus* can survive the dark season in the Arctic Ocean [45], through photoheterotrophic metabolism. Consequently, they may out-compete eukaryotic algae [46] within the autumn and winter seasons in the Arctic Ocean. Therefore, they may be well placed to respond to the continued warming and loss of sea ice [47].

Indeed, it has been assumed that solar radiation offers a bottom-up control on the overall structure and function of Arctic marine ecosystems, and that the lengthy dark period of the polar night represents a quiescent phase in the ecology of the Arctic Ocean. However, with the increased expanse of open water and the warming of the Arctic Ocean's surface, it has become clear that polar night represents a period of microbial activity that has hitherto been largely overlooked [48]. Historically considered synonymous, winter, as a period of sustained low temperatures resulting in extensive sea ice, is now discrete from polar night, as a period of sustained darkness [49]. The implications of this decoupling of polar night and winter beg for further research attention to its consequences for microbial dynamics in a warming Arctic [47, 48]. In summary, understanding the range shifts, genomic adaptations and population structures of important marine primary producers, such as these cyanobacteria [47], will inform our predictions of how the food webs of the Arctic Ocean will respond to a future in which the extent and longevity of sea ice is severely curtailed.

Carbon release from thawing permafrost

Nearly half of global soil organic carbon is found in Arctic soils (1330–1580 Pg C) [18]. Most of this carbon is stored within permanently frozen ground, including permafrost (i.e. soil that remains frozen for 2 or more consecutive years) [50]. However, with warming temperatures across Arctic lands, this accumulated stock of legacy carbon from past climates does not represent a permanent sink of carbon. Indeed, thawing permafrost reinvigorates microbial communities that consume these carbon stores and release greenhouse gases in the form of carbon dioxide (CO_2) and methane (CH_4) to the atmosphere, with potent climate consequences [18, 51]. If our current trajectory of global warming is continued (IPCC

RCP8.5), by the end of this century 30–99% of near surface permafrost in the Arctic will have been degraded [52], with an associated release of 37–174 Pg C due to microbial processes within the thawed soils [18]. Understanding the drivers, rates, mechanisms and extent of microbial carbon cycling in Arctic soils is, therefore, an urgent priority, considering the large uncertainties apparent in the estimated ranges of carbon release [53].

Changes in Arctic lands also affect Arctic rivers and the Arctic Ocean [54, 55]. Thawing and degradation of permafrost will liberate substantial terrestrially derived organic matter (tDOM) for riverine transport to the Arctic Ocean [56]. Approximately 44 Tg organic carbon is released annually to the ocean from coastal and terrestrial permafrost within the Siberian Arctic alone, the bulk of which is predicted to be respired to carbon dioxide [57]. Microbes within coastal Arctic fjords respond readily to the influx of tDOM. Experiments simulating the release of permafrost carbon to a Svalbard fjord showed *Glaciecola* populations expanded aggressively following the addition of terrestrial dissolved organic matter [58]. Since *Glaciecola* is a gammaproteobacterial lineage associated with rapid consumption of organic matter in cold waters [59] following spring phytoplankton blooms, it is possible this effect may have broader impacts on the bacterial processing of organic carbon and food webs in the coastal Arctic [58]. The influence of tDOM extends further than the Arctic shoreline. The first study to develop metagenome-assembled genomes (MAGs) from the Arctic Ocean recently revealed marine *Chloroflexi* populations with the capability for degrading highly aromatic tDOM [20]. It appears that this capability arose through lateral gene transfer from terrestrial lineages, since the aromatic metabolism genes detected within the marine *Chloroflexi* MAGs were closely related to homologues present in terrestrial actinobacteria, acidobacteria and proteobacteria, while the parent lineage of *Chloroflexi* SAR202 has been found in deeper and darker (see the previous section) Arctic waters [60]. While the lateral transfer of carbon between Arctic lands and the Arctic Ocean is well acknowledged [55], the interactions concerning lateral gene transfer between terrestrial and marine Arctic microbial genomes and climate-driven changes in the biogeochemical cycles of the Arctic clearly merit further exploration.

Permafrost itself is considered an unusual microbial habitat [61], since it represents a structurally heterogeneous environmental matrix that combines a long-term deep-frozen store of microbial biomass and genomic diversity from past climates (even >1 million years old) [18], which may become reactivated upon the degradation of the permafrost [62]. Recent chronosequence surveys [63, 64] have examined the survival mechanisms of viable microbiota within permafrost dating from the Pleistocene period (samples that dated to 19 000–33 000 years before the present). These reveal that while endospore-forming taxa are prevalent, viable biomass from some of these taxa remain as vegetative cells [64], underlining the potential for long-term, low-growth-rate survival rather than sporulation as a persistence mechanism within permafrost.

Cold laboratory incubations (reviewed by Nikrad *et al.* [65]) lend further support to the potential for activity and even growth of microbial populations in sub-zero conditions [66]. Analysis of pangenomes from isolate and MAGs from permafrost could, therefore, provide insights to survival mechanisms and microevolutionary processes across geological timescales. However, the urgent questions prompted by the prospect of extensive permafrost degradation relate to the rates and pathways of carbon metabolism in thawing permafrost. In particular, our ability to predict the relative magnitude of permafrost carbon released as either CO_2 or as CH_4, which has an estimated global warming potential ~30 times greater than CO_2 [6], is essential for predicting the role of permafrost in climate warming feedbacks. In-field gas flux measurements or cold-lab incubations alone have not offered an integrative view of microbial contributions to greenhouse gas evasion from permafrost [16]; therefore, recent work has focused on the integration of multi-omics approaches with biogeochemical process measurements of permafrost thaw incubations [67, 68], as well as permafrost cores [69]. Most recently, large-scale genome-centred metagenomics conducted across a permafrost thaw gradient have underlined the importance of linking processes with pathways and taxa by revealing novel fungal pathways for plant polysaccharide degradation and syntrophic interactions resulting in CH_4 production [70]. Furthermore, the potential for viruses to modulate carbon cycling through methanogen infection or lateral gene transfer of carbon processing pathways has been revealed by metavirome sequencing [71].

In determining the magnitude of CH_4 release from thawing Arctic permafrost, a critical question is posed by the capability of CH_4-oxidizing microbes (methanotrophs) to consume CH_4 formed by archaeal CH_4 producers (methanogens) before it can reach the atmosphere. Methanotrophs may modulate the release of between 20 and 60% of the CH_4 formed in tundra wetlands [72–74]. Linking genome-centred metagenomics with metatranscriptomics is revealing the diversity of methane-processing genetic mechanisms present within thawing permafrost. One highlight has been the identification of a resilient, dynamic methanotrophic community that can utilize isozymes with differential affinities for CH_4; thus, aiding their ability to persist through fluctuating CH_4 availability [75]. Understanding the potential for methanotrophy to mitigate methane release from thawing permafrost will require pairing biogeochemical process measurements with the capability to resolve diverse pathways for methane oxidation. These pathways must also be attributed to multiple lineages present within dynamic microbial communities. Pairing biogeochemical process measurements, physical and chemical analyses with genomics seems to offer a promising strategy to resolve the extent to which methanotrophy can offset methanogenesis in thawing tundra over the coming decades. To support accurate predictions of methane release, such approaches must embrace the complexity offered by a net outcome that is the sum of interactions within dynamic microbial consortia in structurally heterogeneous habitats

defined by fluctuating oxygen, methane, terminal electron acceptor and water levels.

Microbial impacts on Arctic glaciers and the Greenland Ice Sheet

Arctic glaciers, ice caps and the Greenland Ice Sheet have started to experience the crippling consequences of Arctic warming, yet the melt that has been experienced to date is a mere fraction of what can be expected as the Arctic continues to warm. The largest of these glacial ice masses is by far the Greenland Ice Sheet, which occupies 1.7 million km^2 and currently sequesters the water equivalent of 7.4 m of sea-level rise [76]. Glacial meltwater is currently the largest contributor to sea-level rise [8], making this relationship a key societal concern and research priority. Models of glacier mass balance presently used to constrain estimates of sea-level rise currently do not incorporate microbiological parameters [8]. Addressing the role of microbiota in the rate and magnitude of Arctic glacial ice loss and climate warming, topics that have historically been overlooked, therefore, are of considerable importance to ensure robust estimations of future sea-level rise and climate change.

That glacial systems are home to abundant and diverse life forms has been long established [77, 78]. This century has seen a refreshed synthesis of evidence for biodiverse microbial ecosystems associated with glaciers and ice sheets, and an acknowledgement that these biomes contribute to global biogeochemical cycles [79]. Like permafrost, glacial ice is a vast, climate-sensitive repository of microbial biomass and genomic diversity [80]. Equally, microbial processes at the surfaces and beds of Arctic glaciers and the Greenland Ice Sheet have the potential to amplify the impacts of climate warming on glaciers.

The subglacial zone beneath glaciers and ice sheets is perennially dark and cold. Microbial ecosystems are apparent here, subsisting on organic carbon washed from the surface [81], relict carbon overridden in the last ice age [82] or existing through chemolithotrophy [83]. Critically, the evolution of anoxic conditions in subglacial habitats can favour methanogenesis [84] through both hydrogenotrophic and acetoclastic pathways [82]. Methane oxidation at the oxygenated glacial margins may mitigate subglacial methane production [85, 86]; however, contrasting results from neighbouring outlets of the Greenland Ice Sheet prompt uncertainty on whether methane oxidation can adequately compensate against methane production [85, 87]. Hitherto, a genomic perspective on subglacial ecosystem structure and function is in its infancy, with very few publicly available metagenomic datasets [88].

In contrast, the surfaces of Arctic glaciers receive abundant solar radiation in summer. This prompts the seasonal development of a range of microbial community types predominantly supported by photoautotrophy [15, 89]. Importantly, the accumulation of microbial biomass replete with photosynthetic and photoprotective pigments and recalcitrant dark organic matter at the glacier surface has the potential to influence the melting rate of the glacier surface [8]. The reduced surface reflectance of glaciers consequent to microbial growth in surface habitats has been termed 'biological darkening' [90] or 'bioalbedo' [91] in recent years. Estimates of microbial contributions to glacier melting are emerging [92–94]; however, integration of microbial-associated parameters in estimates of sea-level rise remains an active research goal.

Snowpacks on Arctic glacial surfaces are vast environments that both support distinctive microbial consortia and are highly sensitive to warming [95–97]. The potential for snowpack bacteria to cycle climate-relevant trace gases has recently been highlighted as an emerging area [98, 99]. However, the growth and pigmentation of green algae in the family Chlamydomonadaceae in discrete patches on snow is particularly apparent. The consequent formation of intracellular carotenoid pigments can modulate local solar energy balance [100] and colour snowpacks bright red. Snow algal productivity can support a diverse range of heterotrophic taxa [101]; thus, subsidizing the development of a snowpack carbon cycle. Similarly, on the bare ice surface, members of the Zygnematophyceae glacier algae [102] form ice-darkening biofilms [103] that are particularly prominent on the southwestern margins of the Greenland Ice Sheet, exhibiting locally structured populations [104]. Glacier algae influence surface reflectivity through the accumulation of dark photoprotective purpurogallin pigments [102], and their expansive spatial coverage can promote both surface ice ablation and carbon cycling [94, 102, 105].

Finally, cryoconite ecosystems are among the most intensively studied habitats on the glacier surface [106]. These collections of granular microbe-mineral aggregates (cryoconite) darken patches of the ice surface, resulting in localized melting and the formation of quasi-circular cylindrical melt holes [106]. Cryoconite granules are maintained at thermodynamic equilibrium depths and generally as single-granule layers at the floor of the cryoconite hole [107]. These responses to solar energy balance and cryoconite debris loads ensures the major primary producers in cryoconite, cyanobacteria, are continually exposed to optimal levels of solar radiation for photosynthesis; thus, promoting high levels of carbon fixation in spite of low ambient temperatures [107–109] Across Arctic glacial surfaces, a single lineage of filamentous cyanobacteria, *Phormidesmis* sp., appears responsible for binding together each cryoconite granule [99, 110–115]. A recent global-scale survey identified a single 16S-23S ITS haplotype of *Phormidesmis priestleyi* predominant upon Arctic glaciers [112]. Since the population structure of *Phormidesmis* sp. and other lineages of glacier cyanobacteria shows increasing fragmentation following the decline in glacierized surface area since the peak of the last ice age, it is likely they have an enduring role as ecosystem engineers of glacier surfaces [112]. Consequently, it is likely that one lineage of glacial cyanobacteria is predominantly responsible for the formation and maintenance of productive island-like microbial ecosystems within the austere environs of Arctic glacier surfaces, making cryoconite holes attractive for studies requiring naturally

Table 1. Key cold adaptations relevant for biotechnology

Target product	Adaptation mechanism	Application
Cold-active enzymes	Amino acid changes that increase enzyme flexibility	Food industry, detergents, molecular biology tools
Polyunsaturated fatty acids	Polyunsaturated fatty acids increase membrane permeability at low temperatures	Dietary supplements for humans, livestock and fish
Ice nucleation proteins	Seed small crystals instead of large damaging crystals	Food industry, synthetic snow
Antifreeze proteins and solutes	Prevent water molecules from forming ice-crystal structure	Cryoprotectants, food industry
Antioxidants and UV pigments	Protect micro-organisms from seasonally high UV irradiation in snow	Biomedical, pharmaceutical, food technology and cosmetics
Exopolysaccharide	Trapping of liquid water, preventing freezing	Biomedical, pharmaceutical, food technology and cosmetics
Antimicrobial compounds	Chemical defences and weapons against competing bacteria in low-resource environments	Pharmaceuticals: antibiotics, antifungals, anti-tumour medications and pesticides

occurring microcosms of community development (cf. the article by Rivett and Bell [116]).

Human dimensions of the changing Arctic: bioprospecting and infectious disease risks

The Arctic has been inhabited by humans for millennia. It is now home to four million people. Moreover, the rapid changes in Arctic climate expected this century are leading to renewed interest in the economic potential of the Arctic as mineral resources, maritime navigation and tourism all become more accessible. This poses microbial risks and opportunities [117].

Firstly, growing commercial and political interests coupled with increased logistical accessibility is likely to stimulate interest in Arctic bioprospecting [23]. Adaptations for life in the cold found within the reservoir of Arctic genomic diversity can be industrially useful [118]. Examples include enzymes with low temperature optima [119], low-alcohol yeast [120], antifreeze and ice-binding proteins [121], and potential antimicrobials [122] (Table 1, fully referenced and expanded in Table S1, available with the online version of this article). To date, the majority of antimicrobial compounds and cold-active enzymes have relied on cultured isolates, which are either screened directly for activity [122] or genome sequenced; followed by the cloning and expression of candidate genes or gene clusters in a heterologous host [123]. These strategies rely on the isolation and genome-sequencing of microbes, which is limiting because: (i) fully sequenced genomes of Arctic strains are limited in number (see below), and (ii) many microbes remain uncultivated. In addition, advances in sequence-based and functional metagenomic approaches [124] offer promising approaches to mine and exploit such potential. For example, specially engineered heterologous expression hosts, such as the ArcticExpress *Escherichia coli* competent cells (Agilent Technologies) and *Pseudoalteromonas haloplanktis* TAC125 strain [123] are noteworthy for being products of cryospheric bioprospecting, and tools by which further functional exploration of this environment can be accomplished. ArcticExpress cells co-express the chaperonin system Cpn60 and Cpn10 from *Oleispira antarctica*, which helps to ensure the proper folding of cold-active proteins and increase the growth rate of *E. coli* at low temperatures [125]. *Pseudoalteromonas haloplanktis* TAC125, however, is of cryospheric origin, and displays increased solubility and secretion of protein products over other Gram-negative expression hosts. Meanwhile, improvements in sequencing technologies have resulted in the assembly of longer contigs, with deeper coverage than ever before, unlocking types of analyses previously available only to whole-genome-sequenced cultured organisms. Tools such as antiSMASH [126], for example, can be used on metagenomic datasets (contigs or MAGs) to detect biosynthetic gene clusters responsible for the synthesis of industrially useful compounds, such as antibiotics, fatty acids, polysaccharides, antioxidants and UV-protective pigments. However, the greatest improvements in bioprospecting will likely come from the synergy of sequence-based and functional methods, because an understanding of the genomic background of the source organism (see below) is vital for the strategic genetic engineering of suitable hosts and the identification of optimal conditions for expression of the desired natural product.

Secondly, the Arctic is not pristine and has not been pristine for some time [127]. There is a long history of human activities that have contaminated the Arctic in many ways, from hydrocarbon exploitation [128, 129] to military activities, including the largest nuclear explosion to date [130]. These have resulted in locally derived contamination of the Arctic. Likewise, long-range atmospheric transport of pollutants and the global distillation effect has led to the deposition of pollutants in the Arctic from the mid-latitudes for at least 3 millennia [127]. The potential roles of microbes in modulating or exacerbating the threats posed by contaminants liberated by Arctic warming is a current focus for researchers addressing radionuclide [131], persistent organic pollutant [132], black carbon [133], mercury [134] and heavy metal

contaminants [135]. Furthermore, both increased access to the Arctic and the potential release of long-frozen hazards is raising the prospect of the liberation of ancient infectious diseases [136, 137]. Such notions seem speculative, for they depend on the release of viable agents able to withstand severe freeze–thaw stresses as they migrate to the actively melting layers of glaciers or permafrost [138]. Hitherto, dedicated efforts to recover pathogens from the Arctic have failed, as human remains have degraded within the active layer of permafrost [139]. However, the release of *Bacillus anthracis* from frozen wildlife carcasses has been invoked as the cause of a recent Siberian anthrax outbreak [140]. A further hypothetical risk is presented by implementing synthetic biology approaches to resurrect poorly described viral genomes from Arctic ice [141], as these may generate highly concentrated infectious materials within laboratories [137]. The ethical debate and moratorium on resurrecting highly pathogenic influenza strains [142] offers a certain precedent for concern within this arena.

Finally, these changes in the accessibility and ecology of the Arctic bring with them pressures for human healthcare [143]. These include increased demand on the limited healthcare services available or the immigration of emerging infectious diseases; for example, as vectors move polewards [144]. This may necessitate enhanced microbiological surveillance and diagnostic capability; and distributed or ubiquitous genomic sensing [145] may prove important in detecting and managing microbial threats as the Arctic experiences disruptive change.

CHALLENGES AND OPPORTUNITIES FOR ARCTIC MICROBIAL GENOMICS

A recurring theme within the preceding sections is that there are significant lacunae in our understanding of Arctic microbial genomics. These constrain both our appreciation of the fundamental properties of Arctic microbial ecosystems, and our ability to predict their interactions with the aggressively changing climate of the Arctic. In a time where microbial genome sequencing in other study areas is all but routine [146, 147], and expensive, expansive efforts to catalogue microbial diversity across the planet yield transformative results [148, 149], why are the diverse and societally relevant genomes of Arctic microbes genomes left out in the cold?

This part of the review will identify some of the salient challenges faced in Arctic microbial genomics and opportunities to address them. These challenges range from conceptual to technical and logistical considerations; thus, there is scope for innovation, which could prove both timely and transformative for Arctic microbial genomics.

Challenge 1 – insufficient data to develop unifying concepts for life in the cold

Most undergraduate microbiology textbooks may define psychrophiles (or cryophiles) in relation to organisms with relatively colder cardinal temperatures for *in vitro* growth [150]; therefore, this challenge may seem surprising. In many cases, it has been assumed that Arctic ecosystems are populated by such psychrophiles. Indeed, there are many organisms from cold regions that are isolated in culture and exhibit growth at low temperatures [151–153]. Furthermore, various well-described traits are linked to growth at low temperature *in vitro*, ranging from changes in enzyme structure to membrane fluidity to stress responses [154]. Table 2 summarizes key genes invoked in cold adaptation in laboratory studies of bacteria, which are then fully detailed in Table S2. These indicate a broad array of mechanisms for cold adaptations, including cold-shock responses to DNA topology modulation, protein synthesis and stabilization, and metabolic processes.

Nevertheless, psychrophily itself almost seems to be a conceptual afterthought defined by contrast to thermophily and mesophily [150]. Critically, whether psychrophily is ecologically relevant remains open to question, for colder optimal growth temperatures in the laboratory do not necessarily translate to increased fitness in low-temperature environments. Recently, Cavicchioli [155] provided a detailed critique of the relevance of psychrophily as a concept for life in the cold. It is clear that not only is *in vitro* psychrophily defined differently by different workers, but also there are striking examples of discordant patterns in the growth optima of organisms prominent within low-temperature environments. Moreover, defining psychrophily on the basis of colder cardinal growth temperatures shown by axenic cultures *in vitro* fails to embrace the diverse range of stresses likely experienced by organisms in Arctic ecosystems. These can include resource and nutrient limitations, energy constraints, UV radiation and reduced water activity [156]; all of which may also act in concert with biotic factors such as competition or predation [157].

Therefore, it could be argued that, by itself, the concept of psychrophily as defined by the growth rates of an axenic culture *in vitro* fails to offer an adequate framework for understanding the adaptations and functioning of Arctic microbes. Yet few ecologically meaningful alternatives have been advanced. Cavicchioli [155] offers the elegant suggestion that the term 'psychrophile' applies to any microbe that is indigenous to a cold environment. While this has a certain utilitarian advantage and is certainly inclusive, it is perhaps overly inclusive. Since our understanding of microbial biogeography remains patchy, with continued debate on the validity of an early 20th century concept on whether all microbes are indigenous everywhere [41, 158–160], and our techniques for detecting the presence of microbes extrapolative, classifying whether microbes (or their phylotypes) are indigenous or transient in a given environment remains problematic. It also fails to recognize the potential for transient and immigratory microbes to make important contributions to Arctic ecosystem functioning [44].

In spite of these limitations, if we define psychrophiles as microbes indigenous to cold environments (*sensu* the paper by Cavicchioli [155]), it is clear we are profoundly limited in our ability to define the genomic basis of psychrophily.

Table 2. Summary of genes implicated in cold adaptation

Gene	Protein	Assigned function
dnaA	DnaA	DNA binding, replication initiation, global transcription regulator
dnaG	DnaG	DNA primase
gyrA	GyrA	DNA cleaving/binding/re-joining subunit of DNA gyrase
hns	H-NS	Nucleoid protein, transcriptional repressor, DNA supercoiling
hupB	Hu-β	Nucleoid protein, DNA supercoiling
recA	RecA	General, homologous recombination, DNA repair, SOS response
cspA	CspA	Cold-inducible RNA chaperone, RNA and DNA binding, anti-terminator, transcriptional enhancer
cspB	CspB	Cold-shock-inducible, RNA binding
cspE	CspE	Cold induced in lag phase RNA chaperone, RNA binding, transcriptional anti-terminator, inhibits PNPase and RNase E, regulation of and expression of stress response proteins RpoS and UspA
deaD	DeaD	ATP-dependent RNA helicase, aids ribosome assembly, possibly involved in RNA degradation
pnp	PNPase	Cold-shock protein required for growth at low temperatures, 3′→5′ exoribonuclease, component of RNA degradosome, purine phosphorylase
nusA	NusA	Transcription termination/antitermination/elongation L factor
infA	IF-1	Protein chain (translation) initiation factor IF-1, RNA binding
infB	IF-2	Protein initiation factor, translation initiation, fMet-tRNA binding, chaperone
infC	IF-3	Protein initiation factor, translation initiation, initiation site selection, RNA binding, stimulates mRNA translation
rbfA	RbfA	30S ribosome-binding factor processing of 16S rRNA (3′→5′ exonucleases)
rnr	Ribonuclease R	Cold-shock induced, ribosome assembly/maturation
yfiA	pY	Protein Y, 30S ribosomal subunit linked, inhibits translation
dnaJ	DnaJ	Chaperone
dnaK	DnaK	Chaperone
hscA	Hsc66/HscA	DnaK chaperone homologue (Hsp70-type protein chaperone)
hscB	HscB	DnaJ co-chaperone homologue (for HscA)
tig	Trigger factor	Multiple stress protein, chaperone, protein-folding, ribosome-binding
aceE	AceE	Pyruvate dehydrogenase E1 component, decarboxylase
aceF	AceF	Pyruvate dehydrogenase, dihydrolipoamide acetyltransferase
lpxP	Palmitoleoyltransferase	Cold-inducible, lipid A biosynthesis
otsA	OtsA	Cold-induced and essential, trehalose phosphate synthase
otsB	OtsB	Cold-induced and essential, trehalose phosphate phosphatase
cspC	CspC	Regulation of growth and the stress response proteins RpoS and UspA
cspD	CspD	Stationary phase induced and nutrient starvation, DNA replication inhibition, biofilm development, persister cell development
cspF	CspF	Very-low-level expression with no detected protein product
cspG	CspG	Cold-inducible, cold-shock protein homologue
cspH	–	Very-low-level expression with no detected protein product
cspI	CspI	Cold-inducible, cold-shock protein homologue
ves	Ves	Cold- and stress-inducible

This is for the simple reason that we lack microbial genomes from the cold environments, including the Arctic. At the time of writing, fewer than a hundred microbial genomes from the Arctic are listed in public databases. Critically, to our knowledge, only one cyanobacterial genome from the terrestrial Arctic is publicly available [115], which frustrates our understanding of the comparative genomics of a major group of primary producers in the terrestrial Arctic. Representative genome sequences for other key microbial groups are also severely limited in their public availability. Importantly, this frustrates any effort to select representative model organism(s) for the genome-centred study of Arctic microbiology.

Inspiration for solving this challenge can be found readily within neighbouring fields of microbial genomics. While high-throughput reconstruction of genomes from deeply sequenced environmental metagenomes offers culture-independent insights to landscape-scale processes in the Arctic (e.g. carbon release from permafrost or marine degradation of tDOM) [20], the potential for experimental validation of the MAGs or exploration of their corresponding phenotypes is curtailed. Furthermore, while the world is turning to the High Arctic to preserve genomic diversity in agricultural crops [161] and open source code (https://archiveprogram.github.com/), there is no corresponding effort to conserve the microbial diversity endangered by Arctic change [162]. Therefore, there is value in the systematic isolation, cultivation, genome sequencing and experimental analysis of Arctic bacteria. This approach offers the advantage of high-quality reference genome sequences coupled with the curation of the source isolate for later experimental verification. The 'Hungate1000' collaboration for sequencing ruminant microbial genomes [163] offers one potential blueprint for community-led sequencing of Arctic microbial isolates. The establishment of a dedicated sequencing and strain curation facility provides an alternative, service-based model. Irrespective of the approach towards the generation of such a resource, systematic sequencing, curation and experimentation with Arctic isolates both conserves Arctic microbial biodiversity and creates an enabling platform. An 'Arctic1000' project would permit for selecting model organisms, testing discrete hypotheses, refining gene annotations and resolving the evolution of cold adaptation [155, 164]. In summary, bringing microbial genomes in from the cold is a necessary but tractable first step in gathering evidence for a unifying concept for psychrophily that will also likely be of relevance for understanding microbial life in other cold regions.

Challenge 2 – phylotypes obscure genomic diversity in Arctic microbes

The profound dearth of available Arctic microbial genomes means that most Arctic microbes known to science are outlined by their phylotypes. Most recently these are viewed through the lens of amplicon sequencing of specific loci [e.g. 16S rRNA genes or 16S rRNA (cDNA) or eukaryotic equivalents] but, historically, community fingerprinting commonly served similar purposes [14, 28, 165].

PCR-dependent amplification and targeted sequencing permits description and comparison of community composition from low-biomass density environments that are vast in scale (e.g. snow or ice across the Greenland Ice Sheet [97, 99] and Arctic air samples [166–168]). However, these applications amplify the challenges typical of amplicon sequencing approaches for cataloguing or comparing microbiomes. A particular challenge for Arctic microbiologists is that many of the taxa prevalent in amplicon sequencing studies of Arctic microbiomes are close relatives of frequently observed contaminants of the amplicon sequencing process. The 'kitome', or the contaminated reagent microbiome [169], typically comprises a range of organisms well adapted to oligotrophic conditions, stresses from low water activity (albeit in high-salt solutions) and cold storage. In short, molecular reagents are often facsimiles of the stresses common in polar environments. Moreover, low-biomass samples are typical of many habitat types across the Arctic, for example, snow, ice or freshwater habitats [15], and the impact of contamination is magnified in such samples [170, 171]. These trends are supported by the coincidence of many authentic members of Arctic microbial communities among blacklisted taxa from microbiome analyses [169] (Fig. 2).

Disentangling authentic from contaminant taxa present in amplicon sequence data, therefore, poses particular challenges for Arctic microbiologists. As well as the type I error (inclusion of contaminants in microbiome profiles), the scope for type II error (the exclusion of authentic taxa) is enhanced. Therefore, rigorous experimental design and management must be emphasized. This can include the implementation of contamination-mitigation practices (cf. the article by Willerslev *et al.* [172]) during sample collection and processing. However, consistently achieving and verifying sterility within the laboratory, let alone during sampling activities in expeditionary conditions, is challenging if not impractical. Therefore, a suite of extraction, reagent-blank and mock community controls [170, 171], which are processed, sequenced and analysed alongside study samples, becomes critical. The use of automated contamination detection software requires careful manual curation, as their application on data from communities dominated by a small core of common taxa or keystone taxa (e.g. cryoconite [114]) may lead to the false negative rejection of those taxa [173].

The imperative for experimental good sense and good laboratory practices has recently been emphasized in microbiome analyses [174], and amplification-dependent studies of Arctic microbiota should be no exception. Furthermore, validation of key experimental trends should be normalized, which should include, as a minimum, identification of the closest environmental and cultured representatives of key phylotypes [175], and ideally orthogonal confirmation of their detection in culture or through phylogenetic staining of samples by fluorescent *in situ* hybridization (FISH). With the development of improved workflows (e.g Anvi'o [176]) for genome-resolved metagenomics, it may be that amplicon sequencing becomes an adjunct to the direct analysis of functional diversity represented within the genomes of Arctic microbes; for

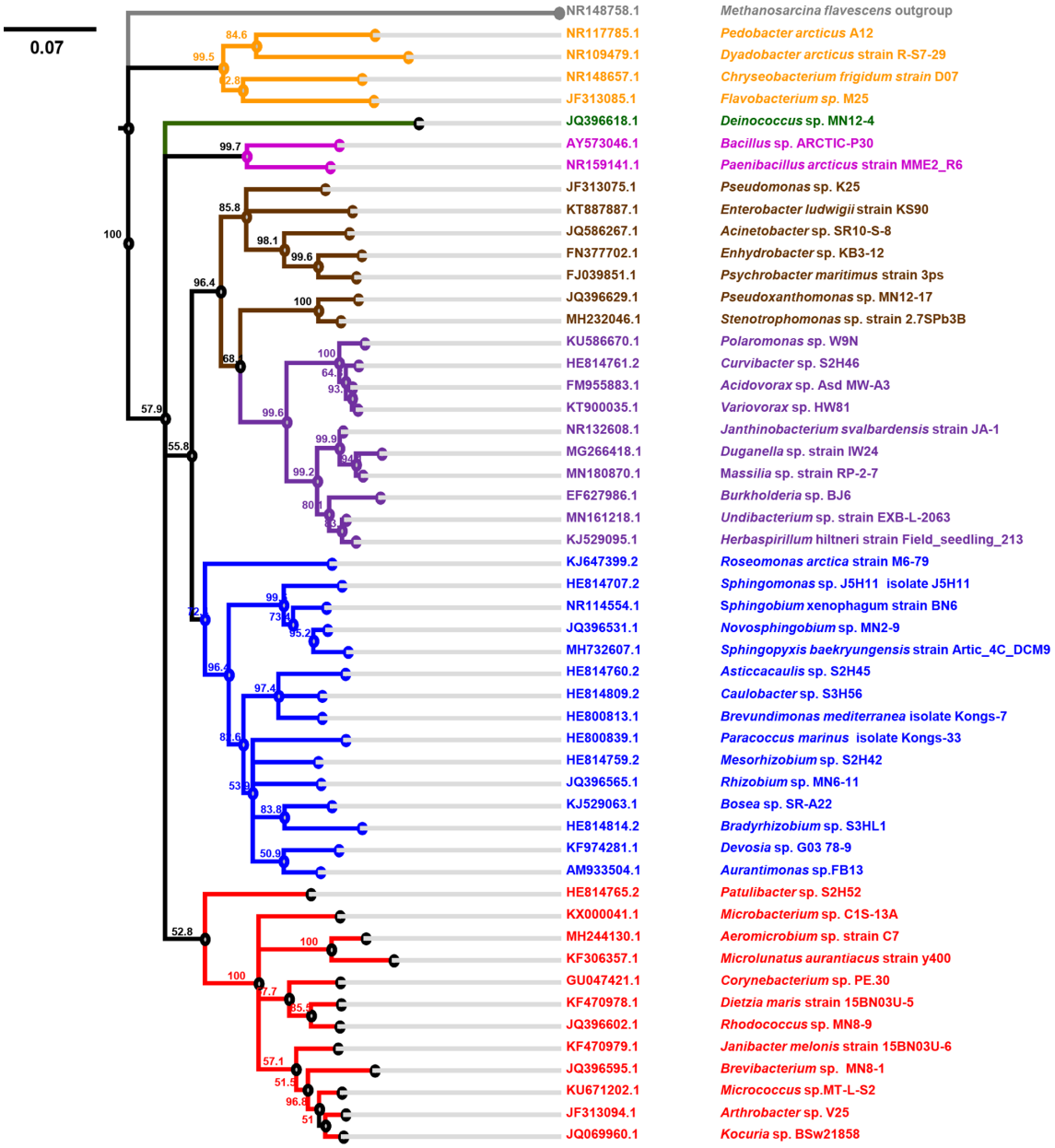

Fig. 2. Neighbour-joining tree of partial 16S rRNA gene sequences from isolates in culture from Arctic habitats that are also reported [169] as frequently occurring contaminants in sequenced negative controls. The tree comprises 52 alignable sequences from the 56 available isolate sequences drawn from the 92 genera named in table 1 of the paper by Salter *et al*. [169]. All seven of the groups with named genera listed by Salter *et al*. [169] are represented in cultures from Arctic environments. *Actinobacteria*, red; *Alphaproteobacteria*, blue; *Betaproteobacteria*, purple; *Gammaproteobacteria*, brown; *Firmicutes*, pink; *Deinococcus-Thermus*, green; and Bacteroidetes, gold. Scale shows nucleotide substitutions per site.

example, by selecting samples for more intensive study by genome-resolved metagenomics on the basis of community profiling.

Challenge 3 – biomolecular stability as a pre-requisite for integrative multi-omics

In spite of the critique of amplicon sequencing presented above, the capability to systematically compare the microbiomes of many samples has led to valuable collaborative efforts for the large-scale mapping of microbiomes [148]. While these efforts capture broad-scale trends in microbial biogeography, they are predominantly focused on the mid-latitudes [148, 149, 177]. Consequently, there is the risk of overlooking genomic diversity within Arctic microbial ecosystems and its interactions with climate change when conducting global-scale analyses [12].

It is likely that the limited availability of samples from high-latitude locations accounts for this bias, for the collection and recovery of microbial samples from the Arctic is non-trivial. Indeed, within the general field of ecology there is a profound station bias in the distribution of studies on Arctic climate change. A recent synthesis showed that 31% of all study citations in 1840 publications on Arctic change relate to work performed in just two locations, Toolik Lake Station in Alaska and Abisko Station in Sweden [178]. Notable gaps in the literature include the microbiology of particularly rapidly changing regions of the Arctic, for example the Canadian Arctic Archipelago or the Russian Arctic coastline [178]. It is likely that the logistical challenges in accessing these vast and important areas are either too costly or practically prohibitive for many investigators. As an approximation, Mallory *et al.* [179] calculated the costs of animal ecological fieldwork are typically eight times greater in the Arctic than comparable work in the mid-latitudes, which increases the barrier for executing studies that then also require costly analyses in the home laboratory in the form of high-throughput sequencing.

The high level of sample integrity required for most forms of microbial 'omics studies exacerbate this challenge. To transcend descriptive inventories of genomic diversity, the systematic capture of transient microbial gene products (transcripts, proteins, metabolites) can offer greater functional insight. Sampling forays straying beyond assured cold-chain archival of samples are, therefore, risky but essential for understanding microbial responses to the Arctic crisis in vast habitats far from the nearest freezer. Indeed, integrating different strands of 'omics methodologies can allow an investigator to reveal how a microbe is contributing to ecosystem function. Such contributions may be through many different routes, with important contributions by active, dormant, damaged or dead microbes in turn [180], which are difficult to disentangle both practically [180] and epistemologically [181]. These challenges are pronounced in harsh environments where microbes may be functioning under prolonged exposure to multiple stresses [156]. Representation within the RNA or protein pool of a habitat is consistent with contemporary contributions to ecosystem function, while the presence of functional genes within metagenomes indicates potential functional contributions, which may be in the past or future. Furthermore, differential extraction of vegetative or spore-associated DNA may indicate the potential for long-term storage of genes within an ecosystem [63, 64] and, finally, even the contribution of lysed microbes to the organic carbon and nutrient pool of an ecosystem can prove critical for foodwebs [182].

Within the realm of multi-omics studies, the paucity of *in situ* metatranscriptome studies of Arctic microbiota presents a notable lacuna in our understanding of ecosystem responses to climate stresses [67, 68, 75]. Multiple challenges must be addressed in implementing such experimental strategies. Firstly, microbial mRNA has a very brief half-life. For laboratory grown bacteria, this is typically in the order of minutes [183], but even assuming the potential for greater mRNA stability in slow-growing cells in low-temperature environments, the turnover of mRNA is likely within hours to a day [184]. Recovering representative high-quality mRNA (RNA Integrity Number >7; [185]) for transcriptome analyses in locations where liquid nitrogen flash-freezing may not be practical or permissible is, therefore, challenging.

Such situations may include research performed at many Arctic stations, research performed over extended field campaigns and research performed in areas only accessible by small chartered aircraft. Furthermore, for aqueous or frozen sample matrices common in the Arctic (snow, ice, meltwater), samples require lengthy pre-processing that can include gentle melting and filtration over hours or days before chemical preservation or deep freezing becomes feasible. Secondly, mRNA is a minor species of RNA when compared to the abundance of rRNA within the cell [186]. Indeed Moran *et al.* [186] estimate that the typical marine bacterial cell may only contain ~200 transcripts at any one time. Using the same allometric assumptions [186] constrained by data on the median cell size of bacteria in glacial meltwater [90], it is possible that only ~40 transcripts are present in a bacterial cell eluted from glacial ice. When an investigator wishes to generate a snapshot inventory of transcripts in a sample, the low abundance of transcripts and the apparent stochasticity of transcription, therefore, pose practical and conceptual problems in quantifying transcripts and their relevance for ecological functions.

Nevertheless, reducing the degradation of biomolecules within the parent environmental matrix appears vital for robust multi-omics studies. Two contrasting approaches are identified as a means of enhancing the fidelity of insights from multi-omics studies. Firstly, researchers have the option of relocating bulk environmental matrices to a controlled laboratory environment for incubation. This approach has the advantage of allowing experimental manipulation under precisely controlled conditions where confounding factors can be minimized, and treatments administered and measured precisely. For environmental matrices that can be sampled and transferred frozen in bulk, this strategy has proven fruitful. A key example is found within experimental studies of permafrost responses to controlled thawing [67, 68], which integrate process measurements of microbial activities with metatranscriptomics of samples incubated under controlled conditions. Such studies address the issue of biomolecular integrity by immediate extraction of nucleic acids (or liquid nitrogen flash-freezing) and have the conspicuous advantage of permitting experimentally replicated application of treatments under controlled conditions. For more labile environmental matrices (e.g. snow or water), habitats where low-activity states are typical (e.g. ice cores) or where replication of field conditions in the laboratory is more challenging [132], bringing the habitat to the laboratory is more problematic. Nevertheless, the establishment of faithful cold laboratory models of Arctic microbial communities or keystone taxa offers one potential route to enhanced functional insights.

Secondly, the opposite strategy of relocating the microbial genomics laboratory to the sample is an increasingly viable

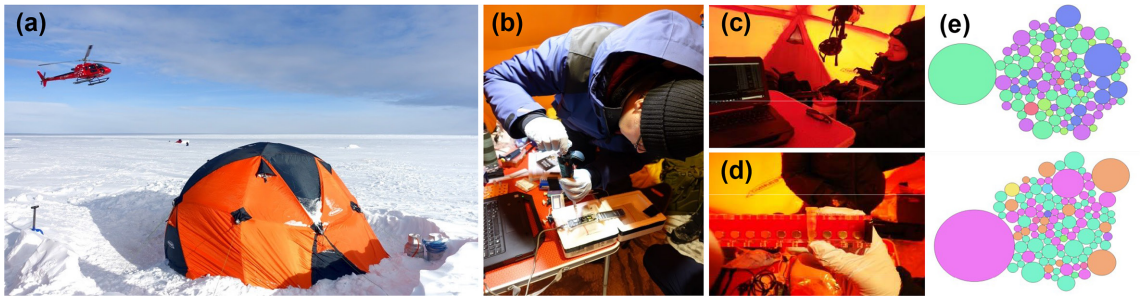

Fig. 3. Example of in-field DNA sequencing and analysis. Working in a remote field camp on the Greenland Ice Sheet during the Arctic winter (a), it was possible to extract nucleic acids and sequence them in ambient temperatures of circa −20 °C by using freeze-dried reagents and adapted protocols for nanopore sequencing (b). *In situ* data processing and analysis (c) permitted a refined experimental strategy (d) for genome-centred metagenomic comparison of glacial habitats (e). Images (a) and (b) by J. M. Cook; images (c) and (d) by A. Edwards. Image (e) is representative of unpublished data from A. Edwards, M. C. Hay and J. M. Cook.

option. The development of third-generation DNA sequencers that are field-deployable in austere conditions is prompting innovation in this area. In particular, nanopore sequencing using MinION from Oxford Nanopore Technologies permits a user to rapidly analyse crude extracts of nucleic acid through shotgun metagenomics or carry out amplicon-based analyses (e.g. 16S rRNA gene sequencing) on a USB-based device. Within the Arctic, this strategy has been implemented in a field station in the Canadian Arctic [187], field camps in Greenland [188] and as part of a student expedition on the Vatnajökull ice cap of Iceland [189]. The selection of miniaturized equipment for battery-powered DNA extraction, simplified library preparation using freeze-dried reagents and sequencing on solar-powered or battery-powered instruments [188, 189] permits on-site characterization of Arctic microbiota, which can focus sampling plans and reduce logistical risks from sample loss or degradation during transport. Moreover, sequencing on-site allows the real-time completion of the scientific method *in situ*, rather than incurring potential delays by returning samples to a remote laboratory (Fig. 3).

In summary, it is likely that a combination of these approaches will offer Arctic microbial genomics researchers the balance between capturing *in situ* processes and the option of precisely controlled laboratory experiments, both of which will be required to advance our knowledge of microbial responses to the warming Arctic.

Challenge 4 – business as usual for Arctic microbial genomics?

The study of Arctic change in the 21st century could be summarized as the measure of a biome-scale response to perturbation on a scale unprecedented in human history. Implicit within this is the knowledge that the resilience of both Arctic ecosystems and Arctic researchers is threatened. Indeed, the burden of ecological grief [190] upon environmental researchers has recently been acknowledged for its impact on their mental health [191]. Climate change impacts are also complicating the study of Arctic microbiology in other critical ways, from the rapid destruction or fragmentation of study habitats [192–194], to anomalous weather patterns [195, 196], to the disruption of infrastructure and logistics. Considering the inherent biases and undersampling of Arctic habitats to date (see the previous section), fieldwork will remain a core requirement for capturing the diversity and dynamics of Arctic microbial genomes across spatial and temporal scales. However, remote fieldwork in the Arctic brings with it additional logistical costs and safety risks [179], and exposure to the increased frequency of extreme weather conditions [8] and their impacts on avalanche, landslide or sea ice disintegration adds to these risks. Furthermore, it must be acknowledged that Arctic microbiology is a carbon-intensive research discipline, with considerable quantities of greenhouse gases released both in regular travel to remote field locations and in the maintenance of cold laboratories and ultra-freezers. Sustainable and innovative ways of exploring the microbiology of the Arctic, while minimizing the contribution of Arctic microbiologists to further environmental change, are required.

The rapid technological developments within microbial genomics have meant that the wealth of data collected and archived in public repositories dates rapidly. Today's ultra-deep sequencing experiment becomes tomorrow's shallow metagenome 'skim' [197]. Welcome increases in capacities for sequencing and data processing [198], enhancements in standards for metadata reporting, and recognition of methodological biases, all contribute to the depreciation or obsolescence of unique datasets harvested with great costs and potential environmental impacts. The Antarctic microbiology research community has recognized the value of preserving community DNA samples in public archives in addition to high standards of data curation [199, 200]. This creates a resource that can be explored retrospectively and shared among researchers to reduce the requirement for additional fieldwork at additional cost and environmental impact. Furthermore, such sample archives provide both a baseline for contemporary genomic diversity and an insurance against habitat loss in the face of Arctic change [162, 199, 200].

Establishing microbial observatories with the capacity for longitudinal studies of changes in Arctic microbial ecosystems is one potential area of development. The Arctic is home to many scientific research stations (https://eu-interact.org/) and research centres, most of which collect and archive a valuable range of environmental measurements, for example meteorological or atmospheric chemistry data [201]. These datasets underpin our quantitative view of the Arctic's present and past; thus, delivering the raw ingredients for forecasting changes in the Arctic and the entire planet [8]. To our knowledge, at present, there is no comparable effort aimed at monitoring Arctic microbiota, in spite of the critical interactions between Arctic microbes and climate change [202]. Creating deployable genomics resources can distribute the task of characterizing and monitoring Arctic microbiota to a network of such stations. By analogy, the inclusion of distributed, real-time pathogen genomics is considered a key data stream for a prospective global surveillance system, which integrates human, animal and ecosystem health [145]. Within the Arctic context, this 'sequencing singularity' [145] would entail the contemporaneous monitoring of Arctic microbiota, along with the physical and chemical environment of the warming Arctic; thus, capturing the complexity of microbe-mediated feedbacks in Arctic change and offering a rich new stream of data to enhance our predictive understanding of Arctic change.

Hitherto, the study of Arctic microbial genomics has typically entailed either laboratory-based analyses, field surveys or plot scale experiments [67, 71, 98]. These are most pertinent for understanding the molecular ecology of the cryosphere at scales of microns to metres, with further insights typically extrapolative. However, it is the emergent macroscale effects of Arctic microbial activity that have relevance for contemporary climate change, requiring microscopic processes to be studied at the scale of entire landscapes. This scale mismatch between processes occurring at the $<10^1$ µm scale and their effects at the 10^1–10^5 km^2 scale (a chasm spanning up to 14 orders of magnitude) makes emergent effects challenging to study.

Scaling down and scaling up both present challenges (Fig. 4). When downscaling, the analysis of biomass crammed into sample tubes or on to membrane filters blurs the fine-scale resolution of microbial interactions and physico-chemical heterogeneity typical of life in the interstices of soil grains or ice crystals [61, 203]. When upscaling, researchers can only cover a limited area and process a finite number of samples, obscuring critical spatiotemporal phenomena and relationships to synoptic scale meteorological, geomorphological, glaciological and hydrological processes. Quantitative predictions of community structure, function and stability at larger scales must integrate the sampling of genomic diversity with measurements of environmental processes and remote sensing to capture emergent macroscale effects.

One example of such an emergent macroscale effect is the biological darkening of glacier surfaces, especially in a 'dark zone' along the western coast of the Greenland Ice Sheet as a result of glacier algae and cryoconite development, as detailed above [102]. These processes must be understood as part of an integrated glacial system, which in turn requires methods for inferring microbial processes at scale using aerial or orbital remote-sensing platforms. Remote detection and quantification of microbial communities, in particular for algae, is routine for lacustrine and oceanic systems. Earlier remote-sensing efforts for cryospheric algae have focused on snow algae [204, 205]. However, the complex and highly spatially and temporally variable optics of glacier ice, combined with the mixing of microbial cells, inorganic light absorbing particles and meltwater, make remote detection of glacier microbes more challenging. Nevertheless, there are several potential footholds that may enable remote biomass quantification and perhaps yield insights into microbial processes distributed over space and time. Glacier algae are discernible by their distinctive pigmentation, and it has been suggested that inversion of radiative transfer models could be used to reverse engineer algal pigmentation from spectral reflectance measurements [94, 206], offering a potential route to unpicking environmental stresses and responses in supraglacial microbial communities. These insights demonstrate that although the existing conceptual models require further empirical support, there are potential emergent phenomena that could be used to infer microbial processes on the ground. As field measurements become more abundant, aerial sensors continue to develop and high performance computing resources become increasingly accessible, our ability to measure, monitor and model the environmentally relevant emergent phenomena related to Arctic microbial processes and their feedbacks to microbial ecology, and to do so at scale, is enhanced. This fusion of genomics and geospatial techniques could enable better-informed climate mitigation strategies, and may well stimulate the next revolution in our understanding of Arctic microbial ecology and its feedbacks to the global climate.

CONCLUDING REMARKS

Climate change is unfolding across the ocean and lands of the Arctic at a pace unprecedented in human history, and its consequences will profoundly affect the Earth and our society. We now appreciate microbes play pivotal roles in the response of the Arctic to anthropogenic warming. Arctic microbial genomics has the potential to inform us of this problem, but this information alone is problematic in that it does not present a solution [207]. Insight into the microbial dimensions of Arctic change can nevertheless support society in its search for solutions to the climate crisis, for example through improving our understanding of carbon sequestration in the Arctic Ocean, aiding models of greenhouse gas release from the permafrost, refining projections of sea-level rise or designing energy efficient catalysis. Within this review, we have identified conceptual and technical barriers, as well as potential routes to surmount these obstacles to progress in Arctic microbial genomics.

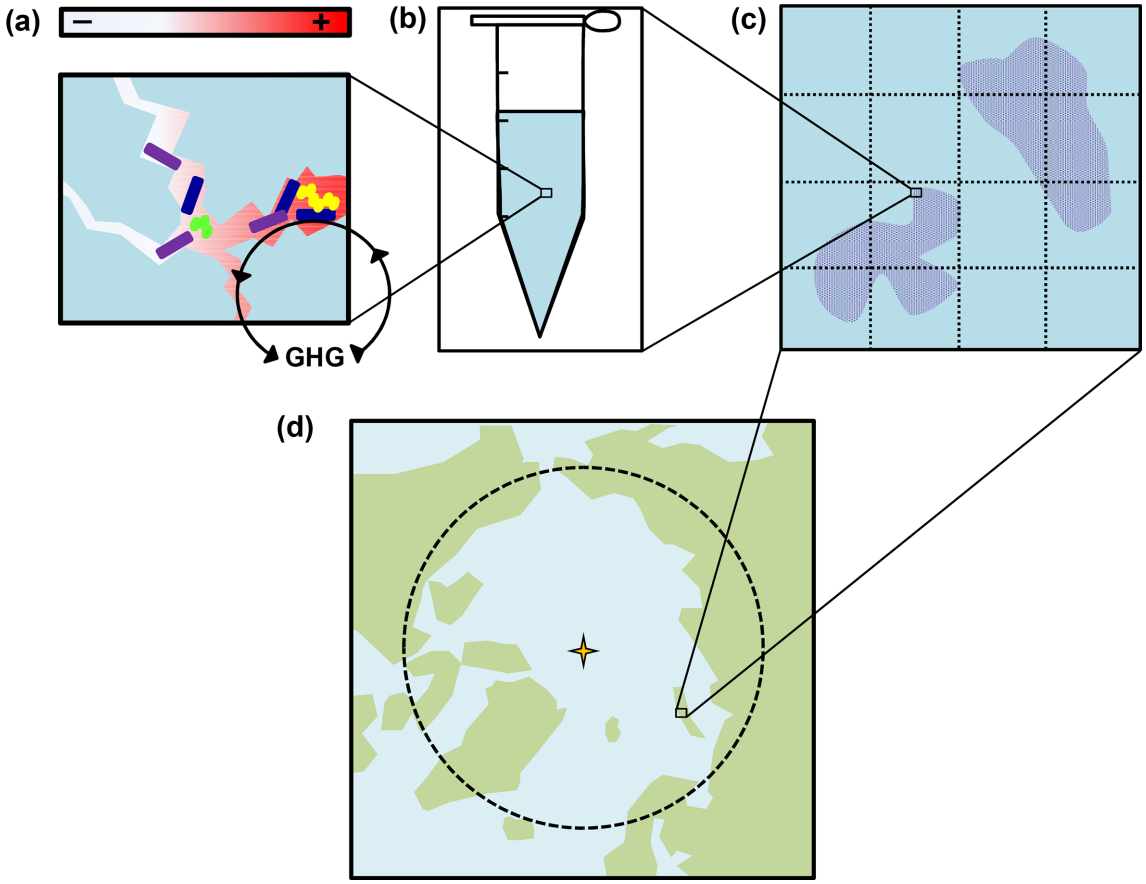

Fig. 4. Arctic genomes across scales. Many critical processes [e.g. greenhouse gas (GHG) cycling] mediated by Arctic microbiota occur within environments that are extremely heterogeneous at the microscopic scale (a). In particular, these include interstitial spaces in otherwise frozen substrates (e.g. sea ice, permafrost, glacial ice) where micro-scale gradients in biomass or physical and chemical conditions are apparent. These are disrupted at the sample scale (b) by the requirement to collect sufficient biomass for (meta-) genomic analysis and the distortion incurred by bulk chemical analyses of substrates. At the plot scale (c), undersampling of spatial heterogeneity at the meso-scale poses a further challenge. Finally, upscaling to the landscape or regional scale from plot-scale studies (d) is hampered by spatial and temporal biases in sampling.

As a priority (Fig. 5), we must couple the pursuit of improved reference data for microbial genomes from the Arctic with capturing the *actualité* of physiological responses and population dynamics in Arctic microbial communities through field-based multi-omics and coupled measurements of processes and environmental parameters. To address this priority, we must gain the clearest insights on how microbes respond to environmental changes through transitioning from inventories of changes in phylotype distribution and relative abundance to studies that primarily focus on genes and their products which are encoded within the genomes of Arctic microbes. Moreover, microbial genomics needs to be better integrated within the interdisciplinary framework and infrastructure of Arctic change science. This will permit robust upscaling and numerical modelling of landscape-scale impacts driven by microbial genomes. Importantly, improving reference data (e.g. high-quality annotated genomes, comparative physiology, laboratory mesocosm studies) will provide crucial context for changes occurring within the field. Meanwhile, insights from real-time integrative studies of geospatial, meteorological, biogeochemical and genomic changes will also provide focus for laboratory-based studies.

Integrating both strands of Arctic microbial genomics research will require effective interaction with other disciplines and research infrastructures. Indeed, at the heart of our blueprint for Arctic microbial genomics is the blurring of traditional disciplinary boundaries, and the necessity of making the border between laboratory and field studies porous. For effective synthesis, accurate inventories of Arctic habitat types and metadata types must be collected, requiring genomics researchers to engage with geographical knowledge. All of these changes will require improved standards and architecture for sample, isolate, data and metadata collection, accessibility and analysis.

By targeting these priorities, we anticipate Arctic microbial genomics would prove agile enough to respond to the contemporary Arctic crisis through providing quantitative

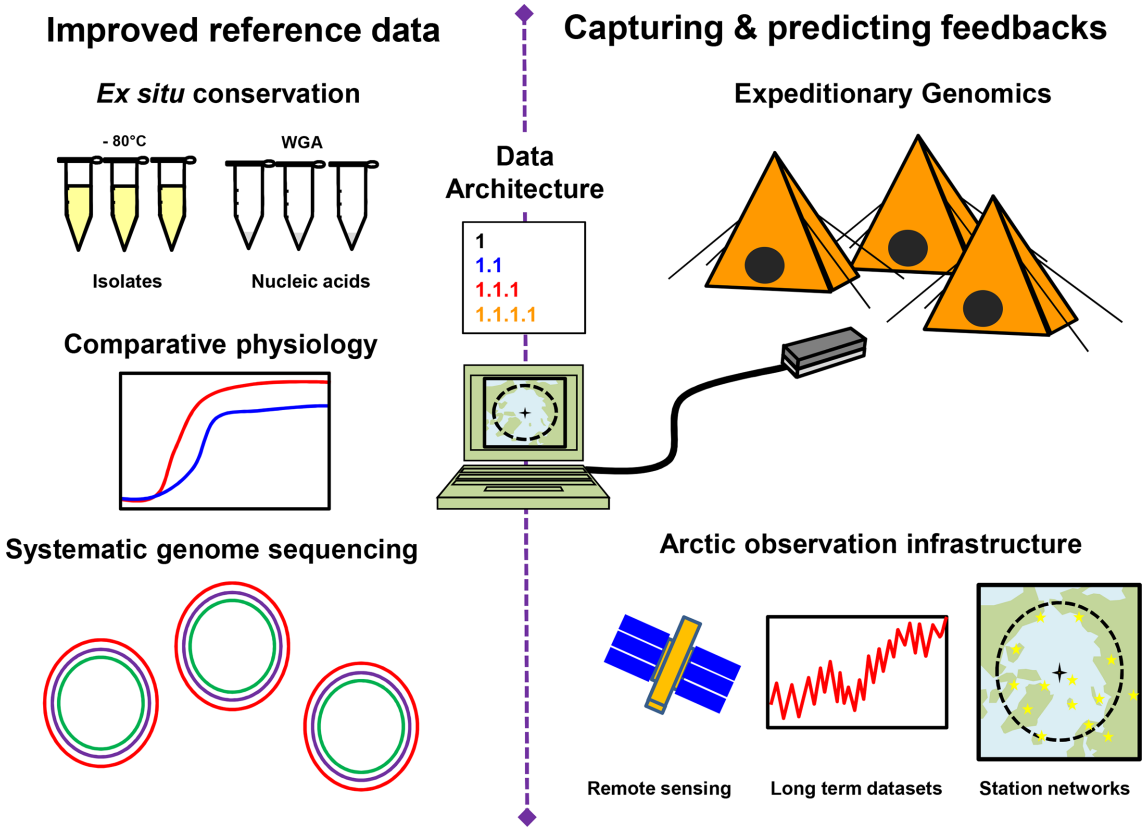

Fig. 5. Arctic microbial genomics will require the fusion of improved reference data and the real-time capture of microbial drivers and responses to changes in the 21st century Arctic. WGA: Whole Genome Amplification.

predictions of microbial feedbacks in the changing Arctic. However, if nothing else, doing so will secure fundamental knowledge and genomic diversity for study by future generations, long after the global consequences of the Arctic crisis have become unequivocally clear for all humans.

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Author contributions
A.E. conceived the review, led its drafting and managed the editing of it. All authors contributed to the drafting of the review and approved the final manuscript.

Conflicts of interest
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