Arctic soil microbial diversity in a changing world

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Abstract

The Arctic region is a unique environment, subject to extreme environmental conditions, shaping life therein and contributing to its sensitivity to environmental change. The Arctic is under increasing environmental pressure from anthropogenic activity and global warming. The unique microbial diversity of Arctic regions, that has a critical role in biogeochemical cycling and in the production of greenhouse gases, will be directly affected by and affect, global changes. This article reviews current knowledge and understanding of microbial taxonomic and functional diversity in Arctic soils, the contributions of microbial diversity to ecosystem processes and their responses to environmental change.

Keywords: microbial functional diversity; carbon cycling; nitrogen cycling; soil active layer; permafrost; global change

1. Specificity of Arctic soils and ecosystems

1.1. Arctic region

The Arctic region includes terrestrial, freshwater and marine environments across northern Asia, Europe and North America. The boundary of the Arctic region is often defined by the Arctic Circle (66°32'N), but the boundary can be drawn far below this, when based on climate, marine or terrestrial environments [1]. When based on temperature, the Arctic is often delimited by the 10 °C July isotherm. However, the Arctic exhibits considerable variation in temperature, precipitation and soil characteristics both spatially and temporally. Arctic terrestrial ecosystems are often divided into three different biogeographical zones with a gradient in environmental conditions from north to south: the High Arctic, Low Arctic and Subarctic (Table 1, Fig. 1a) [1], although various alternative classifications exist. The High

Arctic is the northern part of the Arctic region including Greenland, Nunavut Canadian islands (i.e. Baffin Island, Parry Islands, Queen Elizabeth Islands and Ellsmere Islands), Russian islands (i.e. Franz Josef Land, New Siberia Islands and part of Novaya Zemlya), Severnaya Zemlya and Svalbard (Fig. 1a). The Low Arctic extends mainly from the Arctic continental coastline to the treeline, while the Subarctic boundary starts from the treeline to the closed-canopy of the boreal forest and the southern limits of permafrost (Fig. 1a) [1]. The difference between these zones is seen in the gradient from the High Arctic to Subarctic with changes in temperature, precipitation and plant cover (Table 1). Hence, the growing season increase from the High Arctic to Subarctic as well the size of plants and plant cover, from bare soil and discontinuous cover to continuous (Table 1). The High Arctic is characterised by Polar deserts, corresponding to areas where annual precipitation is <150 mm and the mean temperature of the warmest month is <10 °C (Polar semi-desert is also used for areas with annual precipitation of 150-250 mm) [2].

The Arctic is characterised by cold temperatures, as low as -40 °C in winter (sometimes lower in Siberia) rising to 15 °C (and higher in continental Asia) in summer across the southern Arctic regions [1]. Annual precipitation is low in the Arctic, with less than 500 mm for most of the Arctic with precipitation occurring mostly in the form of snow, increasing from the High Arctic to Subarctic, although the Greenland ice caps (High Arctic) receives precipitation >1000 mm (Table 1). Annual solar radiation received in the Arctic represents a third to a half of the radiation received in temperate and equatorial zones [1], although during at least some of the summer period there is 24 h sunlight within the Arctic Circle but also 24 h darkness in some of the winter period. The growing season, which corresponds to the period of growth of plants, varies between 1 and 2.5 months in the High Arctic, while it can last up to a year in the Subarctic [1].

1.2. Arctic soils

Arctic regions harbour a high diversity of soils. Indeed, 75% of the soil groups defined by the World Reference Base (WRB) [2] are present in the Arctic region. From these soils, about 60% represent cold soils (i.e. soils affected by permafrost). Cryosols are the dominant soil group (27%) and are defined as soils in cold regions where permafrost is present, where water occurs mainly in a frozen form, and cryosols are formed under cryogenic processes (e.g. frost heave, freezethaw cycles, cryoturbation) [2]. Permafrost is defined as ground (including soil, rocks, ice and organic material) that remains at or below 0 °C for at least two consecutive years. Permafrost comprises 24% of exposed land in the northern hemisphere (excluding areas beneath ice sheets; Fig. 1b) [2]. Most of the permafrost was formed during the past ice ages and is divided into four types: continuous, discontinuous, sporadic and isolated patches. Permafrost is found on land but can also be found below the sea (Fig. 1b). The continuous permafrost corresponds to permafrost occurring everywhere throughout an entire region, while discontinuous permafrost covers between 50 and 90% of area, sporadic permafrost covers 10-50% of area and is surround by unfrozen soil, and isolated permafrost covers only 0-10% [2]. Moving from the High Arctic to the Subarctic, the distribution of continuous permafrost decreases, while discontinuous permafrost increases to finish as sporadic and isolated patches (Fig. 1b). The upper part of Arctic soil, named the "active layer", thaws during summer to a depth of between 20 cm and 150 cm and refreezes each winter, but is not part of the permafrost by definition. The thickness of the active layer in summer depends upon local temperature, ground material, soil water content and plant cover, and generally increases in depth from the High Arctic to the Subarctic.

Arctic soils are characterised by a number of key features: multiple soil horizons are often found; low temperatures influenced by air temperature but also by permafrost, snow and plant cover; and soil water contents varying from saturated to dry depending on

precipitation, soil drainage characteristics and permafrost (Table 2). Arctic soils are also characterised by large amounts of organic C, which to 3 m depth are estimated to be more than twice the atmospheric C pool [2-4]. Organic C accumulates because plant material is only partially decomposed in Actic soils due to the low soil temperatures, short periods for biological (microbial) activity and sometimes acidic and/or anoxic conditions [2]. Carbon is accumulated near the surface as plant materials are deposited as litter and in deeper soil horizons due to cryogenic processes that move plant material and soluble compounds down toward the permafrost, where a horizon rich in organic matter can be formed. The migration of organic matter can take thousands of years and enable long-term storage of C [2]. Soils affected by permafrost contain potentially ~50% of the global organic C [2, 3]. In contrast, nutrients such as N and P are considered to be in low abundance in Arctic soils, limiting plant production [5-7] and also the activity of microbial communities [8,9].

1.3. A region under increasing pressure from global change

Arctic environments are facing and will face several threats due to anthropogenic activities. Climate change is predicted to be more pronounced at high latitudes than across other regions on earth [10], in particular with regard to temperature increases, with consequences ranging from decrease in snow cover, glacier retreat, thawing of permafrost and change in plant cover (Table 3). Furthermore, global change will affect soil moisture due to increases in precipitation and changes in hydrological features with the thaw of permafrost and the development of thermokarst landscapes. Atmospheric pollution in Artic regions already leads to increased deposition of compounds and elements, such as N [11] and Hg [12] (Table 3). Atmospheric pollution can originate from localised sources or be transported from lower latitudes [11]. Finally, human activity in the Arctic can have direct effects on the Arctic

126 regions, despite the relative harmony with which indigenous peoples have lived with the
127 Arctic (e.g. increasing pressures from settlements, resource extraction and transport). The
128 number of ships in the Arctic is expected to increase [13] with the Arctic Ocean predicted to
129 be nearly ice-free in the summer by 2050 [14], opening new commercial routes. The
130 reduction of ice on the Ocean, the increase in soil temperature and thaw of permafrost will
131 lead to an increase in petroleum and gas extraction, as well as mining activities (e.g. cobalt,
132 iron ore, nickel, palladium and uranium), which is already important in Alaska, Canada and
133 Russia. All of these disturbances will directly affect Arctic terrestrial ecosystems, with
134 potential increases in microbial activity and changes in microbial diversity due to higher soil
135 temperatures, changes in plant communities, input of nutrients or pollutants and changes in
136 precipitation regimes (Table 3).
137
138 *1.4 Microbial diversity and changes in methodology.*
139
140 Microbial diversity generally referred to the genetic diversity of microorganisms and
141 is defined as the total number of operational taxonomic units (OTUs, richness) and their
142 relative abundance. However, the term “diversity” is often misused in microbial ecology [15].
143 Throughout the review, the terms richness and relative abundance will be used to clearly
144 identify which part of the diversity is discussed; otherwise the term diversity will refer to
145 both richness and relative abundance. Furthermore, the term microbial community structure
146 will refer to microbial community determined by fingerprinting methods (i.e. when there is
147 no information about the OTUs), while microbial gene abundance will refer to the number of
148 genes present in the soil and determined by quantitative-PCR (Q-PCR). The functions of
149 microorganisms can be specifically targeted using genes coding for a part of a process and
150 used as the proxy of the microbial functions. A variety of methods is used to target those

genes, ranging from fingerprinting methods, Sanger sequencing, Q-PCR, metagenomics and also microarray, which have been applied only a few time in the Arctic [16e18]. However, such approach has limitations, mainly because the presence of genes does not reflect the activity of microorganisms [19]. Thus, there is a need to inform microbial functional diversity with direct microbial activity and environmental variables to increase the meaning of functional diversity data. Thus, this review will support microbial diversity data with measurement of microbial activity and environmental variables when possible.

The methods to determine microbial community diversity and changes in composition have evolved greatly over the last two decades. The microbial richness was (and still often is) obtained by Sanger sequencing, but does not provide relevant information on microbial relative abundance. The fingerprinting methods, such as DGGE, T-RFLP and ARISA, were methods of choice to determine changes in microbial community structure. However, they do not give any direct information on which microorganisms changed and the richness and evenness of their genetic profiles do not reflect actual changes in soil microbial diversity [20]. Over the past decade, next generation sequencing developed rapidly, allowing much higher resolution than Sanger sequencing to determine microbial diversity. The era of the fingerprinting methods coupled with Sanger sequencing will slowly leave the place to the era of metagenomics (and metatranscriptomics), although it should be noted that fingerprinting methods were found to have similar ability to determine changes in community structure and to reliably relate those changes to environmental variables [21-23] and metagenomics also present some significant technical and conceptual limitations [19]. Studies focussing on soil microbial communities in the Arctic mainly used fingerprinting methods coupled with Sanger sequencing, although the number of studies using metagenomics is rapidly increasing. The use of Q-PCR to quantify the gene abundance in Arctic soil is also increasing. This variability in the methods used to determine changes in microbial community diversity impact overall

biological conclusions due to the different resolution of the methods, making it difficult to draw overall conclusions in the present review.

2. Ecology of bacteria, archaea and fungi in arctic soils

Until recently, microbial diversity in Arctic soils and more widely in polar regions and high altitude regions, was considered to be low [24,25]. This statement was based on the analogy with plants and animals in which diversity decreases with increases in latitude and altitude. However, recent studies have shown that bacterial community diversity (i.e. richness and relative abundance) in Arctic soils is similar to or higher than in other biomes such as boreal, tropical and temperate forests, grassland, desert or prairie [26-29]. High-throughput DNA sequencing has been used increasingly in recent years to investigate the diversity of bacteria [17,22,29-40], archaea [30,32,35,37,41] and fungi [30,34,36] in Arctic soils.

Key findings were that bacterial diversity was found to differ between different ecosystems in the Arctic, with differences between peat and hummock tundra [37] or between type of tundra (e.g. wet sedge vs. dry heath) [39,42]. Similar findings were reported for archaeal community structure and richness across peatlands [43,44]. Within soils, microbial diversity was found to differ and decrease with soil depth or soil horizons within the active layer (from tundras, to peats) for bacteria [32,35-38,45,46], archaea [35] and fungi [36]. Similarly, the abundance of bacteria [36,37], archaea [36,37] and fungi [36,37] was also found to decrease with soil depth/horizons in the active layer of Arctic soils. When the active layer and the permafrost were investigated simultaneously, bacterial, archaeal and fungal diversity were found to differ between the active layer and the permafrost [30,36,37,47,48] with diversity often lower in the permafrost [36,37,49]. Similarly, bacterial, archaeal and fungal abundances were shown to be lower in the permafrost than the active layer [36,37].

All of these changes in structure, diversity and gene abundance were shown to be driven by different environmental factors such as soil pH across Arctic soils [29,50] and across ecosystems [38,39,45,46,51]. Other drivers of the bacterial and fungal diversity were identified including C/N ratio, NH_4^+ and N concentrations and plant cover [38,39,42,44,46,52]. Phosphorus content was also found to be a dominant driver (after pH) of bacterial and fungal richness, evenness, composition and phylogeny in Arctic soils [50]. Permafrost constitutes a large part of the terrestrial Arctic and represents a unique habitats for microorganisms, some of which are active at sub-zero temperature (see review on the microbial ecology of permafrost by Jansson and Tas, [53]). Overall, these differences in microbial diversity and abundance with soil depth/horizon, between active layer and permafrost and between ecosystems show high heterogeneity of microbial communities, which are likely to respond differently to global changes in relation to their location within the soil, between ecosystems and over time because of the specific biotic and abiotic drivers found (e.g. between soil horizons).

3. Microbial functions in arctic soils

Recent studies showed that the richness and gene abundance related to microbial functions in Arctic soil is similar to other biomes, as found for bacterial diversity. Hence, the bacterial communities involved in N fixation, investigated via the N-cycling functional gene *nifH*, in a High Arctic tundra soil were found to be similar to those from a tropical forest in Venezuela and had higher richness than in uncultivated temperate pastures in North America [54]. Similarly, the abundance of bacterial genes *nirS*, *nirK* and *nosZ* related to nitrite and nitrous oxide reduction (during denitrification), respectively, in Arctic soils (including wet sedge meadows and dry heath tundra) was found to be similar to that in other biomes [55].

Thus, it highlights the complexity of microbial functions in Arctic soils. However, despite the increasing number of studies using high-throughput DNA sequencing to reveal bacterial, archaeal and fungal taxonomic diversity, less attention has been given to the diversity of different microbial functional guilds in Arctic soils.

3.1. Methane cycle and hydrocarbon degradation

The carbon cycle is difficult to study because it cover many different functions involving many genes, such as for the degradation of organic matter in soil. In the Arctic, the main focus of the studies investigating part of the C-cycle is on the production and oxidation of CH_4, due to the importance of CH_4 as a greenhouse gas and the thaw of permafrost that could lead to methane release driving a positive feedback to climate change [56]. Nevertheless, other studies are interested in the diversity of microorganisms capable of bioremediation of organic (e.g. alkane, toluene) and inorganic pollutants coming from the increasing human activity (e.g. petrol extraction). However, there are many other functions related to the C-cycle which are not discussed here, due to the lack of studies.

3.1.1. Methanogens

Methane is produced by archaea and different substrates are used as the source of energy, dividing the methanogens into different metabolic categories namely: CO_2-reduction, acetoclastic and hydrogenotrophic. Methanogenic archaea can be investigated via the *mcrA* gene encoding for the methyl coenzymes-M reductase α-subunit, which is a terminal enzyme catalysing the reduction of methyl group bound to coenzyme-M and ubiquitous of known methanogens [57]. To the best of our knowledge, the diversity of *mcrA* gene in the Arctic was only investigated by two studies that focused on the active layer of peatland (e.g. wet sedge

meadows) [57,58]. The richness of *mcrA* gene in these soils was dominated by *Methanobacterium* (57%), then by *Methosarcina* (15%) and *Methanosaeta* (14%) and to a lesser extent by *Methanocella* (8.8%) and *Methanosphaerula* (4.8%; Fig. 2). The community structure of *mcrA* genes was found to differ between different depths of the active layer of an High Arctic wet sedge meadow (Herschel Island) [57], although the abundance of *mcrA* gene was found either not to vary or increase with depth of active layer at different sites (Herchel Island, Yukon Coast) [35]. Due to the small number of studies that targeted *mcrA* gene, the diversity of methanogens found in studies targeting the 16S rRNA gene was also included in this review (Fig. 2).

Methanogen diversity based on the 16S rRNA gene was dominated by the newly described phylotype [56] candidatus *Methanoflorens* (39.9%), then by the genera *Methanobacterium* (21.6%), *Methanosaeta* (16.6%), *Methanosarcina* (9.0%), candidatus *Methanoregula* (8.5%) and 21 other genera (3.9%; Fig. 2). The newly described phylotype candidatus *Methanoflorens* was found to be dominant in partially thawed permafrost of peat (palsa, bog and fen) in northern Sweden [56,59], but was also found elsewhere in Arctic peat [43] and permafrost [60]. This phylotype was found to be hydrogenotrophic, where H2 is used as an electron donor to reduce CO_2 into CH_4 and may play an important role in methane emissions in the Arctic, although further studies are required to assess the distribution of this phylotype. *Methanobacterium*, producing methane via the hydrogenotrophic pathway, was also found to be an important genus based on both *mcrA* and 16S rRNA genes (Fig. 2). Frank-Falhe et al. [35] found that Methanobacterium were more abundant in the top soil of the active layer in High Arctic wet sedge meadow. However, other studies showed that the dominance of *Methanobacterium* in the top soil of the active layer in peats is not consistent [32,56,59]. The order *Methanosarcinales*, which includes *Methanosaeta* (obligate acetoclastic) and *Methanosarcina* (acetoclastic and hydrogrenotrophic), was often found to

be highly abundant in Arctic soils [30,32,34,35,61], with the relative abundance of *Methanorsarcina* found to increase with depth of the active layer in tundra and peat [32,34,35,56,59]. The widespread distribution of *Methanosarcinales* is often explained by their ability to perform methanogenesis using acetate or hydrogen. *Methanoregula*, found to represent 8.6% of the methanogens in the current survey (Fig. 2), is an acidophilic hydrogenotrophic methanogen and was found in acidic peat in the Arctic [32,34,56,59]. Overall, there is not a specific genus dominating either within a specific ecosystem or soil depth, but there are a small number of methanogenic taxa that could drive methanogen community composition in Arctic soils. It should be acknowledge that such conclusions may reflect the limited number of studies, the different methods used and sites sampled, highlighting the need for further studies. However, the organisms performing the hydrogenotrophic pathway seem to be dominant in term of richness.

The methanogens were often found to be more abundant in deeper parts of the active layer than the top soils of peat soils [32,34,56,59]. In the permafrost, the methanogens were found to be more abundant than in the active layer [30,41] correlated with temperature, soil depth, H_2 and CO_2, or less abundant in the permafrost [17] or even not detected at all in grass and tussock tundra [36,37]. The relatively higher abundance of methanogens in deeper soil was expected because of the production of methane, where higher emissions were found within deeper soils than in the top soil of the active layer [32,57,60]. This is potentially related to the decrease of O_2 and temperature with soil depth, although high CH_4 emissions were also found in the top soil [60]. Similarly, methanogens were found in the active layer of Arctic peat but not in tundra, which was expected because of the CH_4 emissions from peat soils (wet fens) being a source of CH_4, while tundra heath and shrub tundra soils were sinks of CH_4 [62], highlighting the highly variable distribution of methanogens in Arctic soils and the difficulty in predicting their responses to global changes due to this complexity.

3.1.2. Methanotrophs

Methanotrophs use methane as a carbon and energy source via the activity of the methane monooxygenase enzyme. The methane-oxidizing bacteria are divided into three groups: Type I that include the bacterial family *Methylococcaceae* (Gammaproteobacteria), Type II that include the families *Methylocystaceae* and *Beijerinckiaceae* (both Alphaproteobacteria) and a third group within the phylum Verrucomicrobia [63]. The methane monooxygenase enzyme exists in 2 forms. Firstly, the particulate or membrane-bound methane monooxygenase present in all methanotrophs except for *Methyloferula* and *Methylocella* which can be investigated via the *pmoA* gene encoding the α-subunit of the enzyme. Secondly, the soluble or cytoplasmic methane monooxygenase that is present only in type II except for *Methylococcus* and *Methylomonas*, which can be investigated via the *mmoX* gene encoding the active site subunit of the soluble methane monooxygenase.

The *pmoA* diversity in the Arctic was dominated by the genus *Methylocystis* (46%), then *Methylobacter* (14%) and *Methylocapsa* (13%) and several genera with relative abundances of below 10%, such as *Methylosarcina* (6.7%), *Methylomonas* (5.6%), or *Methylococcus* (4.6%), *Methylocaldium* and *Methylomicrobium* (~1%) and the unclassified *Methylococcaceae* (8%; Fig. 3). The diversity of *pmoA* genes was based on studies from peat [57,64,65] and tundra [63,66] active layer soils. The dominance of *Methylocystis* was found in only two studies [57,65] from acidic peat (pH < 5), but the dominant genera vary for each of the other studies without relationship with either soil pH or ecosystem type, suggesting considerable diversity of methanotroph gene *pmoA* in Arctic soils. Similarly to *pmoA*, *mmoX* gene richness was dominated by *Methylocystis* (73.7%), but then by unclassified *Beijerinckiaceae* (24.8%) and *Methylomonas* (1.5%; Fig. 3), although the result is based on a single study from a Siberian Low Arctic palsa peat soil [65]. The community structure of

326 *pmoA* genes was found to differ between different depths of the active layer of an High Arctic
327 wet sedge meadow [57], while *pmoA* genes abundance overall was not found to decrease with
328 soil depth [35,63] or to decrease at specific locations [35]. The abundance of *pmoA* genes was
329 also found to be higher in the active layer of tundra than peat soils [63] and higher in the
330 active layer than permafrost [17].

331 The diversity of methanotrophs was also investigated via targeting 16S rRNA genes
332 and was dominated by *Methylobacterium* (20.1%), unclassified *Methylocystaceae* (18.9%)
333 and *Crenothrix* (14.2%; Fig. 3). Then, 20 genera comprised the remaining diversity (46.8%),
334 also including the order *Methylylacidiphilales* (5.1%), the genera *Methylotenera* (4.9%),
335 *Methylibium* (4.9%), *Methylococcus* (3.8%), *Methylobacter* (3.5%), *Methylosinus* (3.4%),
336 *Methylobacillus* (2.8%), *Methylomonas* (2.8%), *Methylocella* (2.5%), *Methylacidiphylum*
337 (2.1%) and 11 genera and 7 unclassified orders/families with relative abundances of below
338 2% (Fig. 3). The richness of methanotrophs in the Arctic was previously found to be low in
339 comparison to other biomes due to the extreme environmental conditions in the Arctic,
340 suggesting their potential vulnerability to environmental changes [64,67]. However, this
341 review shows high richness of methanotrophs, with 23 genera detected, suggesting
342 considerable functional redundancy. The low richness previously found could be partly
343 explained by the methods used to detect methanotrophs, such as cloning and sequencing [67]
344 and DGGE coupled with cloning and sequencing [64].

345 Type II methanotrophs were most dominant (46.7%), while Type I and other
346 methanotrophs showed similar relative abundances, of 26.7% and 26.6% respectively. The
347 Type I methanotrophs were often reported to be dominant over Type II in the Arctic, based
348 on the number of DNA sequences found [17,64,68,69], or by DNA-SIP studies [66,67], or the
349 number of RNA sequences [34] and their gene abundances [17,66,70]. In contrast, recent
350 studies using next generation sequencing (NGS) showed higher relative abundances of Type

II over Type I [34], often depending on the sampling location [37,56,59,62]. Hence, Type I dominated in a peat (pH 6.6), while Type II dominated in heath and shrub tundra site (pH 6) [62]. Type I was found to dominate in sites with *Eriophorum angustifolium*, while Type II dominated in Sphagnum spp. sites [56,59]. Methane emissions are also directly affected by the plant cover, where ecosystems dominated by sedges have higher emissions because of the stems that channel CH_4 out of the ground, reducing its oxidation [4,71]. At a soil profile scale, Type I were more abundant than Type II in mineral soil horizons in the active layer of a tundra, while Type II dominated in the top soil and buried top soil [37]. This variability in the dominance of Type I vs. Type II may partly be explained by the methods used to assess the diversity (DGGE coupled with cloning sequencing, cloning sequencing, microarray, NGS) that vary in resolution, but also the ecosystems, soil depths and temporality investigated. For example, Yergeau et al. [17] detected Type II in the active layer and permafrost by Q-PCR (~104 and ~103 16S rRNA gene number g^{-1} soil, respectively), but Type II was not detected in the metagenomics libraries, indicating potential methodological limitations. Type I methanotrophs were found to decrease with soil depths within the active layer of tundra while Type II were constant through the soil profile and outnumbered Type I close to the permafrost table [68].

In contrast, other types of methanotrophs were not found to dominate the overall methanotroph diversity, although their relative abundance was often found to be higher than Type I when Type II dominated the relative abundance (data not shown), such as the putative methanotrophic Verrucomicrobia [37]. In conclusion, the diversity of methanotrophs seems to be complex and variable and the dominance of one group (or family/genus) over others is not clear and needs further research to determine the biogeographical patterns and drivers of the diversity of methanotrophy across ecosystems, soil depths and horizons.

3.1.3. Petroleum hydrocarbon degraders

The receding Arctic ice sheet facilitates the intensification of petroleum exploration, production, storage and transportation in the Arctic region, increasing the risk of hydrocarbon contamination of soil (Table 3). Decontamination of polluted sites in the Arctic is difficult due to the remote location and harsh environmental conditions. Since a variety of native microorganisms are capable of petroleum degradation [72], bioremediation was proposed as an effective method to reduce pollution of Arctic soils. However, low temperature directly affects the rate of biodegradation, as well as the physical nature and chemical composition of hydrocarbons. The persistence of hydrocarbons in cold soils is less subject to evaporation and photo-oxidation, indicating slow in situ rates of hydrocarbon degradation [73]. It has been shown that some psychrophilic bacteria are still active during winter, with microbial respiration recorded at temperatures as low as -15 °C [49]. However, hydrocarbon degradation is known to occur above 0 °C [74].

The identification of microorganisms able to degrade hydrocarbons is based on genes encoding for proteins involved in biodegradation of hydrocarbons as polycyclic aromatic hydrocarbons (PAHs) and volatile aromatics collectively indicated as BTEX (benzene, toluene, ethylbenzene, xylene). Numerous petroleum-degrading bacteria have been isolated and characterised from contaminated polar soils [73]. Genes encoding catabolic enzymes involved in the degradation of representative fractions of petroleum hydrocarbons, including n-alkanes and aromatic and polycyclic aromatic hydrocarbons (PAHs), appear to be widespread in Arctic soils [75,76] and Alaskan sediments [77]. These microorganisms harbour genes encoding hydrocarbon degradation, such as *alkB, alkM, alkB1, alkB2, xylE, ndoB, nidA* [78].

Alkane hydroxylases play an important role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives and many other compounds for C5 to C12 n-alkanes.

401 Alkane monooxygenases catalyse the initial terminal oxidation of the alkane. The alkane
402 monooxygenases have been described for only a small number of bacteria in Arctic systems.
403 For example, *Pseudomonas oleovorans* is known to carry *alkB* involved in C5-C12 n-alkanes
404 degradation [79]. In general, alkane-degradative psychrotrophs contain at least four alkane
405 monooxygenase homologues *(Rh alkB1,Rh alkB2,Rh alkB3* and *Rh alkB4*) [75,76,80].
406 *Acinetobacter sp*. strain M-1 contains two *alkB*-related alkane hydroxylases, named *alkMa*
407 and *alkMb*, which are differentially regulated depending on the alkane content in the medium
408 [81] *alkB, alkM* primers sets were used in culture-independent studies to identify [16,82,83]
409 and quantify [31,84] *alkB* genes and to determine their prevalence in Arctic contaminated
410 soils. An increase in *alkB* gene expression has been observed after contamination with diesel
411 spill in an ex situ experiment including N amendment and soil aeration, while an in situ
412 experiment did not show any increase in *alkB* gene expression [16]. The use of metagenomics
413 methods has also shown that *Caulobacter, Pseudomonas* and *Rhodococcus* could be involved
414 in alkane degradation in Arctic soils [31,84].

415 The first step in the microbial degradation of PAHs is the action of the dioxygenase,
416 which incorporates atoms of oxygen at two carbon atoms of a benzene ring of a PAH
417 resulting in the formation of cis-dihydrodiol. Aromatic-ring-hydroxylating dioxygenases
418 generally consist of a terminal dioxygenase (an iron sulphur protein) and a reductase chain,
419 which transfers electrons from NAD(P)H to the terminal dioxygenase. The reduced terminal
420 dioxygenase, catalyses the direct insertion of molecular oxygen into the substrate to form
421 cisarene diols. Eriksson et al. [85] showed that PAH degradation at a low temperature
422 occurred in anaerobic conditions. Phylogenetic studies of amino acid sequences of the
423 proteins involved in the initial oxidative attack of PAHs and BTEX and in their ring cleavage
424 show significant sequence homology, indicating a common ancestry that allowed the design
425 of group-specific primers. In contrast to alkane degradation genes present in the cold-tolerant

bacteria described above, the genes used for aromatic degradation by psychrotolerant and psychrophilic bacteria do not appear to differ significantly from those identified in mesophilic isolates. Whyte et al. [86] found that catabolic genes from several aromatic-degrading psychrotolerant strains had homology to those described in mesophilic bacteria (although other isolates appeared to have novel genes). For PAH degradation, the main genes used to characterise or quantify biodegradation pathways are *ndoB* and *nidA*, encoding for naphthalene dioxygenase and pyrene dioxygenase, respectively. Bacterial community structure and composition of Arctic soils are known to be influenced by different plant species and diesel contamination. Ferrera-Rodrıguez et al. [83] reported that *Eriophorum scheuchzeri, Potentilla rubricaulis, Oxyria digyna, Salix arctica* and *Puccinellia angustata* not only modified the abundance of hydrocarbon-degrading bacteria but also their community structure. The community structure of a hydrocarbon degrader was found to differ between the rhizosphere of different plant species [83]. Hence, *Puccinellia angustata* was found to have a high phytoremediation potential because it was the only species that harboured a rhizosphere containing *alkB, ndoB* and *xylE* genes simultaneously [83]. Yergeau et al. [31] found a positive correlation between the abundance of *Pseudomonas* hydrocarbon-degrading genes and soil hydrocarbon content. Correlations between soil hydrocarbon content and *Pseudomonas alkB* and *ndoB* genes and *Rhodococcus sp alkB1* and *alkB2* were also mentioned by Yergeau et al. [31]. The *ndoB* gene encoding the a-subunit of the iron sulphur protein of naphthalene dioxygenase is less widely distributed than *alkB* in the rhizosphere [83]. PAH degradation and horizontal gene transfer between strains have been found in Antarctica [87], but not yet in the Arctic.

3.2. Nitrogen cycle

451 The nitrogen cycle is probably the biogeochemical cycle that is most accessible to
452 study due to the well documented number of genes that can be targeted to investigate the
453 different steps of the N-cycle. Nevertheless, only a few studies have assessed their diversity
454 in Arctic soils.

455 The fixation of N can be investigated using the *nifH* gene encoding for the Fe protein
456 subunit of nitrogenase reductase. The bacterial phyla carrying *nifH* genes in Arctic peat [65],
457 tundra soil aggregates [54] and the rhizosphere [88], include unclassified bacteria (68%),
458 Alphaproteobacteria (16%), Gammaproteobacteria and Firmicutes (5% each) and
459 Betaproteobacteria and Deltaproteobacteria (3% each) (Fig. 4). At the genus level, organisms
460 such as *Rhodopseudomonas*, *Bradyrhizobium* were found in shrub tundra [54,88], while in
461 peat, mainly methanotrophic organisms were found, suggesting that methane-oxidizing
462 bacteria play an important role in both C and N-cycles [65]. However, further studies are
463 required to confirm the differences in *nifH* richness between peat and tundra ecosystems.
464 Studies using fingerprinting methods (e.g. RFLP, T-RFLP) showed that different plant
465 species (i.e. *Dryas integrifolia*, *Salix arctica*, *Cassiope tetragona*) [88], different tundra (e.g.
466 sedge meadow, heath or shrub tundra) and different soil depths within the active layer [89]
467 harboured different *nifH* gene community structures, while *nifH* gene abundance was found
468 to decrease with soil depth in a tundra active layer [35], but was higher than in the permafrost
469 [17]. Overall, plant composition, soil characteristics, soil moisture and temperature were
470 identified as the main drivers of *nifH* gene composition [88,89].

471 Nitrification, i.e. the oxidation of NH_4^+ into NO_3^-, is comprised of two steps: the first
472 step corresponds to the oxidation of NH_4^+ into NO_2^- (ammonia oxidation) and the second step
473 corresponds to the oxidation of NO_2^- into NO_3^- (nitrite oxidation). The oxidation of NH_4^+ into
474 NO_2^- is performed by both bacteria and archaea and is investigated by targeting the *amoA*
475 gene encoding the first subunit of the ammonia monoxygenase enzyme. In the Arctic tundra

(shrub, tussock, dry moss tundra) and peat, the *amoA* archaeal genes were found exclusively within the phylum *Thaumarchaeota*, while at the genus level, *Nitrosospharea* [79,80], *Nitrosotalea* [90] were the most dominant archaeal ammonia oxidisers in Arctic tundra [79,80] and peat [79] (Fig. 4). Archaeal *amoA* genes were found to be more abundant than bacterial *amoA* genes in Arctic tundra (shrub, tussock, polygons, dry moss tundra) and peat soils [17,35,91], as was previously found for many other biomes [92], although the dominance of archaea was found to be lower in permafrost than in the active layer [17], and bacterial *amoA* gene abundance was also found to be higher for some specific tundra (tussock grass) [82] and fen peat sites [91,93]. The abundance of bacterial and archaeal *amoA* genes was also found to decrease with soil depth in the active layer [35] and to be lower in the permafrost [17]. Soil moisture, pH and NO_3^- concentrations were found to be important drivers of archaeal richness [91]. In contrast to archaeal *amoA* gene diversity, the diversity of bacterial *amoA* genes or the *nxrA* gene targeting the second step of nitrification (encoding for the subunit of the nitrite oxidoreductase in *Nitrobacter* species) have not yet been directly investigated in Arctic soils.

Denitrification is the anaerobic reduction of NO_3^- to N_2 through several steps, producing the greenhouse gas N_2O as an intermediate. The first step of denitrification is the reduction of NO_3^- into NO_2^- and can be targeted via *narG* (encoding for the catalytic a-subunit of the membrane-bound nitrate reductase) and *napA* (encoding for the catalytic subunit of the periplasmic nitrate reductase) genes. In peat soils in the Arctic, *narG* gene diversity was dominated by Actinobacteria (56.2%), Alphaproteobacterium (38.4%) and in lower relative abundances by Betaproteobacteria (5.1%), Epsilonproteobacteria and unclassified bacteria (~0.1% each) (Fig. 4) [94,95]. However, no diversity data were available for *napA* genes, although a few metagenomics reads (19 in total) were assigned to *napA* genes in Arctic peats [34]. *narG* genes showed similar diversity patterns between

501 cryoturbated (i.e. soil mixed by cryogenic processes in tundra) and non-cryoturbated peat soil
502 but *narG* gene abundance was higher in cryoturbated soil, where higher nitrate-dependent
503 denitrification occurred at higher rates than in non-cryoturbated peat soils [94]. Different
504 *narG* gene diversity were found between the soil 0-20 cm and soil 20 cm e permafrost layers,
505 with higher diversity in upper layers [95].

506 The second step of denitrification is the reduction of NO_2^- into NO, which is catalysed
507 by two different metalloenzymes, a copper reductase and a cytochrome *cdl*-nitrite reductase
508 which are encoded by *nirK* and *nirS* genes, respectively. The diversity of *nirS* genes in Arctic
509 soil (peat) was dominated by Betaproteobacteria (76%), then Alphaproteobacteria (19%) and
510 unclassified bacteria (5%), while that of *nirK* genes in peat (same studies as *nirS*) was
511 dominated by Alphaproteobacteria (98%) and unclassified bacteria (2%) (Fig. 4) [94,95]. The
512 diversity (i.e. Shannon diversity index and species evenness) of *nirK* genes was found to be
513 higher in an Arctic peat soil from 0 to 20 cm than 20 cm-permafrost, while *nirS* gene
514 diversity was higher in the deeper soils, but the abundance of both genes was higher in the
515 0e20 cm soil, suggesting higher nitrite reductase activity in top soils and their composition
516 different between depths [95]. The *nirS* and *nirK* gene richness and evenness (Shannon-
517 Weaver index) was found to be lower in cryoturbated than non-cryoturbated peat soils and
518 the *nirS* and *nirK* gene community compositions were different between soils [94].

519 The third step in denitrification is the reduction of NO into N_2O, which can be
520 investigated via the *norB* gene encoding for a subunit of the nitric oxide reductase. However,
521 no analysis of *norB* sequences in Arctic soils are reported in the literature, although a few
522 metagenomics reads (14 in total) were assigned to *norB* genes in Arctic peats [34] and lower
523 richness in the nitric oxide reductase community was found in the permafrost than in the
524 active layer [17]. The final step of denitrification is the reduction of N_2O into N_2, which can
525 be targeted via the *nosZ* gene encoding for a subunit of nitrous oxide reductase. Similarly to

nirK, *nosZ* gene diversity in Arctic (peat) soils was nearly exclusively dominated by Alphaproteobacteria (99.5%) and then Betaproteobacteria (0.4%) and Gammaproteobacteria (0.1%) (Fig. 4) [94,95]. The diversity and abundance of nosZ *genes* between different soil depths (0-20 cm vs. 20-permafrost) and between cryoturbated and non-cryoturbated in peat were found to be similar [94,95], while permafrost harboured lower richness of nitrous oxide reductase community [17]. In contrast, the *nosZ* gene community structure (investigated by T-RFLP) differed between different tundra (i.e. sedge meadows, *Cassiope tetragona* heath and shrub tundra), and with soil depth of the active layer of sedge meadow and alkaline shrub tundra, but these differences were not consistent across all the sites [89].

Overall, the diversity of N functional guilds remains poorly understood in Arctic soils, with only seven studies targeting those genes, showing different diversity between the different steps of the N-cycle, but also similarities in their diversity patterns (e.g. between *nirK* and *nosZ* genes; Fig. 4) and variability between ecosystems, soil horizons/depths indicating that the microorganisms involved in the different steps of the N cycle are unlikely to respond similarly to environmental/global changes.

4. Vulnerability of arctic ecosystems to environmental change

4.1. Temperature

The effects of increases in temperature are typically investigated in situ using open top chambers that increase the air (1-4 °C) and to a lesser extent, top soil (0.7-2 °C) temperatures [9,89,96,97], or ex situ using microcosms incubated at controlled and usually much higher temperature than the climate change models predict (Table 3). More rarely, heating lamps and soil warming cables have been used to simulate warming in Arctic

551 environments [98]. Soil fertilisation has also been used in field plot or microcosm
552 experiments to simulate higher nutrient availability (due to increased mineralization rates or
553 permafrost thawing and higher plant activity) expected with global warming [99]. However,
554 as for plants [7], experimental studies in the Arctic have shown that fertilisation and warming
555 can have substantially different impacts on microbial communities [9], and findings from
556 fertilisation studies should not be extended to the effects of warming. Hence, fertilisation was
557 shown to have stronger and sometimes antagonistic effects compared to warming on carbon,
558 nitrogen and phosphorus microbial biomass in Subarctic tundra heath (e.g. increase in C
559 microbial biomass with fertilisation and decrease with warming) and the bacterial community
560 structure differed more importantly between tundra soil under open top chambers and
561 fertilisation treatment than with respect to the control plots [9].

562 CO_2 emissions were found to increase with temperature in peat active layers and
563 permafrost [41,100], with addition of acetate increasing emissions at 22 °C in comparison to
564 4 °C in active layer and permafrost, while methanol addition increased emission only in the
565 permafrost [41]. Anaerobic conditions in Arctic peat increased CO_2 emissions at 20 °C but
566 not at -0.5 °C, while anaerobic conditions showed lower CO_2 emissions at 20 °C only [100].
567 The Q10 (i.e. increase of CO_2 emission or carbon mineralisation for a 10 °C increase in
568 temperature) of Arctic soils has been reported to average 1.3, which will translate into a
569 substantial increase in CO_2 emission as the temperature rises in the active layer and
570 permafrost, and is somewhat a conservative estimate as compared to literature values relative
571 to soils from other regions [101]. Increases in temperature often resulted in an increase in
572 CH_4 emissions in the active layer of Arctic soils (peat, polygonal peat), with optimum
573 temperatures of between 20 and 25 °C [41,58,60,61,100,102-104], although some studies
574 have found no effect of increasing temperature on CH_4 emissions depending on the location
575 (between peats and between peat and tundra) and coupled with hydrology, soil organic C,

permafrost and vegetation [41,100]. Addition of H_2, CO_2 or methanol was found to result in increased CH_4 emissions in the active layer and permafrost of peat or tundra at 15-20 °C in comparison to 4-5 °C (with a stronger effect of H_2 in most peats), while addition of acetate decreased emissions in the active layer and permafrost [41]. Anaerobic conditions were also found to increase CH_4 emissions (while aerobic conditions showed lower emissions) after 30 days of incubation at 20 °C but not at -0.5 °C [100].

The methanogenic community was also found to be affected by increases in temperature. Hence, the relative abundance of methanogens increased with temperature in the peat active layer [41,61,102], from 28% to 58% of the overall archaeal diversity at 20 °C in comparison to 5 °C [102]. The diversity of methanogens was found to be affected by temperature increases [41,102], with an increase in relative abundance within the peat active layer of *Methanomicrobiales* (from 16% at 5 °C to 32% at 20 °C) and *Methanosarcina* (from 2% to 14%), while *Methanobacterium* decreased (from 10% to 6%) [102]. Methanogen relative abundance was found to increase in the active layer and in permafrost of Arctic peat incubated at 22 °C coupled with acetate, methanol or CO_2 addition, with higher abundance of hydrogenotrophic methanogens, while acetocalstic methanogens were adapted to lower temperatures [41], suggesting that different methanogens respond differently to increases in temperature [41,102]. In contrast, thawing of permafrost at 5 °C did not increase the abundance of *mcrA* genes [30].

Increases in temperature were also found to affect the oxidation of CH_4 and the methanotroph community. Oxidation of CH_4 increased in top soil incubated at room temperature in comparison to 4 °C [66] and in thawing permafrost incubated at 5 °C in comparison to frozen one [30]. Liebner and Wagner [68] showed that the effect of temperature on the potential of methane oxidation varies with soil depth in low-centred icewedge polygons. The highest potential methane oxidation in soil close to the permafrost

601 was found at 4 °C, but not at higher temperatures (12 or 21 °C) or at 0 °C, while in the top
602 soil, the optimum methanotrophic potential was reached at 21 °C, but also showed relatively
603 high methane oxidation at 28, 12, 4 and 0 °C. Hence, the methanotrophic community close to
604 the permafrost is likely to be dominated by psychrophiles, while in the top soil, mesophiles
605 and psychrotolerants may dominate. Similarly, thawing of permafrost at 5 °C increased the
606 abundance of Type II methanotrophs and of *pmoA* genes, confirmed by an increase in the
607 oxidation of CH_4 [30]. The community structure of *pmoA* was also found to be affected by
608 incubation of top soil at room temperature in comparison to 4 °C, with the presence of
609 *Methylobacter* detected only at room temperature; *Methylotenera* and *Methylophilus* were
610 mainly detected in samples at room temperature, while *Methylosarcina* were found at both
611 temperatures [66].
612 Overall, an increase in temperature is expected to increase CH_4 and CO_2 emissions in
613 the Arctic, especially in thawing permafrost, but also to increase the temperature sensitivity
614 of respiration, linked to soil with high C/N releasing more GHG [4,105,106]. However, the
615 balance between sources and sink of GHG remains unclear. Although CH_4 emissions can
616 increase via higher activity of methanogens, it remain unclear how methanotroph activity will
617 be affected and mitigate CH_4 emissions at the same specific location, as such balance will
618 depend on the plant cover, the soil water content (anaerobic/aerobic conditions) and soil
619 properties [4]. Furthermore, effects of an increase in temperature could occur in different
620 phases: a rapid response of microbial communities to changes in temperature from a couple
621 of months to a decade, and then a second phase over a longer period related to the change in
622 vegetation (the “greening” of the Arctic), which will be more productive and provide greater
623 C supply to microbial communities. The potential response of microbial communities and
624 their feedback to GHG is likely to be more complex than a simple increase in activity with
625 temperature, highlighting the need for studies taking into consideration all those parameters.

The effects of increases in temperature of the N functional guilds have also been investigated in Arctic regions. Warming experiments using open top chambers showed changes in the community structure of *nifH* genes within the organic horizon of the High Arctic shrub tundra following 5-6 years of warming [96]. At the same location, but at different sites, Walker et al. [89] also found that the structure of *nifH* and, to a lesser extent, of *nosZ,* genes significantly changed following warming within both the upper and lower part of the active layers in the High Arctic tundra (wet sedge meadow, acidic shrub tundra, willow tundra), but the response varied with location (no effect in Cassiope tetragona heath tundra or alkaline shrub tundra) and was more pronounced in wet sedge meadows, with a reduction in complexity of the community structure (i.e. reduction in the richness of T-RFLP profiles). In contrast, 16 years of warming with open top chambers in High Arctic tundra heath did not affect the abundance or community structure of either *amoA* bacteria or *nosZ* genes in the upper soil horizon, although the use of a low resolution fingerprint method (DGGE) and the single sampling time point in the upper soil horizon may explain the absence of effects on microbial communities [97]. Microcosm experiments showed that thawing of permafrost at 5 °C decreased the abundance of *nifH* genes, while *narG* gene abundance increased with thawing of permafrost, but *nosZ* gene did not change [30]. Increases in N_2O emissions and NH_4^+ consumption were found only at 20 °C, but not at 4 or 28 °C [91]. Overall, N fixation seems to be negatively affected by warming, while the effect of warming on nitrifiers and denitrifiers showed variability (i.e. presence or absence of effect) which could be related to differences between sites and soil horizons investigated, but also the different increase in temperature simulated either in situ or ex situ.

The effects of increasing temperature have been identified for CH_4 and CO_2 emissions and, to a lesser extent, for N_2O, but the effect on microbial diversity remains unclear. Different communities of methanogens and methanotrophs can respond differently to similar

increases in temperature depending on their soil depth, but also to soil organic C, hydrology, plant cover and permafrost [68,100]. Finally, most studies have investigated effects of large temperature increases, up to 30 °C, rather than the smaller increases expected due to climate change. There is a clear need for studies simulating what will happen in situ.

4.2. Soil moisture

Changes in soil moisture are expected due to the increase in precipitation and thaw of permafrost, that could lead to the development of thermokarst landscapes, where higher soil moisture and greater flooded areas are found, when other are drained with lower soil moisture. However, the effects of increased soil moisture on Arctic microbial communities have rarely been directly investigated via the simulation of greater soil moisture, but have more often been assessed indirectly via natural gradients in soil moisture across landscapes. Soil water content was found to be important for the abundance of methanogens within active layers of High Arctic tundra, with methanogens and methane emissions present only in soils permanently wet, such as peat, but not present in heath or shrub tundra sites [62,107], while methanotroph abundance tends to be lower in wetlands [62]. Denitrification was found to be controlled by soil moisture and organic C in High Arctic wet sedge meadows and heath tundra soils [55], with positive correlations between a nitrous oxide reductase assay and soil moisture, while at the same site, N_2O emissions were found to be insensitive to changes in soil moisture [108]. Abundances of *nosZ* and *nirK* genes were found to be 10 times higher in top soils from wet sedge meadow sites, with soil moisture of ~60% in comparison to sites (bare soil, upland sedges) with a soil moisture of ~20%, while neither *nirS* nor *amoA* archaea abundances were affected [108]. In contrast, previously at the same sites, nosZ gene abundance was not found to vary between sites [109] or was found to be more abundant at

low soil moisture ~20% [55], while *nirS* gene abundance was lower at ~60% soil moisture. This indicates variability in the spatial distribution of nitrogen functional guilds and over time, making it difficult to conclude confidently on the effects of soil moisture and hence on the effects of increases in precipitation. The *amoA* bacteria and archaea, *nirS, nirK* and *nosZ* genes in top soil were not found to be affected by soil moisture across a natural soil moisture gradient (from tussock grass tundra to fen) in Subarctic Alaska [93]. Addition of water in situ over 16 years increased CO_2 emissions in High Arctic tundra shrub tundra, but did not affect the abundance or structure of *amoA* bacteria and *nosZ* genes in the upper soil horizon [97]. However, use of the low resolution fingerprint method DGGE and the single sampling time point in the upper soil horizon may again explain the absence of effects on microbial communities [97].

4.3. Atmospheric and terrestrial pollution

The effects of N deposition (Table 3) on microbial functional guilds has been rarely investigated and studies have often tended to focus on fertilisation effects, rather than to simulate atmospheric N deposition which typically occurs at lower rates than in fertilization studies, which also often include co-input of P and K. This results in a limited knowledge of the effect of N pollution on microbial communities in Arctic ecosystems.

Among the studies that have taken place in High Arctic shrub tundra, the community structure of *nifH* genes was found not to be affected by 5-6 years of NPK addition (solution 5 g m^{-2} yr^{-1}) within the organic horizon over the summer [96]. Similarly, the addition, over 16 years, of 10 or 50 g NPK m^{-1} yr^{-1} (solution of NPK) with or without warming did not affect the structure or abundance of *amoA* bacteria or *nosZ* genes in the upper soil horizon of High Arctic shrub tundra [97]. However, with 10 g NPK m^{-1} yr^{-1}, the tundra was a source of

methane (regardless of warming), while it was a sink for methane for the other treatments (i.e. control, warming, high fertilisation and treatments in interaction) [97]. Similarly, fertilisation was found to increase N_2O emissions in Subarctic palsa peat active layers, especially with 20 mM $NaNO_3$ and, to a lesser extent, by 20 mM NH_4Cl [95]. Fertilisation also dramatically increased N_2O rates in mesic birch hummock tundra (Canadian low Arctic) in early spring, after 2 years of addition of 10 g $NH_4NO_3^-$ m^{-2} yr^{-1} [110]. The lack of effects of N addition on the abundance and structure of N-cycling functional guilds is surprising considering the long-term addition of large amounts of N. However, sampling only in the organic horizon, where larger amounts of nutrient are present in comparison to the mineral horizon or bare soil, coupled with sampling only once or twice in a year, may partly explain the absence of treatment effect.

One of the climate feedbacks from Arctic warming is that the thawing of sea ice will inexorably facilitate the exploitation of Arctic resources by man, with petroleum and gas being the most targeted, and the probability that contamination of soil will increase. Mining activities are also important in the Arctic (especially in Alaska, Canada and Russia) including, for example, cobalt, iron ore, nickel, palladium and uranium, and are likely to increase. With increasing temperature, the period in which soil temperatures exceed 0 °C should also increase. Thus, we hypothesize that hydrocarbon degradation rates will be enhanced, as has been suggested in studies of warmed soils [111]. However, the literature related to the effect of temperature variation on the remediation of Arctic contaminated soil is scarce. The temperature sensitivity of hydrocarbon mineralisation in Arctic soils is still largely unknown. In an enrichment cultures inoculated with samples from Arctic soils, Eriksson et al. [112] showed that low temperature (7 °C) severely limited PAH biodegradation under aerobic conditions, but not under nitrate-reducing conditions. A study made on a High Arctic permafrost site indicated that without other limiting factors, the active

biodegradation period can be extended to about 6 months despite periods with subzero soil temperatures [113]. Among limiting factors, including O_2 depletion or substrate availability, nitrogen has been shown to be a major limiting factor in Arctic bioremediation [111]. For this reason, biostimulation (e.g. addition of nitrogen) is commonly applied to hydrocarbon contaminated polar soils for in situ treatment [73]. This biostimulation may occur naturally with increased N deposition. However, very few studies have been conducted on the effect of nitrogen on hydrocarbon-degrading microorganisms in Arctic soils to date. Using a 15N-based DNA-SIP approach, Bell et al. [84] determined which taxonomic groups most readily incorporated nitrogen from monoammonium phosphate added to contaminated and uncontaminated soil. Their results suggest that nitrogen uptake efficiency differs between bacterial groups in contaminated soils. A better understanding of how temperature, nutrient availability and plant interactions in soil affect hydrocarbon degraders should help to improve bioremediation treatments.

5. Conclusions

The Arctic is a major world biome of high conservation value which plays an important role in regulating the global carbon balance and Earth's climate. Across this biome, microbial taxonomic and functional diversity in Arctic soils has proven to be highly diverse and complex, yet comparable in many ways to that in other biomes despite the harsh environmental conditions. As with other biomes, microbial diversity was found to vary with soil horizons/depths, between ecosystems (e.g. tundra, peat) and with plant cover. Furthermore, an important feature of many Arctic soils, the permafrost, presents a significant potential of microbial functions and diversity, showing that despite the frozen state of permafrost, microbial activity can occur therein. Thawing of permafrost due to global

751 warming will directly affect these communities, especially at the interface of soil active
752 layer/permafrost where thawing takes place. This review has focussed on the most-studied
753 functional guilds in the Arctic, but many other microbial functions require attention, as many
754 other functions/genes have been detected in the Arctic, such as chitinase (*chiA* gene),
755 homoacetogenesis (*fthfs* gene), [FeFe] hydrogenase (*hydA* gene) and sulphate reduction
756 (*dsrA* gene) [17,34].

757 The knowledge of the diversity of microbial functional guilds in Arctic soil and their
758 responses to global change therefore remains limited and further research is needed (Table 4).
759 Some potential threats such as the effect of mercury deposition on microbial diversity are not
760 yet understood. There is also a real need to consider the spatial (vertical and horizontal
761 distribution of microorganisms) and temporal (over the entire year) variations of Arctic
762 microbial diversity and function. The understanding of changes in microbial diversity and
763 function over time is limited, being mainly studied in summer and usually at a single time
764 point. Similarly, there is a need to perform in situ experiments to more closely mimic global
765 changes, across soil horizons/depths/ecosystems and over short (a few days) and long
766 (months-years) periods of time, as different responses of microbial diversity are expected
767 within the vertical and horizontal distribution over time. Additionally, interactions between
768 temperature, hydric regime and pollution require more investigation, at least using laboratory
769 experiments. These future research areas may help to predict the real effect of global change
770 on microbial communities and their subsequent impact on sinks and sources of greenhouse
771 gases in the Arctic.

772

773 ## Conflict of interest

774 The authors declare they have no conflict of interest.

775

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1243 **Table 1**: Description of the three main arctic regions: High Arctic, Low Arctic and Subarctic

1244 (based on Jones et al. [2]).

	High Arctic	**Low Arctic**	**Subarctic**
Limit	Northern part of Arctic region	Arctic continental coastline to the treeline	From the treeline to the upper latitudinal limit of the boreal forest
Growing season	1-2.5 months	3-4 months	3.5-12 months
Temperature	Annual <-15 °C Mean July 4-8 °C	Annual -15 to -10 °C Mean July 4-11 °C	Large fluctuations Annual -20 to 5 ºC (even below -20 °C in Siberia, where the average in January is -50 ºC)
Precipitation	< 250 mm or between 250-500 mm (only Greenland ice cap receives > 1000 mm)	< 500 mm and often < 250 mm	Large variations with annual precipitation varying from < 250 mm, up to 750 mm
Plant cover	Large areas of bare soil Discontinuous plant cover Bryophyte and lichen 50-80%, vascular plant 0-20%	Plant cover increases to 80-100% Decrease in bryophyte and lichen density	Increase in vegetation height due to shrub dominance. Plant cover 100%
Ecosystems	Polar desert[a] and polar semi-desert[b], tundra,	Increase of tundra area and peatland; decrease in polar semi-desert area	Transition zone between arctic tundra and boreal forest; large areas of wetland

1245 [a] Polar deserts are areas where annual precipitation is < 150 mm and the mean temperature of the warmest

1246 month is < 10 °C [2].

1247 [b] Polar semi-desert is also used for areas with annual precipitation of 150-250 mm [2].

1248 **Table 2:** Soil characteristics and drivers in Arctic regions.

	Characteristics	Drivers
Soil horizons	Organic horizon at the soil surface. From few cm in High Arctic to 40 cm in peat (up to several meter in Subarctic) Mineral horizons mix of organic & mineral material	Plant cover determines the presence and the thickness of the organic horizon. Mineral horizon can contain organic matter via cryogenic processes
Temperature	-30 up to nearly 10°C. Soil temperature is directly related to air temperature	Always lower than the air temperature in summer due to the presence of permafrost and insulation from plant cover. Conversely, the soil temperature in winter is substantially warmer than the air temperature due to snow and plant cover which insulates the soil from the air temperature
Water	Arctic soils are: - poorly drained (85 - 90% of the Low Arctic, and some wetlands of High Arctic) - well drained which is common in the High Arctic [1]	- Precipitation - Soil draining characteristics: annual runoff in High Arctic soil can reach 80 - 90% over two or three weeks during the snowmelt period leading to extremely low water availability during the growing season [1] - Permafrost: barrier to water drainage, source of water from its melting
Carbon content	Total C (1672 petagrams) described as being more than twice the atmospheric C pool and especially of organic C [3,2]	Input of organic C is low due to small plant inputs from low annual production leading to C-input ranging from 1 – 50 g C m^{-2} yr^{-1} [1,2]

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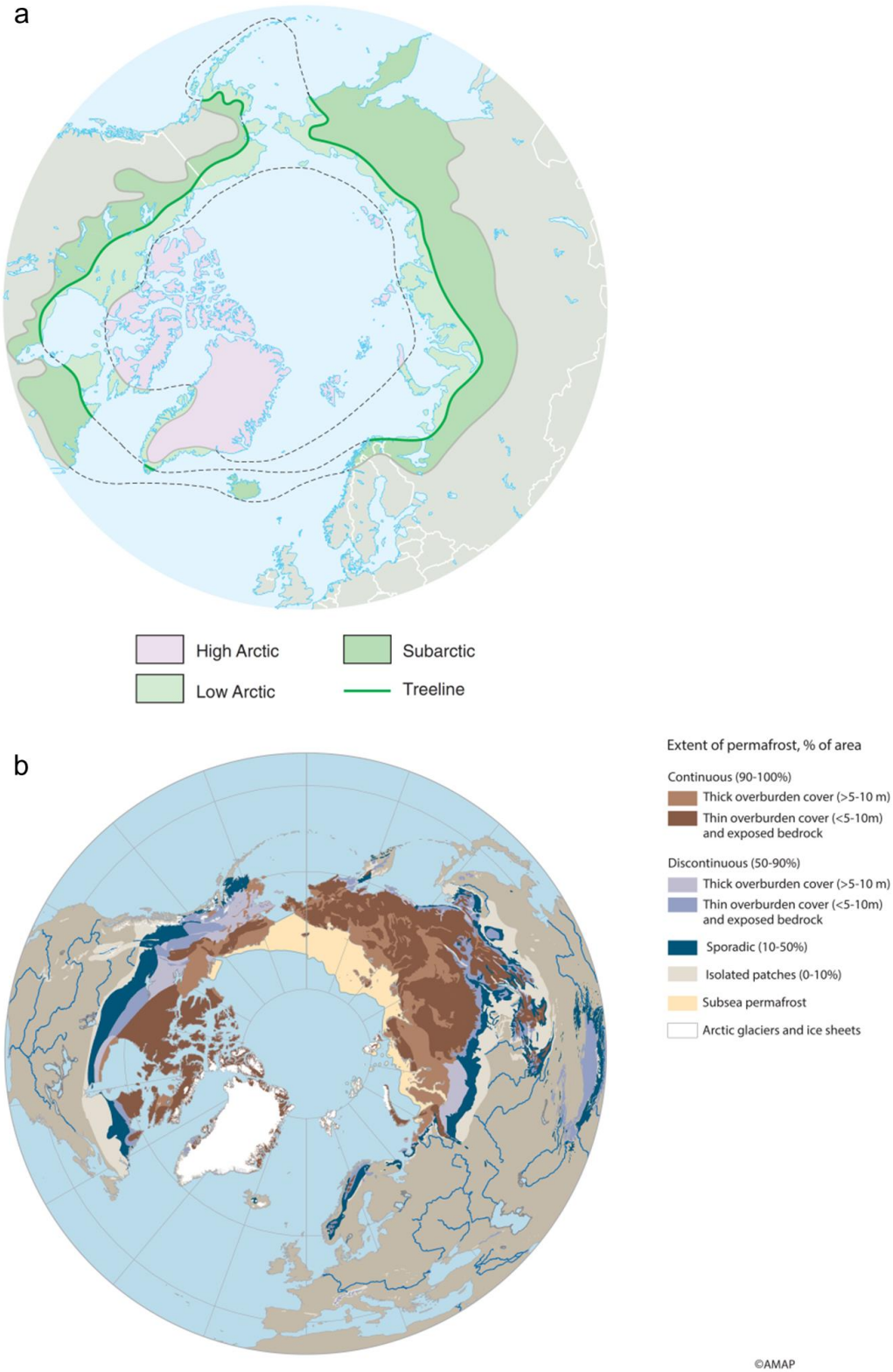
Table 3: Anthropogenic environmental threats to the Arctic regions that could directly affect arctic soils and microbial communities.

	Threats	Consequences
Temperature [14]	Increase of 3-6 °C by 2080 Increases higher in autumn and winter than summer, and lower over land (2-3 °C) Increase in permafrost temperature (0.5 - 2°C)	• Decrease in overall snow cover • Decrease in duration of snow cover by 10-20% by 2050, increasing the summer period • Glacier retreat: new land revealed (glacier forefield), release of water and nutrients • Thawing of the permafrost: increased depth of active layer, release of organic matter to decomposition, , increase of water (due to the melt of ice) and decrease (due to greater drainage). Increase in therrmokarst landscapes (i.e. formation of depression, mound, lake due to thaw of ice in the permafrost). • Change in vegetation diversity, increase in size, root growth deeper, treeline moving northward.
Precipitation [14]	Increase in precipitation, higher in autumn and winter than in summer, but does not prevent dry periods without precipitation, more precipitation falling as rain rather than snow.	• Increased probability of rain in winter, leading to more frequent ice cover on land • Change in soil moisture (both dry and wet conditions) • Change in vegetation diversity and increase in size
Atmospheric pollution	N deposition (1 – 5 kg N ha^{-1} yr^{-1} across the Arctic) [11] Increasing Hg deposition [12]	• Source of nutrients for biotic activities in this nutrient poor region. • Potential to increase in primary productivity • Change in plant and microbial activity and diversity • Select microorganisms resistant to Hg [114] • Change in vegetation diversity and increase in size
Human activity [10,13]	Increase in ships and aircraft Increase in human presence Increase in mining, hydrocarbon and gas exploration	• Fuel combustion leading to atmospheric deposition, and fuel spillage • Direct change/destruction of habitat via settlement development, resource extraction, transport

Table 4: research priorities for the study of microbial communities in arctic soils.

General	Specific
Spatial variability*	The horizontal and vertical distribution of microorganisms should always be taken into consideration. Different soil horizons, rhizosphere, bare soil, plant cover, active layer, permafrost directly influence microbial community and are unlikely to respond similarly to global changes. Not taking into consideration such variability will lead to miss understanding on the effect of global changes and the feedbacks from microorganisms.
Temporal variability*	Too often studies in the Arctic did only one sampling point and nearly exclusively in summer time. However, studies showed that microbial community can change rapidly over few days [52], over season [115,116], and still active in subzero temperature [53]. There is a need to study winter, snow melt period, the summer period throughout the changes in plant activity, and performed multi point sampling over few days and months. There is also a need for long term experiment over years.
In situ multi-factorial experiments	*In situ* experiments realistically mimicking global changes are crucial to determine effects on microbial diversity and functions. Furthermore, multi-factorial experiments (**interactions between temperature, hydric regime, plant cover and pollutions**) are also crucial to gather understanding of the real effect of global change on microbial communities and their subsequent impacts such as on sinks and sources of greenhouse gases in the Arctic.
Going beyond richness to understand biogeochemical cycles	Until recently, most studies focused on the microbial richness due to methodological limitations. Thus, there is a need for full diversity data (i.e. richness and relative abundance) and their interactions. These data should be directly link to spatial and temporal variability and environmental variables as well as with microbial activity to directly understand their effect on biogeochemical cycles.
Plant-microorganisms interactions	Global changes directly affect plants distribution and activity, with for example the northward move of the treeline. Plant cover has a direct effect on microbial community [39]. There is a need to understand how the changes of plant community will affect (and be affected by) microbial communities and how this will affect biogeochemical cycles (see Opinion article from Wullschleger et al [117]).
Functional diversity and threats	This review has focused on the most-studied functional guilds in the Arctic, but many other microbial functions requires attention, as many other functions/genes have been detected in the Arctic, [16,18,34]. Furthermore, some potential threats such as the effect of mercury deposition and other heavy metals, or mining on microbial diversity are not yet understood.

* Spatial and temporal variability should always been takin into consideration, and such approach in microbial ecology is fundamental for any biomes [19].



1257

1258 **Fig. 1.** Maps a) of the three ecological zones in the Arctic region, based on floristic data
1259 (AMAP [1,118]), and b) of the distribution of permafrost in the Northern Hemisphere
1260 (AMAP [14]).

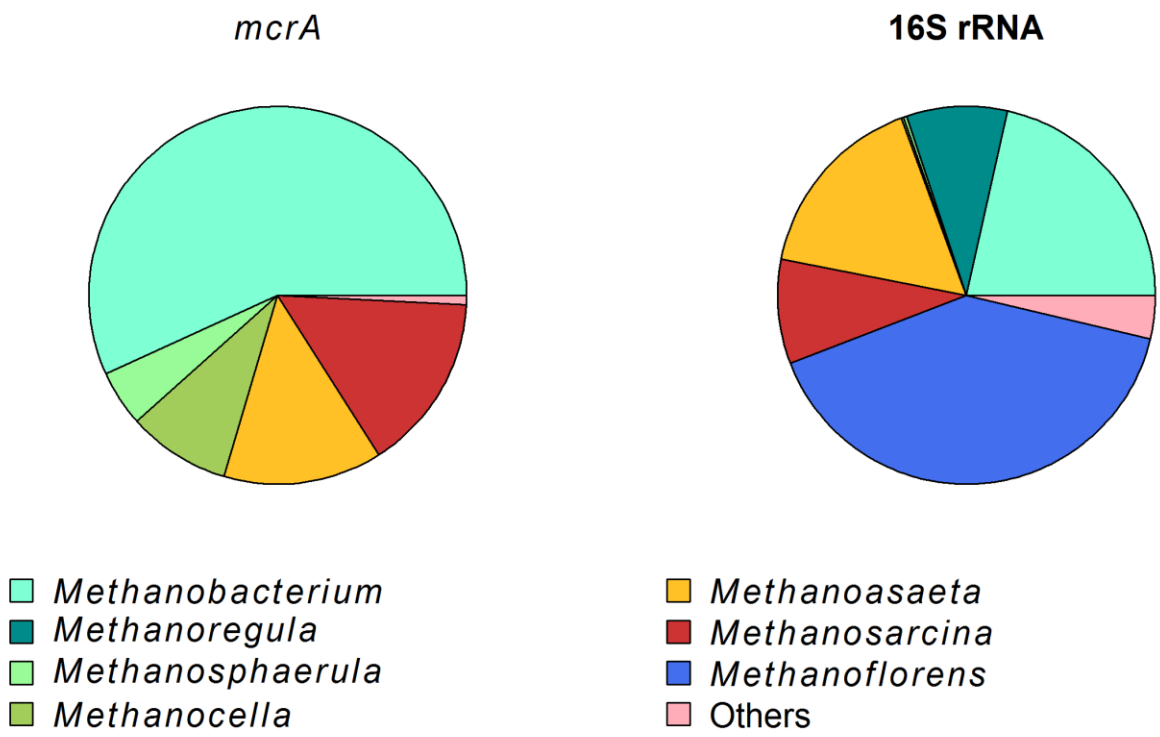


Fig. 2. Overall methanogen taxon composition in arctic soils. The relative abundances of methanogen genera is shown, based on sequences from published studies in the Arctic and investigating *mcrA* genes (encoding for the methyl coenzymes-M reductase α-subunit) and 16S rRNA genes (including only methanogen related sequences). The *mcrA* gene diversity was based on 125 sequences from the active layer of peat soils [58,57]. 16S rRNA gene diversity was based on 103,379 sequences originating mainly from active layers of peat soils [34,43,44,56,58–61,102,107] but also the tundra active layer [42], and permafrost [119,120]. Note that the diversity of methanogens based on the 16S rRNA gene is dominated by studies using next generation sequencing [34,56,59,62].

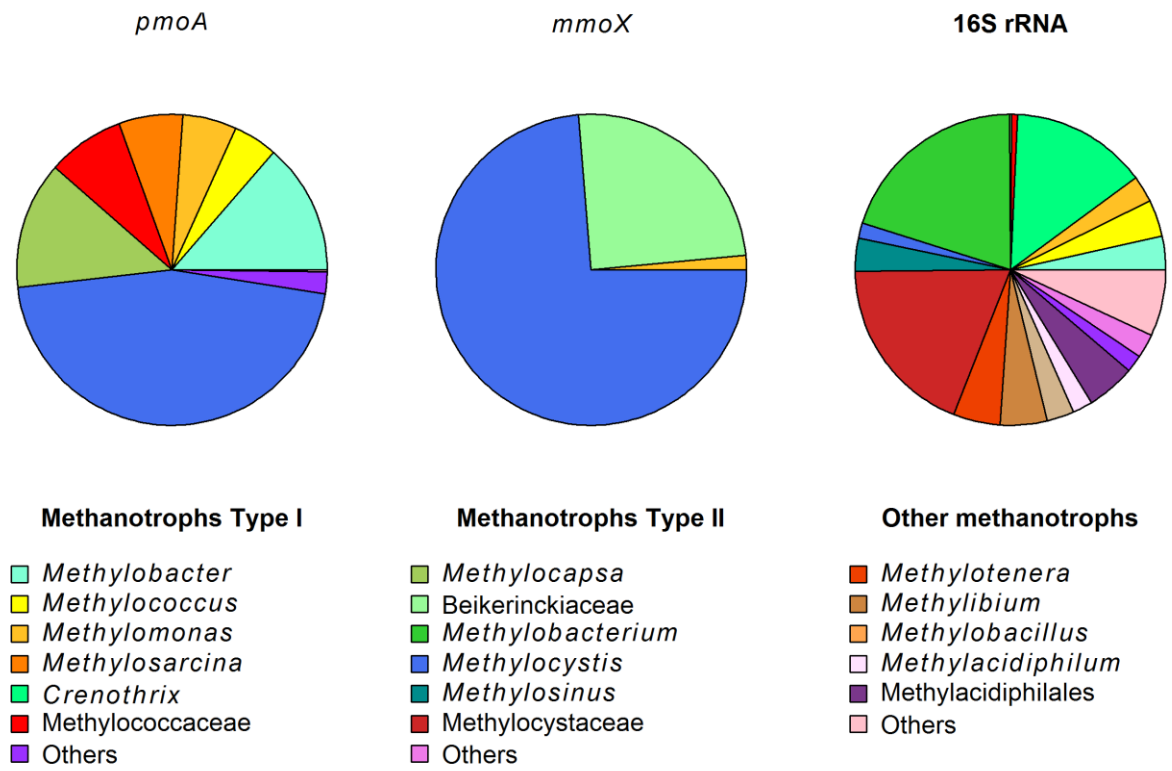
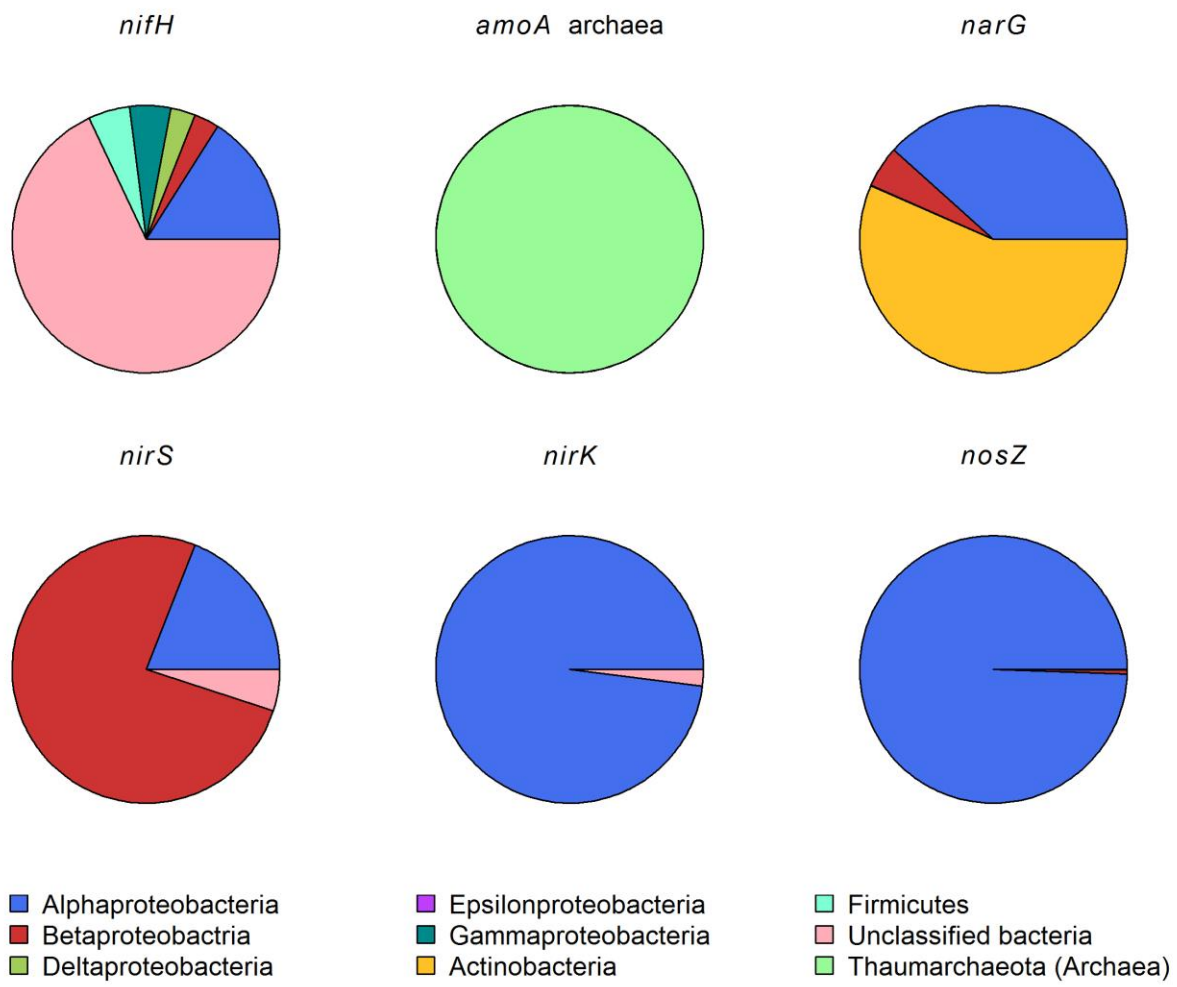


Fig. 3. Overall methanotroph composition in arctic soils. The relative abundances of genera (otherwise order or family when sequences not assigned to a genus) composition of methanotrophs is shown, based on sequences from published studies in the Arctic and investigating *pmoA* genes (encoding the α-subunit of the methane monooxygenase), *mmoX* genes (encoding the active-site subunit of the soluble methane monooxygenase) and 16S rRNA genes (including only methanotroph related sequences). The *pmoA* gene diversity was based on 522 sequences from the active layer of peat soils [57,64,66,121] and tundra [63]. The *mmoX* gene diversity was based on 133 sequences from a single study investigating the active layer of peat soils [121]. The 16S rRNA gene diversity was based on 25,517 sequences originating from active layers of peat soils [34,56,59,62,66,67,121,122] but also tundra active layers [62]. Note that the diversity of methanogens based on the 16S rRNA gene is dominated by studies using next generation sequencing [34,56,59,62].



1286

1287 **Fig. 4.** Overall phylum and class (Proteobacteria) - level composition of nitrogen-cycling
1288 genes in arctic soils. The relative abundances of different phyla and/or classes of the N-
1289 functional genes is shown based on sequences from published studies in the Arctic. *nifH:*
1290 *nitrogen* fixation (133 sequences) [54,88,121], *amoA* archaea: ammonia oxidizing archaea
1291 (4,476 sequences) [90,91], *narG:* reduction of NO_3^- into NO_2^- (16,145 sequences) [94,95],
1292 *nirS*: reduction of NO_2^- into NO (4,145 sequences) [94,95], *nirK* reduction of NO_2^- into NO
1293 (34,018 sequences) [94,95], *nosZ*: reduction of N_2O into N_2 (17,233 sequences) [84,85].

1294