A database of glacier microbiomes for the Three Poles

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Abstract. Glaciers cover 10% of Earth's land area and are a pool of carbon and nitrogen for downstream ecosystems. Microbes, including bacteria, fungi, algae, and other microeukaryotes, are the primary inhabitants of glacier ecosystems and are key drivers of carbon and nitrogen transformation. Here, we present a dataset on supraglacial bacterial and archaeal (referred to as prokaryotic hereafter) communities across the Antarctic, Arctic, Tibetan Plateau, and other alpine regions. The dataset comprises 2,039 amplicon sequencing data, 999 cultured bacterial genomes, and 208 metagenomes, covering ice, snow, and cryoconite habitats. The dataset contains 64,510 amplicon sequencing phylotypes, with a higher prokaryotic diversity in the Tibetan glaciers than in the Antarctic and Arctic glaciers, which were respectively enriched with Gammaproteobacteria, Bacteroidota, and Alphaproteobacteria. The dataset also contains 62,595,715 unique genes and 4,501 prokaryotic genomes, a 35.5% expansion from previous publications. Genes were annotated for those associated with carbohydrate-active enzymes, nitrogen cycling, methane cycling, antimicrobial resistance, and microbial virulence, revealing the dynamic microbial functions in glacial habitats. This comprehensive dataset provides standardized prokaryotic diversity, taxonomy, community structure, and genetic functions of glacial microbiomes. The data can be leveraged to elucidate ecological principles governing the distribution of microorganisms, to gain insights into the key functional genes for supraglacial microbiomes, to build mechanistic models, and to identify any potential biohazards for policymakers to make informed decisions regarding climate change. The dataset is available at the Global Glacier Genome and Gene Database (https://nmdc.cn/4gdb/).

1 Introduction

Glaciers cover 10% of Earth's land area (Cauvy-Fraunié and Dangles, 2019) and are mainly distributed in the Antarctic, Arctic, and Tibetan Plateau (the Three Poles) (Qiu et al., 2008). Glaciers store approximately three-quarters of Earth's freshwater (Boetius et al., 2015) and are also a pool of carbon and nitrogen. It has been estimated that six Pg of carbon are stored in global glaciers. These carbon may be released into downstream ecosystems with glacier runoff (Hood et al., 2015), influencing key elemental cycling in downstream ecosystems. Before carbons and nitrogen are released, they undergo extensive biological transformation (Guo et al., 2022), primarily microbial-driven. Microbes, including bacteria, fungi, algae, and other microeukaryotes are the main inhabitants of glacier ecosystems (Cauvy-Fraunié and Dangles, 2019). These microorganisms employ strategies to survive the glacial conditions, such as strong UV radiation, low temperature, and low carbon and nitrogen nutrients (Ciccazzo et al., 2016). As microorganisms are the key driver of carbon and nitrogen transformation in glacier ecosystems, knowledge of their biogeography and functions can greatly enhance our understanding of the biogeochemical cycling in glacial ecosystems and aid in predicting the impact of climate change.

The glacier as a habitat is not homogeneous and is divided into supraglacial, englacial, and subglacial ecosystems. Compared with other glacier-related habitats, the microorganisms in supraglacial ecosystems are the most active, due to their exposure to external environment and ambient temperature. Supraglacial ecosystems can be further separated into snow, ice, and cryoconite holes (cylindrical depressions formed by the preferential melting of dark debris into the surface, typically comprising surface water and cryoconite at the bottom) (Cook et al., 2016), each of which has distinct microbial composition (Anesio and Laybourn-Parry, 2012). Algae and Cyanobacteria are the primary producers in supraglacial ecosystems, with other heterotrophic microorganisms participating in the transformation and degradation of endogenous and exogenous nutrients (Hotaling et al., 2017, Anesio et al., 2017). Active metabolism is reported in glacial ecosystems; for instance, cryoconite is a source of methane but a sink of carbon dioxide, with a rate of 4.60 μmol $m^{-2}d^{-1}$ and −1.77 μmol $m^{-2}d^{-1}$, respectively (Zhang et al., 2021). Furthermore, organisms with photosynthesis, nitrification, and denitrification functions are also widespread in glacier cryoconite (Cameron et al., 2012; Stibal et al., 2020).

It was estimated that the mean microbial abundance in glacier surface meltwater is 10^4 cells mL^{-1} (Stevens et al., 2022), this quantity may further increase with the enhanced climate warming (Segawa et al., 2005). Some of these naturally occurring microorganisms are known as emerging contaminants, which are not commonly monitored in the environment but have the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects (Taheran et al., 2018). A previous study cultivated hemolytic bacteria from Spitsbergen glacier meltwater with potential pathogenicity (Mogrovejo-Arias et al., 2020). Other emerging contaminants in glaciers such as antibiotic resistance genes and microbial virulence factors have also received increased attention (Mao et al., 2023).

Here, we present a glacier dataset on glacial prokaryotes (bacteria and archaea) across the globe. This dataset includes amplicon sequencing data from 2039 samples, 999 cultured bacterial genomes, as well as shotgun metagenomic sequencing from 224 samples (**Fig. 1**). From an ecological perspective, this dataset with standardized microbial diversity, taxonomy, and community structure can improve understanding of the ecological principles governing the distribution of microorganisms across glaciers, as well as their partitioning across the various habitats in the supraglacial ecosystem. From a geochemical cycling perspective, the database can provide insights into the key functional genes for supraglacial microbiomes, which can be used to better comprehend carbon and nitrogen cycling and allow the building of a model to anticipate glacial carbon and nitrogen dynamics in the future. The dataset archives glacial-specific microorganisms and unique genes in digital form, thus representing an invaluable resource for bioprospecting. Additionally, the dataset can be employed to identify any potential biohazards (pathogens and emerging contaminants) of glaciers and evaluate the impact of glacier melting on downstream ecosystems from a biosafety perspective, thereby assisting policymakers in making informed decisions regarding climate change.

2 Materials and methods

2.1 Data acquisition

Amplicon data: Based on the keywords of "glacier" OR "snow" OR "ice" OR "cryoconite" with sample type being DNA and instrument of Illumina, we retrieved 225,378 SRA initially. Then the results were filtered manually to remove non-supra-glacier habitats (such as glacier forefield, subglacial sediment, proglacial lakes, ice cave), metagenome data, and primers that do not amplify the V4 region of the 16S rRNA gene (i.e., those amply V3V4, V4, and V4V5 were retained) (**Table S1** and **S2**).

Metagenome data: All articles containing the keyword "glacier metagenome" were retrieved using the Web of Science (searched on the 1st of December 2022). Only studies with sequenced ice, snow, or cryoconite samples with raw sequence data uploaded on the NCBI Short Read Archive were kept. Additionally, a few metagenome data without published articles were added from IMG/M database and NMDC database based on keyword search by terms "ice", "snow" and "cryoconite". In addition to metagenomes from the Antarctic, Arctic, and Tibetan Plateau, metagenomes from the Andes and Alps were also downloaded. (**Table S3**).

Cultivated bacterial genome data: 883 isolate genome data of Tibetan Plateau glaciers were obtained from the TG2G dataset (Liu et al., 2022) and other 116 genomes of bacterial isolates from glaciers beyond the Tibetan Plateau were downloaded from the NCBI Genome database based on keyword search by terms "Antarctic", and "Arctic". After careful manual curation, only samples from ice, snow, and cryoconite habitats were kept (**Table S4**).

2.2 Amplicon sequencing data processing

Sequencing data were processed using the USEARCH v11 pipeline (Edgar, 2010) on a sequencing project basis. For each NCBI sequencing project, the reads associated with the project were first merged and quality screened with a max expected error threshold of 0.5, while single-end reads were directly quality screened using the same threshold. The quality-filtered reads from each bioproject were aligned against the SILVA reference alignment (release 128) to ensure that the V4 hypervariable region is covered, using the align.seqs command in Mothur. After removing any sequences that do not cover the

V4 region (using screen.seqs in Mothur), the remaining sequences were dereplicated. After this pre-processing was completed for all bioprojects, the dereplicated sequences of different bioproject were combined, and dereplicated again. Then, these further dereplicated sequences were clustered with 97% identity and chimeric sequences were identified and removed using the cluster_otus command in USEARCH. The representative sequences were used as the references for OTU table construction. Additionally, the phylotype representative sequences were taxonomically classified using the Bayesian classifier against the Silva database (release 132) (Quast et al., 2012). Then mitochondria, chloroplast, and eukaryotic sequences were removed from the OTU table. After removing samples with less than 5000 reads, the final OTU table comprises 2,039 samples and 64,510 OTUs. The sequencing depth (number of reads) ranges from 5036 to 1492659 per sample. We retained two datasets, one without rarefaction (**Table S5**) and another were subsampled to 5036 reads (**Table S6**).

We calculated the Shannon diversity, richness (number of phylotypes), evenness, and Good's Coverage indices for both original and subsampled data using R. The alpha diversity indices (richness, evenness, and Shannon diversity) and the relative abundance of dominant taxonomy lineages were compared using Kruskal-Wallis one-way ANOVA by region (Antarctic, Arctic, Tibetan Plateau, and other alpine regions) and habitats (snow, ice, and cryoconite), multiple testing was performed based on the Dunn's post-hoc test using FSA package in the R environment (Ogle et al., 2022). The community structure variations were visualized using an NMDS ordination plot based on the Hellinger-transformed Bray-Curtis distance matrix. Permutational analysis of variance (PERMANOVA) was used to test the significance of community differences in samples by region and habitat (Anderson, 2017) using the "vegan" package in R with 999 permutations. PERMDISP analysis was performed using the betadisper command in the vegan package. Core phylotypes were defined as occurring in more than 55% of the samples with average relative abundance > 0.1% in each habitat-region pair (Delgado-Baquerizo et al., 2018). If a phylotype was identified as a core phylotype for all habitats of a region, then it was designated as the core phylotype for the region. This classification was modified from, so that the dominant phylotype designation is less affected by the unbalanced samples for each habit-region pair.2.3 Metagenome data processing

Metagenome data processing has been described previously (Liu et al., 2022). Briefly, it includes raw data quality filtering, assembly, open reading frames prediction, and genome binning. Gene open reading frames (ORFs) for the metagenomic assemblies were predicted using Prodigal (Hyatt et al., 2010), and dereplicated by clustering at 80% aligned region with 95% nucleotide identity using MMseqs2 (Steinegger and Söding, 2017) with parameters: easy-linclust -e 0.001, --min-seq-id 0.95, -c 0.80.

Metagenomic assemblies were binned using MetaBAT 2 (v2.12.1) (Kang et al., 2019), MaxBin 2 (v2.2.7) (Wu et al., 2016), and VAMB (v2.0.1) (Nissen et al., 2021) separately. The resulting bins (or MAGs) were then refined using RefineM (v0.0.20) (Parks et al., 2017) by removing contigs with divergent GC content, coverage, or tetranucleotide signatures. Then only MAGs meeting the medium and higher quality of MIMAG (Bowers et al., 2017a) were retained (completeness > 50%, contamination <10%). The obtained MAGs were combined with the isolate genomes, and these genomes were dereplicated using the thresholds of 10% aligned fraction and a genome-wide average nucleotide identity (ANI) threshold of 95%. They were then taxonomically annotated using the Genome Taxonomy Database Toolkit (GTDB-Tk, v2.4) (Chaumeil et al., 2019) against the GTDB release R220.

2.4 Gene function annotation

The functions of the dereplicated genes were also annotated using eggNOG-mapper (Huerta-Cepas et al., 2017) and the eggNOG Orthologous Groups (OGs) database (v5.0) (Huerta-Cepas et al., 2019). This includes the KEGG functional orthologs (Kanehisa et al., 2017), the carbohydrate-active enzymes database (CAZy) (Levasseur et al., 2013), and the COG categories (Tatusov et al., 2003). Antibiotic resistance genes (ARGs) were annotated against the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017) and Resistance Gene Identifier (RGI v3.1.4) (Alcock et al., 2020) with the loose model (--include_loose). Virulence factors were annotated by aligning gene sequences against the Virulence Factors Database (VFDB 2019) (Liu et al., 2019) with DIAMOND blastp (Buchfink et al., 2021) (e-value threshold of $1e^{-5}$).

3 Results

3.1 Amplicon-based dataset

A total of 2,039 glacier-related samples were retained after quality filtering, comprising 1077 from cryoconite sediment, 601 from snow or ice, 216 from glacial melting water (including cryoconite hole meltwater and supraglacial melting water), 79 from ice core, 34 from snow during algal blooming (Algal material), and 32 from subglacial basal ice (**Table 1**). Spatially, 29% of all samples (n = 574) were from the Antarctic, 28% (n = 574) from Arctic glaciers, 16% (n=335) from the Tibetan Plateau (n = 335), and 27% from other alpine regions (n = 545, the Alps, Keniya, Japan, and Montana Glacier National Park).

Table 1 Number of samples by habitat and region.

	Snow	Ice	Cryoconite	Supraglacial meltwater	Ice core	Algal material	Basal ice
Antarctic	119	58	335	59	0	1	13
Arctic	107	74	286	59	6	23	19
Tibetan Plateau	67	8	172	30	58	0	0
Other non-polar glaciers	89	79	284	68	15	10	0

3.1.1 Prokaryotic diversity

The amplicon sequencing dataset comprised 64,510 phylotypes. Due to the large variation in sequencing depth among samples, here we only presented patterns that are consistently observed in the original and subsampled OTU tables (**Tables 2**, **3**, and **Table S7**). Across all habitats, the number of OTU observed (prokaryotic richness) was significantly lower in other non-polar glaciers than those in the three poles (Antarctic, Arctic, and Tibetan Plateau). This pattern is also significantly observed for the alpha diversity indices including the Chao1, ACE, and Gini Simpson, but not in the Shannon diversity. Specifically, the prokaryotic Shannon diversity of the Arctic glaciers was not significantly different from non-polar glaciers. Across habitats (**Table 3**), supraglacial meltwater had the highest prokaryotic diversity compared with other habitats (except the ice core). These alpha diversity indices typically follow orders of supraglacial meltwater > snow > ice core > ice > cryoconite > algae-influenced snow > basal ice. This may reflect the strength of environmental filtering among the habitats.

We further compared the alpha diversity indices of the same habitats across different regions (**Table S8**). Due to the bias in the number of samples across habitats, here we only compared the cryoconite, snow, ice, and supraglacial water. For cryoconites, their prokaryotic diversity (Shannon diversity and Gini Simpson indices) was the highest in the Antarctic, which was followed by the Arctic, Tibetan Plateau, and other non-polar glaciers. For snow, its prokaryotic diversity (Richness, Chao1, and ACE indices) was the highest in Tibetan glaciers, followed by Antarctic, Arctic, and other non-polar glaciers. For ice, the diversity indices were not significantly different across all regions, with only significantly higher values of diversity being observed in the Antarctic than those in the Arctic and Tibetan Plateau. For supraglacial meltwater, other non-polar glaciers demonstrated the highest diversity (Shannon diversity and Gini Simpson diversity indices, all $P < 0.05$), while the Antarctic had the lowest values. In summary, the biogeographic pattern for prokaryotic diversity is the same habitat differed among regions.

Table 2 Diversity metrices of the bacterial community in glacier microbiomes of global glaciers.

Region	Antarctic	Arctic	Non-polar glacier	Tibetan Plateau
Original dataset				
Species observed	1025.1±722.5^{b}	981.4±784.4^{b}	868±676.3^{c}	1185±825.9^{a}
Chao1	1400±912.5^{b}	1492.4±1308.7^{b}	1223.2±941.8^{c}	1768.2±1279.3^{a}
ACE	1422.5±948.1^{b}	1526.9±1340.8^{ab}	1232.3±959.5^{c}	1767.5±1295.4^{a}
Shannon diversity	4.3±1.03^{a}	3.97±1.05^{b}	4.17±1.19^{b}	4.2±1.3^{a}
Subsampled dataset				
Region	Antarctic	Arctic	Non-polar glacier	Tibetan Plateau
Species observed	475.7±232^{a}	467.9±275.9^{a}	468.1±384.2^{b}	507.1±317.3^{a}
Chao1	809.6±458.3^{a}	847.9±575.3^{a}	777.5±679.4^{b}	884.4±622^{a}
ACE	825.7±482.6^{b}	881.6±619.9^{ab}	796.6±715.3^{c}	903.4±651.8^{a}
Shannon diversity	4.23±1^{a}	3.9±1.02^{b}	4.1±1.16^{b}	4.11±1.25^{a}

Statistical test is based on Kruskal-Wallis one-way ANOVA, multiple testing is performed based on the Dunn's post-hoc test. Different letters indicate significant differences at $P = 0.05$.

Table 3 Diversity metrices of the bacterial community in glacier microbiomes of different habitats.

Habitats	Algal material	Basal ice	Cryoconite	Supraglacial Ice	Ice core	Snow	Supraglacial meltwater
Original dataset							
Species observed	596.3±451.7^{ab}	489.6±306.3^{a}	904.4±532.6^{c}	929.2±695.9^{bc}	999.9±438.3^{cd}	1178.8±1121.6^{c}	1344.3±910.3^{d}
Chao1	873.5±648.2^{ab}	646.8±379.1^{a}	1316.9±786.1^{c}	1285.8±962.3^{bc}	1452.8±618.7^{cd}	1726.4±1736.5^{bc}	1898.4±1307.7^{d}
ACE	905.1±682.1^{ab}	641.8±388.3^{a}	1335.5±806.4^{c}	1296±979^{bc}	1430.8±599^{cd}	1760.1±1777.7^{bc}	1914.2±1339.1^{d}
Shannon diversity	3.18±1.38^{a}	3.4±0.99^{a}	4.21±0.84^{b}	3.67±1.09^{a}	4.6±0.8^{c}	3.8±1.5^{a}	5±1.2^{c}
Subsampled dataset							
Species observed	317.5±293^{ab}	239.2±129.5^{a}	464.8±223^{c}	356.1±205.1^{ab}	521.7±207.9^{cd}	471.9±386.5^{bc}	710.1±458.2^{d}
Chao1	568.6±544.6^{abc}	357.4±201.4^{a}	798.9±424.1^{d}	613.7±405.5^{b}	799.5±325.4^{de}	883.2±807.7^{cd}	1176.4±836.6^{e}
ACE	588.9±581.2^{abc}	353.4±207.7^{a}	818.5±442.6^{d}	626.1±419.2^{b}	795.6±339.9^{de}	921.9±868.7^{cd}	1207.6±888.5^{e}
Shannon diversity	3.14±1.35^{a}	3.37±0.99^{a}	4.14±0.82^{b}	3.6±1.06^{a}	4.5±0.8^{c}	3.7±1.4^{a}	4.9±1.1^{c}

Statistical test is based on Kruskal-Wallis one-way ANOVA, multiple testing is performed based on the Dunn's post-hoc test. Different letters indicate significant differences at $P = 0.05$.

3.1.2 Prokaryotic taxonomy composition in the glaciers of the Three Poles

We identified 53 bacterial and archaeal phyla across the dataset. The glacier microbiomes had similar taxonomy composition, dominated by Proteobacteria (averaged 37.8%), Cyanobacteria (22.2%), Bacteroidetes (20.3%), and Actinobacteria (9.2%) (**Fig. 2** and **Tables S9**). At the class level (**Tables S10**), the microbiome was dominated by Gammaproteobacteria (22.7%, Proteobacteria), Oxyphotobacteria (22.1%, Cyanobacteria), Bacteroidia (22.2%, Bacteroidetes), Alphaproteobacteria (13%, Proteobacteria), and Actinobacteria (class) (8.1%, Actinobacteria).

3.1.3 Bacteria community structure in the glaciers of the Three Poles

NMDS ordination plot revealed distinct prokaryotic communities among habitats (**Fig. 3a**). PERMANOVA analysis showed significantly different prokaryotic community structures among Antarctic, Arctic, Tibetan Plateau, and other non-polar glaciers ($P < 0.001$). The influence of location exhibited a higher R^2-value (0.10535) than the influence of habitat ($R^2 = 0.06299$), suggesting that spatial location may have a greater influence in shaping glacier microbiomes. PERMDISP analysis showed that snow samples exhibited a significantly (at the threshold of $P = 0.05$) higher dispersal from centroid (67.4%) compared to cryoconite (65.1%). Comparatively, those of ice core and algae were lower (57.9% and 58.9%, respectively), possibly due to the low sample numbers.

We identified the top three most abundant phylotypes for each habitat-region pair (**Fig. 3b** and **Table S11**). These abundant phylotypes were mainly affiliated with Proteobacteria, Cyanobacteria, and Bacteroidetes. The distribution of these abundant phylotypes clustered predominantly by geographical location, reflecting strong spatial effects. We then attempted to identify ubiquitous phylotypes for each region-habitat pair, defined as those present in more than 55% of samples with a relative abundance greater than 0.1%. However, we found no phylotypes that were common across any regions or habitats (**Table S12**). The number of ubiquitous phylotypes varied, ranging from nine in Arctic ice to 129 in Tibetan-supraglacial meltwater. Notably, we did not identify any ubiquitous phylotypes in Arctic-snow or other non-polar-snow. Most of the ubiquitous phylotypes were associated with Gammaproteobacteria (29% of the total), followed by Bacteroidetes (19%), Alphaproteobacteria (16%), Cyanobacteria (13%), and Actinobacteria (11%). This distribution may indicate their ability to disperse and adapt to different environmental conditions.

3.2 Metagenome- and genome-dataset

We acquired 226 glacier metagenome data (**Table S2**) and 999 bacterial genomes from glacial environments (**Table S3**). After quality filtering and assembly, 63,294,073 unique Open Reading Frames (ORFs) were obtained. Of these dereplicated ORFs, 47.8% (29,947,128) were functionally annotated using eggNOG.

3.2.1 Overall features glacier metagenome-assembled genomes

After binning, the dataset generated 3,502 metagenome-assembled genomes of medium quality (Genome completion ≥ 50%, contamination < 10%) and higher (Bowers et al., 2017b). After combining the genomes of cultivated glacier bacteria (999), this expanded the total genome number to 4,501 from the previously published 3,322 (Liu et al., 2022) (**Table S13**), a 35.5% increase. The median genome size was 3.46 Mb ranging from 0.42 Mb to 10.49 Mb; the GC% was 60%, ranging from 30% to 76%. The MAGs were taxonomically affiliated with 33 phyla, 79 classes, 154 orders, 271 families, and 549 genera (**Fig. 4**). Additionally, 3470 (77.1% of all MAGs) were unable to be classified at the species level, reflecting substantial genomic novelty in the glacier microbiome. These genomes were dereplicated into 1400 genomic OTUs (gOTUs), which are typically considered species.

3.2.2 Key functional genes

Carbohydrate-active enzymes: The dataset contains 1,082,125 genes encoding carbohydrate-active enzymes (CAZY, **Fig. 5a**), i.e., those enzymes involved in the metabolism of glycoconjugates, oligosaccharides, and polysaccharides (Zerillo et al., 2013). Genes associated with carbohydrate hydrolysis and biosynthesis were the most abundant, accounting for 45.2% and 44.4%, respectively. In contrast, those genes associated with non-hydrolytic cleavage of glycosidic bonds, hydrolysis of carbohydrate esters, and assisting in degrading biomass substrates were relatively scarce, accounting for 0.8%, 3.1%, and 0.2% of the predicted CAZY, respectively. This indicates that the glacier microbiome is competent in a diverse range of carbon transformation processes, mediating the delivery of carbon to downstream ecosystems.

Nitrogen cycling: The dataset contained 138,421 unique genes associated with nitrogen cycling, most of which (99.3%) were associated with nitrate reduction and/or denitrification pathways (**Fig. 5b**). These genes included the *nirB* gene responsible for the nitrite reduction to ammonia in the assimilatory nitrate reduction pathway, the *narB* and *nirA* genes responsible for sequential nitrate reduction to ammonia in dissimilatory nitrate reduction pathways, and the *nirK* gene responsible for nitrite reduction to nitric oxide in denitrification pathway. This suggests that microbial-driven nitrate reduction is widespread in glacial habitats for both nitrogen assimilation and energy supply, highlighting their potential roles in NO_x formation. In comparison, genes involved in nitrogen fixation (*nifH*) and nitrification (*hao*) were relatively rare, with only 678 (0.49% of the nitrogen cycling-related genes) and 240 (0.17%) unique genes identified, respectively. This suggests that microorganisms capable of these high-energy demand processes only account for a small fraction of the glacial microbiome, which is consistent with the low nitrogen fixation rates reported in glacier-related habitats (Telling et al., 2011).

Methane cycling: The dataset contained 154 methane cycling-related genes. Of these, 93 were the soluble form of methane oxidase (*mmoX*), accounting for 61% of the total methane-cycling genes identified (**Fig. 5c**). More *mmoX* genes were identified from cryoconite (34.4% of the total methane-cycling genes identified) than from ice (20.8%) or snow (5.2%). Conversely, genes associated with the particulate form of methane oxidation (*pmoA*, *pmoB*, and *pmoC*) were more frequently identified from ice (21.4%) than from cryoconite (5.8%) and snow (3.2%). The different partition of soluble- and particulate-form methane oxidizers likely reflects their distinct environmental selection process. Only six unique methanogenesis-related genes (*mcrA*) were identified, almost exclusively in cryoconite metagenomes. This is consistent with the cryoconite as a methane source in the literature (Zhang et al., 2021).

Antimicrobial resistance genes: Using thresholds of 80% identity and 80% sequence coverage, we identified 1166 ORFs that exhibited high sequence similarity to 224 antibiotic resistance genes (ARG). Of these identified ARGs, *MexF*, beta-lactamase, and *mexK* were the most abundant, accounting for 8.1%, 4.2%, and 3.7% of the ARGs identified, respectively (**Table S14**). The predominant antibiotic resistance mechanisms were antibiotic efflux and antibiotic inactivation, accounting for 44% and 41% of the total ARGs identified, respectively. These ARGs were predicted to confer resistance against 30 different antibiotics, with penam, tetracycline, and macrolide being the most commonly encountered resistant targets. Additionally, 54% of the identified ARGs provided multiple drug resistance, with the *OprM*, *CpxR*, and *tolC* genes conferring resistance to 16, 15, and 15 types of antibiotics, respectively. ARGs were identified in 566 genomes (13% of total genomes obtained). This low proportion of ARG-bearing genomes suggests that the glacier habitats are only weakly affected by antibiotic contamination. Of the genomes containing ARGs, 48.6% and 34.1% were affiliated with Proteobacteria and Firmicutes, respectively (**Fig. 5d**). However, the resistance mechanisms exhibited by these two bacterial phyla were markedly distinct, with antibiotic efflux (*MexF*) and antibiotic target alternation (*vanZf*)/inactivation (*FosB*) being the most common mechanisms for Proteobacteria and Firmicutes, respectively. Most of the genomes (n=224) carried only a single ARG, while seven genomes possessed more than ten ARG genes, with *Pseudomonas aeruginosa* genomes hosting up to 48 ARGs.

Virulence factors: Using thresholds of 80% identity and 80% coverage, the dataset contains 66,822 virulence factors, accounting for 0.11% of the total ORFs identified (**Table S15**). Virulence factors were predominately associated with adherence, motility, and immune modulation functions, while those associated with toxin production accounted for only 0.48% (**Fig. 5e**). We did not detect any toxin genes from the genomes obtained using the same thresholds, with only those associated regulation, effector delivery systems, and metabolic factors being identified from Proteobacteria, Actinobacteria, and Deinococcota genomes. Nevertheless, 878 potential toxin genes were identified from the genomes if the criteria were loosened, with sequence identified ranging from 20.1% to 67.8%, these genes may represent novel toxins without references in the dataset, or non-toxin genes homologues to known toxin genes. These candidate toxin genes were most abundantly identified in Gammaproteobacteria, followed by Bacteroides (15.9%) and Alphaproteobacteria (10.0%).

4 Data availability

The data introduced here is the first step in archiving global glacier microbial data. For this purpose, the data is deposited into the Global Glacier Genome and Gene Database (4GDB, https://nmdc.cn/4gdb/) and the National Tibetan Plateau Data Center (https://doi.org/10.11888/Cryos.tpdc.300830, Liu, et al., 2023), which provides a comprehensive solution for glacier microbial studies, featuring amplicon sequencing phylotype table, representative sequences, taxonomic annotations, metagenomic raw sequences, assembled contigs, annotated gene sequences, sequences of metagenome-assembled genomes, and the growth characteristics of cultivated microorganisms, into a user-friendly website. The 4GDB website is mainly structured into three sections, comprising amplicon sequencing, metagenome/genome sequences, and function prediction. The user-friendly web interface allows data filtering based on sample type, sample location, habitat type, gene type, and taxonomy, enabling seamless download of the filtered results. In conclusion, 4GDB (https://nmdc.cn/4gdb/downloadtemp) provides an open-access genome- and gene-orientated resource platform that is regularly updated to include newly published and in-house generated sequence data.

Author contributions

YL conceptualized the paper, SH, YL, TY, ZZ, YC, KL, PL, JL, and MJ analysed the data, TY, SG, QS, GF, LW, and JM developed the website, MJ and YL prepared the manuscript with contributions from all authors.

Competing interests

The contact author has declared that none of the authors has any competing interests.

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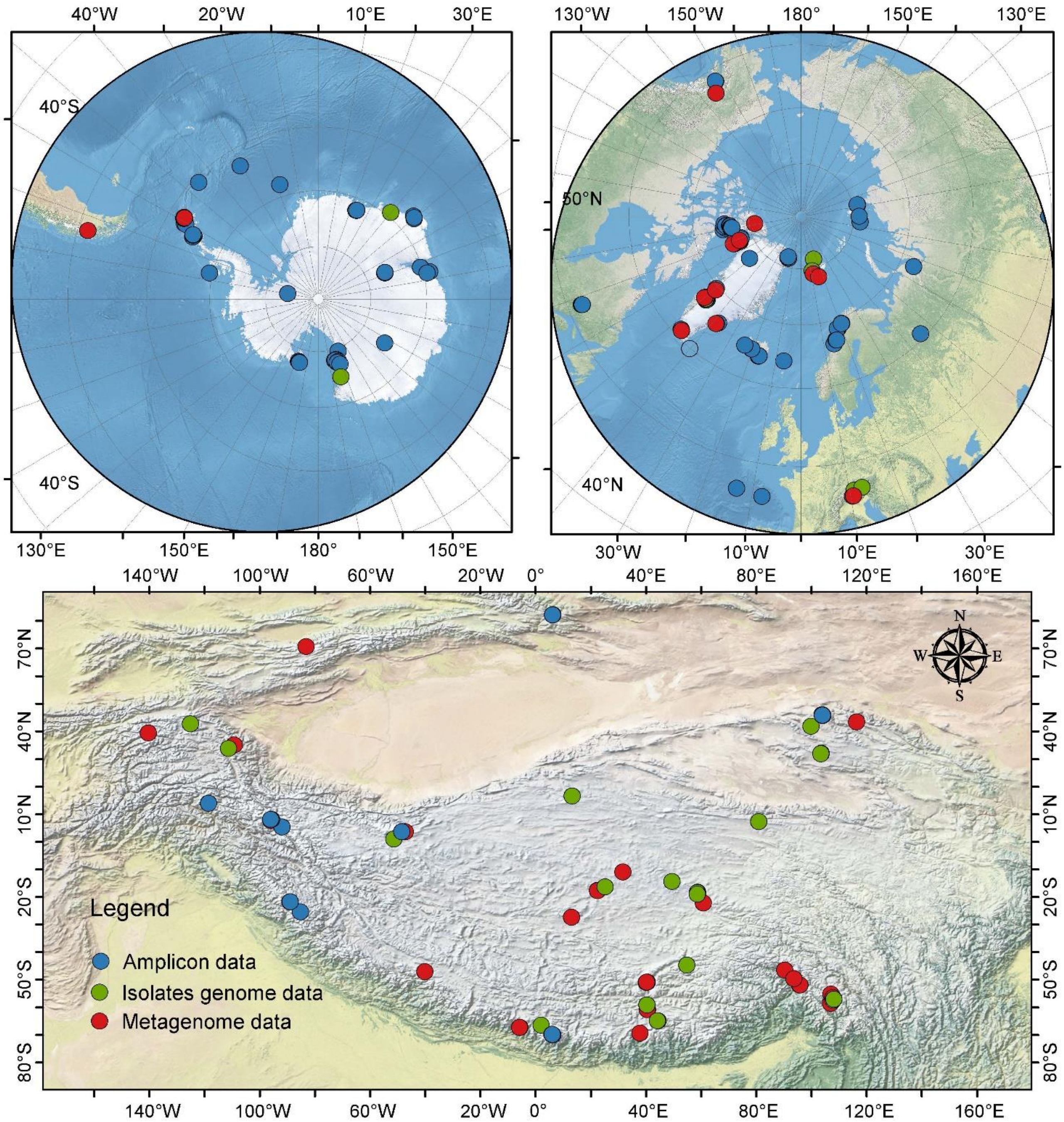

Fig. 1 The location of glacier samples across the Antarctica, Arctic, and Tibetan glaciers

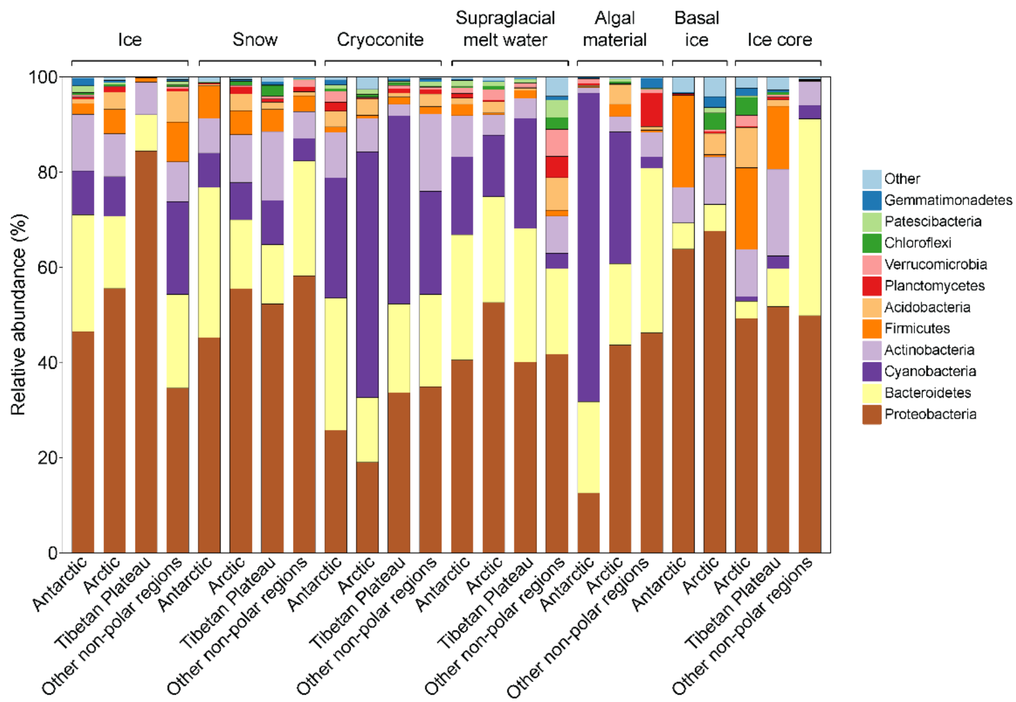

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Fig. 2 Taxonomy composition of supraglacial ecosystems in glaciers of the Antarctic, Arctic, Tibetan Plateau, and other alpine regions.
Taxonomy is inferred based on amplicon sequencing results.

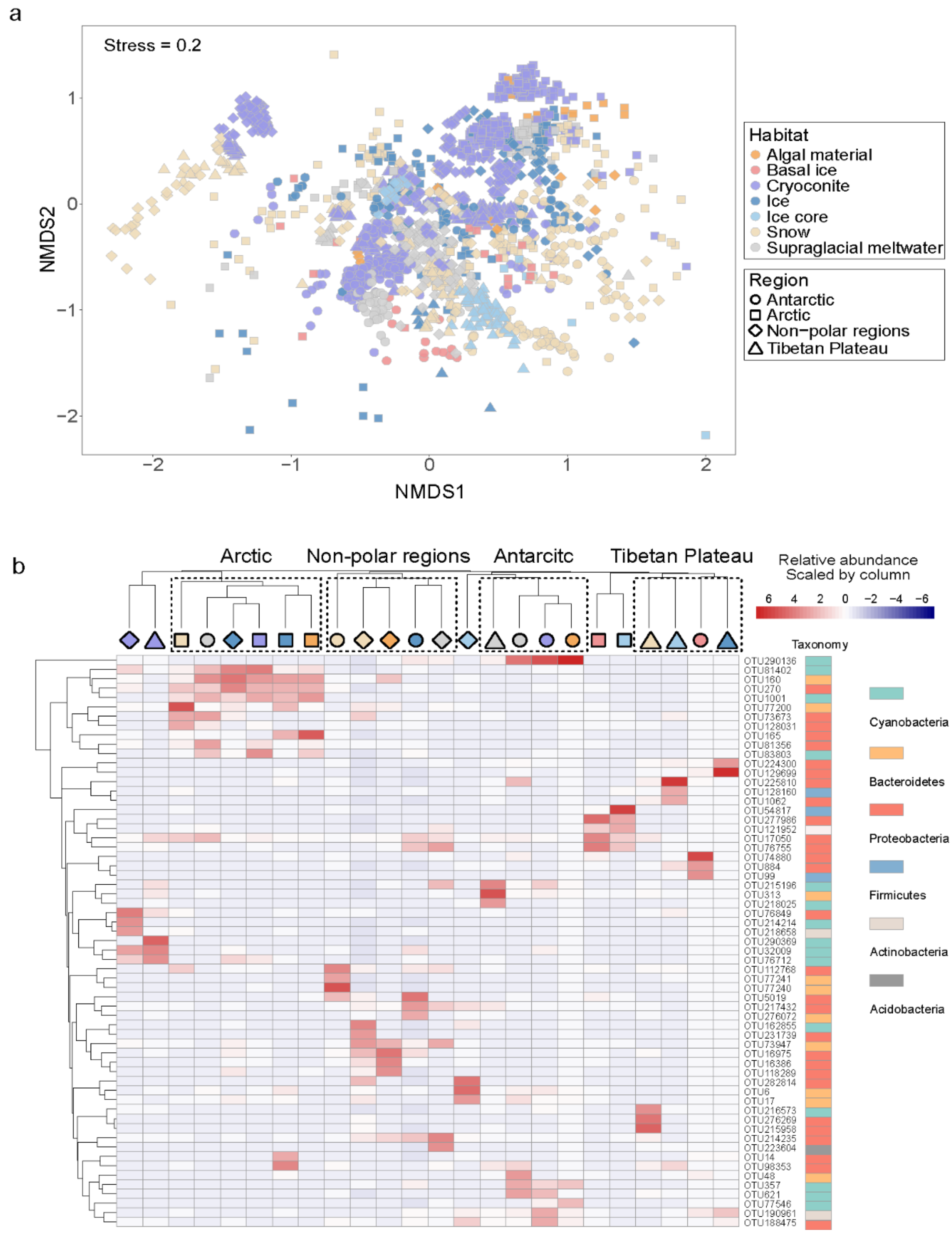


Fig. 3 The community structure of glacier microbiomes across the Antarctica, Arctic, and Tibetan Plateau.

(a): Microbial community structure differences visualized using the non-metric multidimensional scaling ordination plot; (b): The heatmap highlights the distribution pattern of dominant phylotypes for each habitat-region pair.

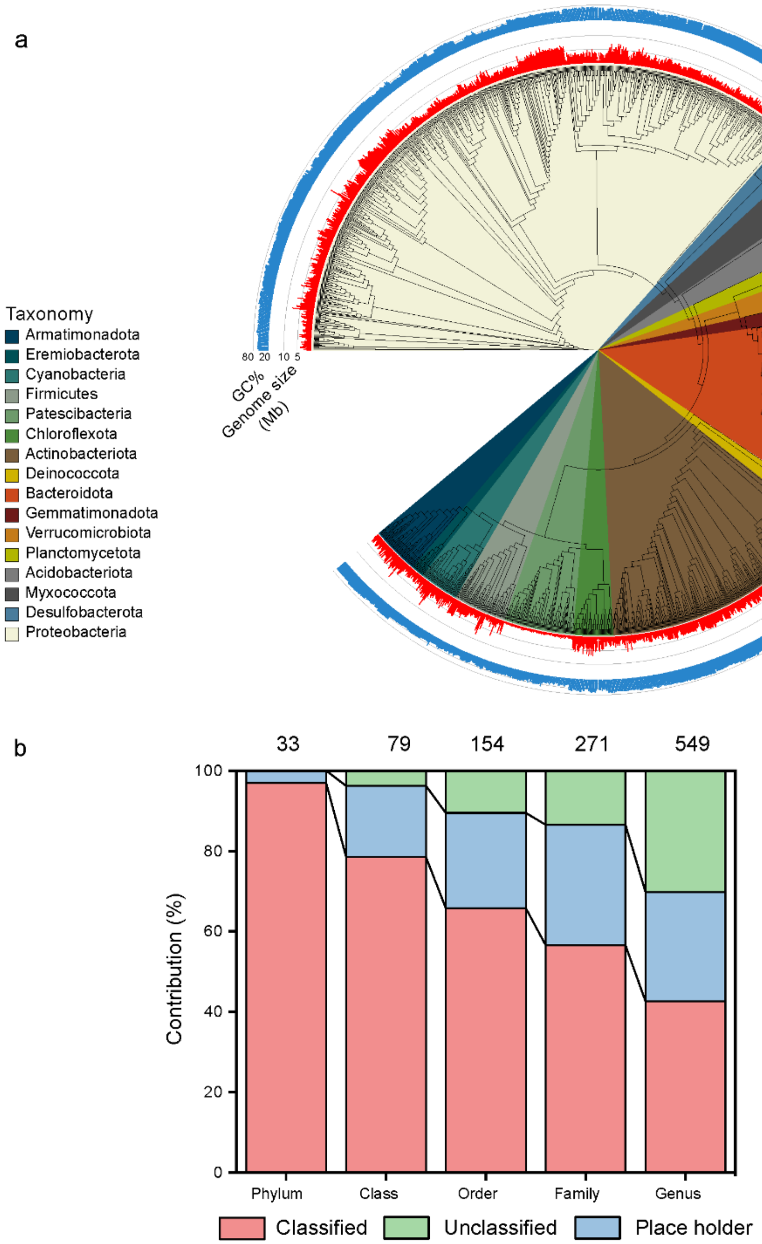
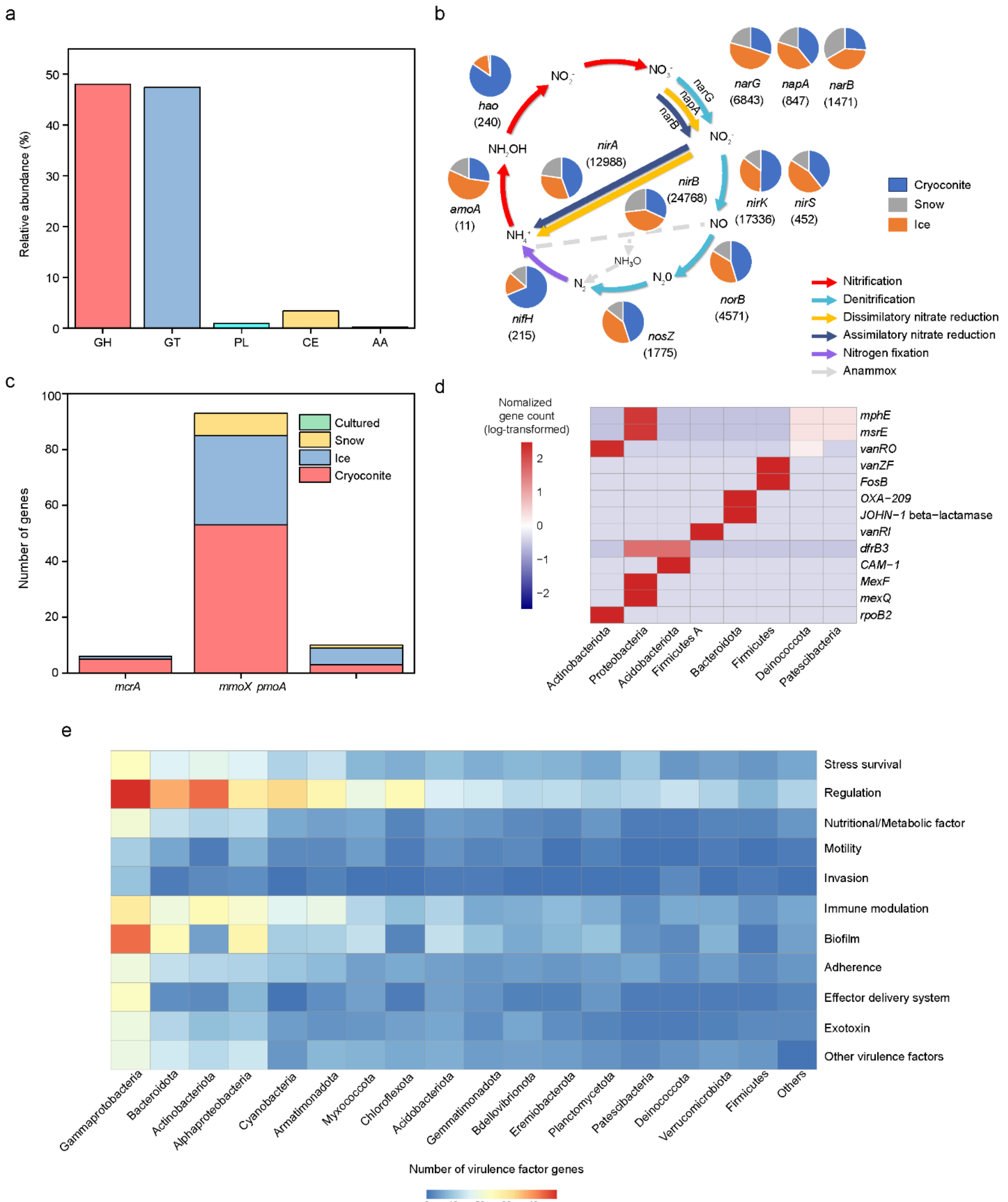


Fig. 4 Taxonomy classified of the obtained prokaryotic genomes

A: The phylogenetic tree contains the representative bacterial genomic OTUs with genome size and GC% being shown; b: The taxonomic classified of the obtained genomes. Classified refers to valid taxonomic classification, place holders are genomes that have been deposited in the GTDB R220 database, while unclassified are genomes that have not been deposited in the GTDB database.



a
Relative abundance (%)
0
10
20
30
40
50
GH
GT
PL
CE
AA
b
NO_2^-
NO_3^-
narG
napA
narB
NO_2^-
NH_2OH
hao
(240)
amoA
(11)
nirA
(12988)
nirB
(24768)
NH_4^+
NO
NH_3O
N_2O
N_2
nifH
(215)
nosZ
(1775)
norB
(4571)
narG
(6843)
napA
(847)
narB
(1471)
nirK
(17336)
nirS
(452)
Cryoconite
Snow
Ice
Nitrification
Denitrification
Dissimilatory nitrate reduction
Assimilatory nitrate reduction
Nitrogen fixation
Anammox
c
Number of genes
0
20
40
60
80
100
Cultured
Snow
Ice
Cryoconite
mcrA
mmoX pmoA
d
Nomalized gene count (log-transformed)
2
1
0
−1
−2
mphE
msrE
vanRO
vanZF
FosB
OXA−209
JOHN−1 beta−lactamase
vanRI
dfrB3
CAM−1
MexF
mexQ
rpoB2
Actinobacteriota
Proteobacteria
Acidobacteriota
Firmicutes A
Bacteroidota
Firmicutes
Deinococcota
Patescibacteria
e
Stress survival
Regulation
Nutritional/Metabolic factor
Motility
Invasion
Immune modulation
Biofilm
Adherence
Effector delivery system
Exotoxin
Other virulence factors
Gammaproteobacteria
Bacteroidota
Actinobacteriota
Alphaproteobacteria
Cyanobacteria
Armatimonadota
Myxococcota
Chloroflexota
Acidobacteriota
Gemmatimonadota
Bdellovibrionota
Eremiobacterota
Planctomycetota
Patescibacteria
Deinococcota
Verrucomicrobiota
Firmicutes
Others
Number of virulence factor genes
0
10
20
30
40

Fig. 5 Features of the functional genes across the glacier metagenomes from the Antarctica, Arctic, and Tibetan Plateau.

a: Genes associated with carbohydrate-active enzymes (GH: Glycoside hydrolases; GT: glycosyl transferases; PL: Polysaccharide lyases; CE: Carbohydrate esterases; AA: Auxiliary activities); b: nitrogen-cycling (The numbers indicate the number of genes identified, gene of the anaerobic ammonium oxidation pathway are not identified in the glacier metagenomes); c: methane cycling (*mcrA* gene is responsible for methanogenesis; *mmoX* gene is the soluble form methane oxidation gene, while *pmoA* is the particulate form methane oxidation gene, both of which are associated with methane oxidation); d: genes associated with antibiotic resistance; and e: the number of genes associated with virulence factors, the numbers have been square root-transformed.

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