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Supraglacial microbial communities from an isolated high mountain glacier in Türkiye

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Abstract. The biodiversity of glaciers has been studied in polar regions and mountain ranges, yet isolated high mountain peaks covered by glaciers in temperate regions - islands of ice - are virtually unknown. These systems are particularly at risk of rapid change and disappearance as temperatures increase, thus understanding their biological diversity and the potential for organisms to survive as the landscape alters is time-critical. In this study, we assessed the community composition of a glacier surface in Türkiye, located on the extinct volcano Ağri Dağı (Ararat Mountain). We collected samples of sediment (cryoconite) from water-filled depressions (cryoconite holes), then analysed samples through culturing, microscopy, metabarcoding (16S and 18S rDNA), metagenomics and metatranscriptomics. We cultured and identified 158 bacterial strains belonging to 11 genera (*Cryobacterium*, *Polaromonas*, *Flavobacterium*, *Arthrobacter*, *Massilia*, *Devosia*, *Paeniroseomonas*, *Deinococcus*, *Glaciihabitans*, *Knoellia*, and *Rugamonas*) and 41 species. Nine species of *Cryobacterium*, *Flavobacterium* and *Knoellia* did not grow above 10 °C, indicating particular vulnerability to climate warming. Amplicon-based community structure was dominated by Cyanobacteria, with the genus *Tychonema* particularly abundant, while cultured isolates represented only a small fraction of amplicon diversity but captured phenotypically diverse, cold adapted heterotrophs. Functional potential in metagenomes revealed that cyanobacteria dominated bacterial primary production, whereas other phyla specialized in nutrient scavenging, which was further apparent in metatranscriptomic analysis. Transcriptomic analysis demonstrated disproportionate dominance of Cyanobacterial transcripts across core metabolic pathways. Amplicon analysis of 18S rDNA and rRNA reads from metatranscriptomics showed that eukaryotes were active, with communities dominated by Ciliophora, while metazoan grazers identified in other cryoconite systems (such as tardigrades and rotifers) were not detected in either molecular data or microscopy, suggesting a simplified, protist-dominated food web. The combination of different microbiological and molecular approaches demonstrates that this rapidly changing glacier hosts cyanobacteria-dominated, bacterial–protist assemblages with absence of metazoan grazers, showing that isolated low latitude glaciers are a viable microbial habitat under threat.

1. Introduction

In high mountain environments, glaciers and ice caps support wider ecosystems by releasing water, sediments and dissolved solutes, including nutrients, during melt, but they are also ecosystems which sustain generalist and unique, psychrophilic organisms (Carey et al., 2017; Hawkings et al., 2025; Losapio et al., 2025). Biological activity on glacier surfaces peaks during summer, when meltwater provides the liquid phase necessary for microbial metabolism and growth. In recent years, investigation of the biodiversity of glaciers and adjacent systems increased has demonstrated that they host a variety of organisms, some of which are glacier specialists and are endangered due to glacier loss (Gobbi et al., 2026; Valle et al., 2021; Zawierucha et al., 2026). The most species-rich habitats on glacier surfaces are cryoconite holes. They serve as biogeochemical

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factories, reservoirs of organic matter and hotspots of glacial biodiversity (Anesio et al., 2017; Buda et al., 2020). Cryoconite holes are formed by the reduction of albedo by dark, fine mineral–organic material on the glacier surface, known as cryoconite. Often, cryoconite at the bottom of holes forms a granular-shaped bioaggregate consisting of mineral particles, organic matter, and organisms such as bacteria, algae, fungi, protists and invertebrates (Rozwalak et al., 2022; Wejnerowski et al., 2023). Invertebrates such as tardigrades and rotifers structure the microbial food web through grazing (Jaroměřská et al., 2020; Zawierucha et al., 2022). The presence or absence of metazoan grazers may influence bacterial community structure, yet food web studies on low- to mid-latitude glaciers are virtually absent (Crosta et al., 2025).

Cryoconite holes are characterized by stable summer temperatures around 0.2 °C, with the presence of grains of mineral substrate for biofilm formation and organic matter, both autochthonous and allochthonous (Buda et al., 2020; Shamurailatpam et al., 2023; Zawierucha et al., 2019), forming a stable and relatively hospitable niche in an otherwise extreme environment. Low temperatures decrease rate of decomposition of organic matter and preserve DNA; therefore, distinguishing what is resident and active from what is merely "wind-blown" in cryoconite holes is crucial for estimating the true glacial inhabitants (Chen et al., 2025; Pittino et al., 2023a; Poniecka et al., 2020). Differentiating between active and 'accidental' inhabitants requires a combination modern and classical microbiological approaches, including metatranscriptomics and culturing of organisms at a range of temperatures. Such studies are particularly important since, in recent years, glaciers and their biota have been recognized as endangered. Each year, glaciers lose hundreds of gigatons of ice, along with organisms that are yet to be classified and catalogued (Gobbi et al., 2026; Losapio et al., 2025; Simoncini et al., 2026). The most endangered glacial systems are those in low-latitude mountains; the best examples are glaciers on isolated mountain peaks, such as volcanoes (Baldasso et al., 2019; Sarıkaya and Tekeli, 2014; Zemp et al., 2026). Despite ongoing climate warming and consequently glacier melt, few studies have focused on microorganisms inhabiting the remaining isolated glaciers that persist at low latitude and high altitude, and even fewer have studied cryoconite communities (Kuja et al., 2023; Pittino et al., 2023b).

The bacterial community composition on glacier surfaces is shaped by an interplay between atmospheric and local (wind-blown) deposition and the core microbiome of glacier. Biogeographic studies have demonstrated some similarities with polar and other mountain glaciers, and that bacterial communities can change both seasonally and annually (Darcy et al., 2018; Pittino et al., 2018). The dominant phyla in cryoconite are Cyanobacteria, Proteobacteria, Bacteroidota, and Actinobacteriota; autotrophic, heterotrophic, chemolithoautotrophic, and both aerobic and anaerobic organisms may also be present (Ambrosini et al., 2017; Liu et al., 2017; Zarsky et al., 2013). Cyanobacteria, particularly filamentous oscillatorialean taxa, act as ecosystem engineers by producing extracellular polymeric substances (EPS) that bind mineral particles into granular bioaggregates, driving primary production and structuring the physical habitat of cryoconite holes (Wejnerowski et al., 2023). Psychrophilic microorganisms associated with the cryosphere had to develop numerous adaptations to survive the multiple stresses in the extreme environment. These include adaptation to UV (Paredes Contreras et al., 2025; Silva et al., 2019), low temperatures (Hassan et al., 2020; Singh et al., 2014a) and freeze-thaw cycles (Mumcu et al., 2023; Perez-Mon et al., 2020). Such adaptations suggest the presence of unique metabolites, which can be used in biotechnology applications (Alvarado-Cuevas et al., 2015; Gallardo et al., 2014). Therefore, cataloguing and genotyping the disappearing psychrophiles is crucial not only for conservation purposes but also applied studies.

Metatranscriptomics complement DNA-based surveys in cryoconite holes, where preserved extracellular DNA and allochthonous wind-blown cells can decouple community structure from metabolic activity. On Forni glacier (Italian Alps), RNA sequencing showed that only a fraction of the total DNA-characterized community was actively transcribing, with several taxa—such as anaerobic Chloroflexi and fermentative lineages - detected exclusively via RNA (Pittino et al., 2023a).

Here we focus on the microbial communities in cryoconite holes on the high-altitude glacier located on an isolated mountain peak in continental climate, in Türkiye. Such glaciers persist as isolated icy islands and are at risk from both local tourism and global warming. Previous work on Turkish glaciers has not applied integrated microbial approaches, and on Ağri Dağı (Ararat Mountain) only the bacterial diversity of snow has been investigated (Azzoni et al., 2018). Therefore we combine culturing,

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metagenomics and transcriptomics to understand: (i) whether the bacterial and eukaryotic community composition on an isolated temperate mountain peak reflects the global cryoconite taxa or shows distinct signatures; (ii) whether metatranscriptomics shows differences from the DNA-based community composition; (iii) which culturable heterotrophs are obligately psychrophilic and thus most vulnerable to habitat loss under continued warming, and (iv) show the basic structure of the cryoconite food web, based on the composition of eukaryotes and microscopic observation.

2. Materials and Methods

2.1. Study Site and Sampling

Ağrı Dağı (Mount Ararat), located in the easternmost part of Türkiye near the borders with Iran and Armenia, is the largest and highest volcanic complex on the Anatolian Plateau. The complex, with Greater Ağrı reaching 5,137 m a.s.l. and Lesser Ağrı 3,896 m a.s.l., is a dormant compound volcano and represents one of the most important glacierised areas in the region (Azzoni et al., 2017; Yılmaz et al., 1998). The lithology is dominated by calc-alkaline to mildly alkaline basaltic andesites and dacites (Yalcin, 2020). Present-day ice bodies cover an area of 7.749 ± 0.47 km² and include a small ice cap (4.892 ± 0.294 km²), six debris-covered glaciers (2.240 ± 0,13 km²) and four valley glaciers (0.617 ± 0.04 km²), flowing down the flanks of the volcano. Ongoing climate-driven retreat, where Ararat glaciers lost 47% of their area in 46 years (Kaya and Karataş, 2025), is spatially heterogeneous and controlled by topographic factors including elevation, aspect, and slope (Canpolat et al., 2026). Samples were collected on 30.08.2024 from Ağri Dağı glacier at 5000 m a.s.l [39°42'09"N, 44°17'37"E] on the ice cap near the summit (Fig. 1). Three cryoconite holes were chosen randomly within a 100 m radius, then sampled by chipping frozen cryoconite into sterile falcon tubes using a sterilised ice axe. Samples were transported to the base camp in temperature-controlled conditions (in ice) and fully frozen within 24h hours, prior to temperature-controlled transport to University of Warsaw, Poland.

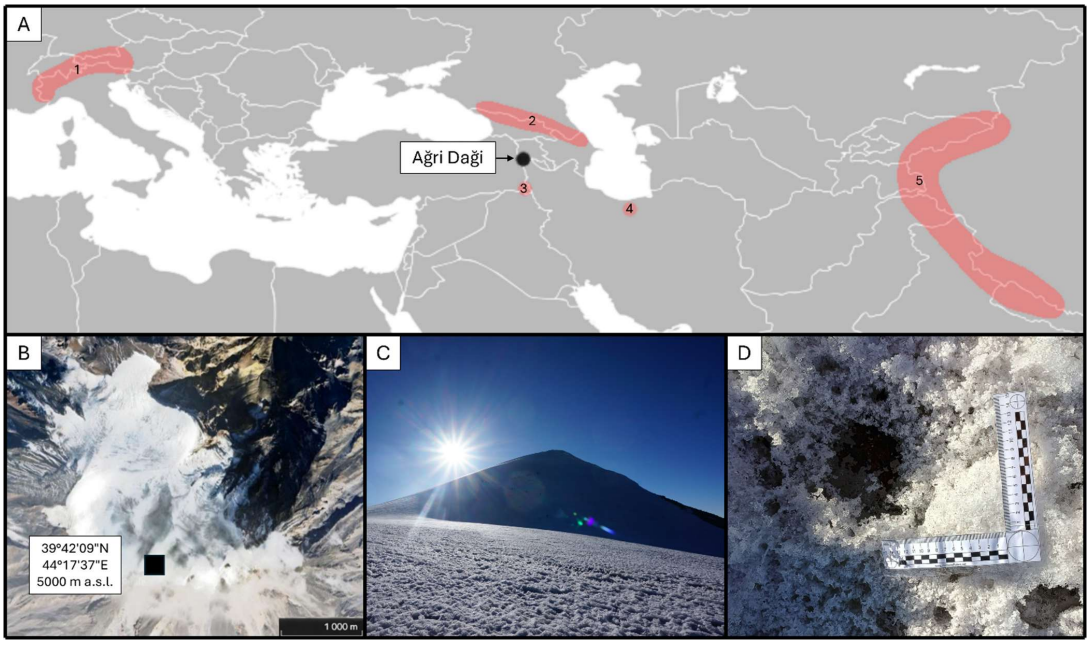

Figure 1: Location of study site: A. Ağri Dağı (black dot) and the nearest regions with glaciers (red areas, 1: The Alps, 2: Caucasus Mountains, 3: northern Zagros Mountains, 4: Mound Damavand, 5: Himalayas, Karakoram, Hindu Kush, Pamir and Tien Shan Mountains,); B. Sampling location on Ağri Dağı ice cap; C. Ağri Dağı summit; D. Cryoconite hole.

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2.2. Sample characteristics

Organic content of samples was quantified by loss-on-ignition (LOI) after drying (overnight at 105 °C) and combusting subsamples for five hours at 550 °C. Total carbon and nitrogen concentrations were determined using an Elementar Vario Pyro Cube Analyser. To assess presence of microfauna, each sample was screened under a light microscope at multiple magnifications. Additionally, we measured size and visualized cryoconite granules by using OLYMPUS equipment: CKX53 microscope, DP28 camera connected with cellSens Entry 4.2 software.

2.3. Isolation of culturable bacteria, temperature growth range and identification by 16S rRNA gene sequencing

Culturable bacteria were isolated following the methods of Singh et al. (2014)(Singh et al., 2014b). Cryoconite samples were serially diluted in sterile physiological saline, plated on preselection media and incubated at 0.2°C and 5°C for up to eight weeks. Representative colonies were cultured on R2 Agar (R2A) and nutrient agar (NA) (Biomaxima) for DNA extraction and temperature growth tests. Temperature range was assessed on R2A plates incubated in the dark at 0.2°C, 5°C, 10°C, 15°C, 20°C and 25°C for up to 4 weeks. Colony formation was considered as sign of viability in tested temperatures.

Genomic DNA from the cultures was extracted by heating (95°C for 2 min) according to Dillon et al. (2005) (Dillon et al., 2005). Quality of the DNA was assessed fluorometrically and by gel electrophoresis. A nearly full-length 16S rRNA gene fragment of ~1,484-bp was amplified with the universal primers 8F (5′-AG AGTTTGATCATGGCTCAG) and 1492R (5′-CGGTTACCTTGTTACGACTT) proposed by Weisburg et al. (1991), enabling accurate taxonomic classification (Weisburg et al., 1991). PCR reactions (25 µL) contained 1× buffer (1.5 mM $MgCl_2$), 0.25 µM primers, 0.2 mM dNTPs, 1.25 U OptiTaq polymerase (EURx), and 2 µL DNA template. PCR cycling conditions were: 94 °C for 3 min, 30 cycles of 94 °C for 45 s, 47 °C for 45 s, 72 °C for 1 min 30 s; with a final extension at 72 °C for 3 min on C1000 Touch™ Thermal Cycler (Bio-Rad)). PCR products were verified on 1.5% agarose gels, purified and sequenced using a 3130xl Genetic Analyzer (Applied Biosystems). The obtained 16S rRNA gene sequences were assembled using BioEdit (v. 7.7.1) software and identified by BLASTn searches with 97% cutoff of similarity to species level. Sequences of all isolates described in this study were deposited in NCBI (National Center for Biotechnology Information) under the accession numbers PZ164965-PZ165122.

2.4. Amplicon Sequencing (16S and 18S rRNA genes)

DNA was extracted from cryoconite sediment using DNeasy PowerSoil Pro Kit from Qiagen according to manufacturer's instructions. Samples were then processed by Genomics Core Facility (GCF) at the Centre of New Technologies (CeNT) at the University of Warsaw, Poland for both amplicon and metagenomic sequencing.

Samples were normalized to 5 ng/µL and used for PCR amplification with 16S Amplicon PCR Forward Primer/Reverse Primer (Illumina, Part # 15044223 Rev. B) and 18S primers TAReuk454FWD1/TAReukREV3 (Stoeck et al., 2010). The reactions (25 µL each) were prepared in triplicate using 5× Phusion™ HF Buffer (1×), 10 mM dNTPs (200 µM each), forward and reverse primers (0.3 µM each for 16S or 0.4 µM for 18S), Phusion™ High Fidelity DNA Polymerase (0.02 U/µL), and 5 µL (16S) or 1 µL (18S) of template DNA. Cycling conditions for 16S rDNA were: 98 °C for 4 min; 20 cycles of 98 °C for 20 s, 50 °C for 30 s, 72 °C for 10 s; final extension 72 °C for 5 min; hold 12 °C. Cycling conditions for 18S rDNA were: 98 °C for 30 s; 12 cycles of 98 °C for 10 s, 53 °C for 30 s, 72 °C for 30 s; then 15 cycles of 98 °C for 20 s, 48 °C for 30 s, 72 °C for 10 s; final extension 72 °C for 10 min; hold 12 °C.

Amplicons were visualized on 1.5% agarose gel stained with GelGreen. Remaining volumes were purified using KAPA Pure Beads (ratio 1:0.8). Adapter ligation and enrichment PCR (8 cycles) were performed using Illumina Nextera DNA UD Indexes Set D and KAPA HiFi HotStart Ready Mix. Following fluorometric quantification, libraries were pooled equimolarly. Fragment size distributions were analyzed with a TapeStation system using High Sensitivity D1000 reagents, and concentrations were verified by qPCR. Sequencing was performed on an Illumina NovaSeq 6000 platform using paired end 2 × 250 bp reads, following the manufacturer's standard workflow.

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Sequence preprocessing and denoising were performed following the protocol described by Ezzat et al., 2024, using the QIIME2 (v.2020.8) framework (Bolyen et al., 2019; Ezzat et al., 2024). Briefly, raw paired-end reads were trimmed with cutadapt, quality filtered, denoised, and chimera-checked using Deblur (16S) or DADA2 (18S) to obtain amplicon sequence variants (ASVs). Taxonomic classification was assigned against the SILVA v138 database (Quast et al., 2013).

2.5. Metagenomics

DNA samples were fragmented by sonication using a Covaris S220 system to achieve an average fragment size of ~450 bp. Libraries were constructed from 200 ng of fragmented DNA using the KAPA Hyper Prep Kit and KAPA UDI Adapters Kit 15 µM, following manufacturer protocols with 7 cycles of enrichment PCR.

Fragment size selection (~500 bp) was performed using KAPA Pure Beads in a two-step cleanup process (sample:bead ratios of 1:0.55 and 1:0.7). Library fragment length distributions were assessed using an Agilent TapeStation 4150 with High Sensitivity D1000 Reagents, and concentrations were quantified by qPCR with the KAPA Library Quantification Kit.

Sequencing was conducted on an Illumina NovaSeq 6000 platform using NovaSeq 6000 S1 Reagent Kit v1.5 in paired-end 2 × 150 bp mode, with 1% PhiX spike-in, following standard manufacturer procedures.

Metagenome-assembled genomes (MAGs) were generated from raw paired-end reads using the MAGsGeneration pipeline (https://github.com/michoug/MAGsGeneration). Reads were trimmed with Trim Galore (v. 0.6.10) (Krueger et al., 2012), human reads removed using strobealign (v. 0.14) (Sahlin, 2022) and samtools (v. 1.9) (Li et al., 2009) and assembled into contigs with MEGAHIT (v. 1.2.9) (Li et al., 2015). Coverage was calculated using strobealign and CoverM (v. 0.7.0) (Aroney et al., 2024) to support binning. Per-sample binning was performed with MetaBAT2 (v. 2.15) (Kang et al., 2019), Rosella (v. 0.5.4) (Newell et al., 2023), TaxVAMB (v. 5.0.4) (Kutuzova et al., 2024), CONCOCT (v. 1.1.0) (Alneberg et al., 2014) and COMEBin (v. 1.0.3) (Wang et al., 2024), followed by consensus binning with DAS Tool (v. 1.1.7) (Sieber et al., 2018) to obtain non-redundant bins. Bins were refined using Rosella. All bins across samples were combined, and quality was assessed with CheckM2 (v. 1.1) (Chklovski et al., 2023) (completeness ≥70%, contamination <10%). Passing bins underwent dereplication with Galah (v. 0.4) (Aroney et al., 2026). Final MAGs were evaluated for quality (CheckM2) and taxonomy (GTDB-Tk) (Chaumeil et al., 2020). Read mapping against final MAGs was conducted with CoverM, and functional annotation performed using prodigal and eggNOG-mapper (v. 2.1.9) (Huerta-Cepas et al., 2019; Hyatt et al., 2010). Microbial life-history strategies were inferred from MAG sequences using MicroTrait (v. 1.0) (Karaoz and Brodie, 2022), that extracts ecologically relevant functional traits from microbial genomes using profile hidden Markov models and maps them onto the YAS (Yield–Acquisition–Stress) framework.

2.6. Metatranscriptomics

RNA was extracted using RNeasy PowerSoil Total RNA Kit and rRNA was depleted with the NEBNext® rRNA Depletion Kit (Bacteria) according to manufacturer's instructions. Samples were then processed by Genomics Core Facility (CeNT UW, Warsaw, Poland).

Libraries were constructed from 7.5 µL of RNA samples using the KAPA RNA HyperPrep Kit with KAPA Universal Adapter and KAPA UDI Primer Mixes. Samples underwent fragmentation (half at 85°C for 3 min, half at 65°C for 1 min), the portions were pooled, and library preparation continued per manufacturer protocols with 16 cycles of enrichment PCR.

Library fragment length distributions were assessed using an Agilent TapeStation 4150 with High Sensitivity D1000 Reagents, and concentrations were quantified by qPCR with the KAPA Library Quantification Kit. Sequencing was conducted on an Illumina NovaSeq 6000 platform using NovaSeq 6000 S2 Reagent Kit v1.5 in paired-end 2 × 150 bp mode, with 1% PhiX spike-in, following standard manufacturer procedures.

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Raw metatranscriptomic reads were first depleted of rRNA sequences using SortMeRNA (v. 4.3) (Kopylova et al., 2012). Remaining non-rRNA reads were trimmed for adapters and low-quality bases with Trim Galore (v. 0.6.10), retaining reads ≥15 bp after trimming.

Transcript abundance was then quantified against the MAG catalog using Kallisto (v0.48.0)(Bray et al., 2016). A species-level index was created from all MAG fasta files, and expression was estimated in paired-end mode with bootstrap analysis (99 replicates), yielding transcript-per-million (TPM) values for downstream differential expression analysis. Downstream analyses were conducted in R Statistical Software (v4.4.2).

3. Results

3.1. Sample characteristics and microscopic observations

Mean organic matter content in the samples was 7.48 ± 2.08 %, with a C/N ratio of 7.44 ± 0.15. The material was in form of irregular granules ranging in size from 114×144 to 675×901 µm, with organic particles and cyanobacteria visible in fluorescent microscope. Between irregular granules, mineral particles were also common (Fig. 2). Inspection of the samples under stereomicroscopes did not reveal presence of microscopic invertebrates.

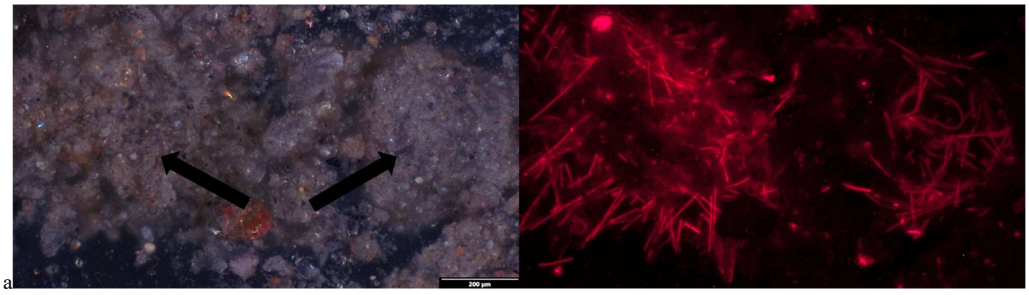

Figure 2: Cryoconite under light (left) and fluorescent microscope (right). Arrow indicates cryoconite granule.

3.2. Cultivation-Based Diversity

Total number of bacteria (colony forming units, CFUs) on preselection media ranged from 4.84×10^6 to 2.05×10^7 at 0.2 °C and 2.05×10^7 to 3.6×10^7 at 5 °C. Bacterial colonies were pigmented (colourless, white, cream, yellow, orange, pink, red and brown) and varied morphologically (glossy or matte; transparent or opaque; jagged or smooth edge; smooth, raised or cratered elevation) (Supplementary Fig. 1).

A total of 158 bacterial strains belonging to 11 genera and 41 species were isolated and identified. The genus *Cryobacterium* was the most numerous, with 92 isolates assigned to 13 species, among which *Cryobacterium breve* and *C. ruanii* were the most abundant (26 strains each). The genera *Polaromonas*, *Flavobacterium*, *Arthrobacter*, and *Massilia* comprised 21, 20, 9, and 8 isolates, respectively. Within *Polaromonas, P. glacialis* was the most abundant, with 14 isolates. Among *Flavobacterium, F. frigoris, F. pectinovorum,* and *F. xueshanense* each had 4 isolates. The remaining genera were represented by lower number of strains. Within *Arthrobacter*, six species were detected. *Massilia aquatica* and *M. timonae*, as well as *Devosia psychrophila*, *Paeniroseomonas aquatica*, *Deinococcus marmoris*, *Glaciihabitans tibetensis*, *Knoellia subterranea*, and *Rugamonas rubra*, were all represented by one or two isolates (Supplementary Table 1; Supplementary Fig. 2).

3.3. Growth of bacteria across different thermal gradients

All tested isolates grew at 0.2 °C, 27.8% of the strains survived at 25 °C, the highest tested temperature, and 45.6% grew at 20 °C. Isolates belonging to nine species, *Cryobacterium. luteum*, *C. luteum/ruanii*, *C. melibiosiphilum*, *C. ruanii*, *Flavobacterium. aquariorum*, *F. frigoris*, *F. fructosi*, *F. pectinovorum*, *F. xueshanense* and *Knoellia subterranea*, did not show growth above 10 °C. Variation in maximum growth temperature was observed among strains identified as the same species (Fig. 4). For example, among the *C. breve* isolates, 14 did not grow above 15°C, whereas ten showed growth at

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20 °C and two at 25 °C. Furthermore, many of the isolated strains matching to single ASV exhibited the phenotypic diversity in colony color or growth temperature spectrum. For example, *C. breve* maximum growth temperature ranged from 15 to 25°C, and the colony color from yellow, through orange and pink to red. Similarly, *P. glacialis* maximum growth temperature ranged from 20 to 25°C with colony colors: transparent, white, cream, light-yellow, yellow, orange-red and orange.

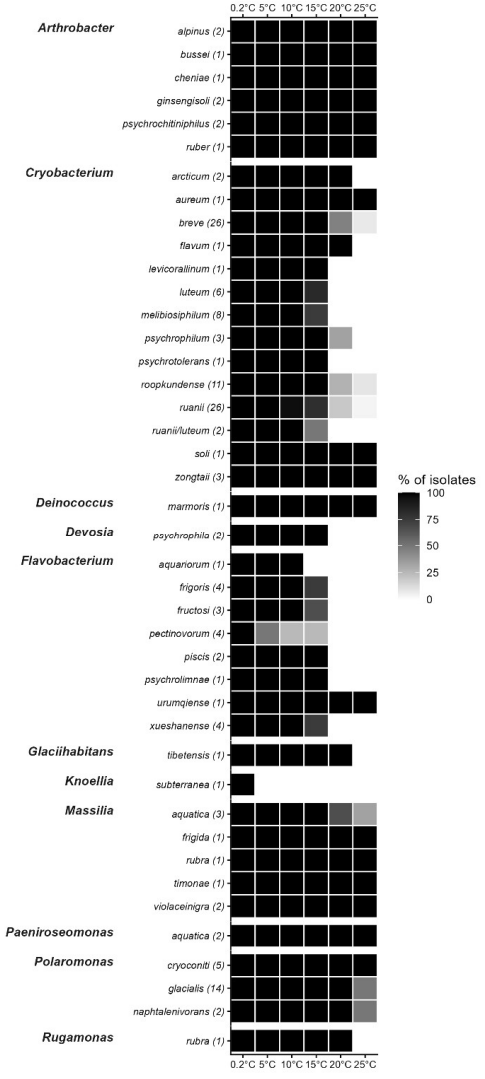

Figure 3: Growth of bacteria strains in temperature gradient.

3.4. Amplicon-Based Community Composition

The bacterial community of cryoconite holes on Ağri Dağı Mountain was dominated by Actinobacteriota (27.6%), Proteobacteria (23.4%) and Cyanobacteria (21.5%), followed by Bacteroidota (13.7%), Verrucomicrobiota (4.4%) and Patescibacteria (2.3%) (Fig. 4.). At the genus level, a single filamentous cyanobacterium assigned to *Tychonema* was particularly abundant (21.4%), followed by *Nocardioides, Oryzihumus, Polaromonas, Ferruginibacter* and *Sphingomonas*.

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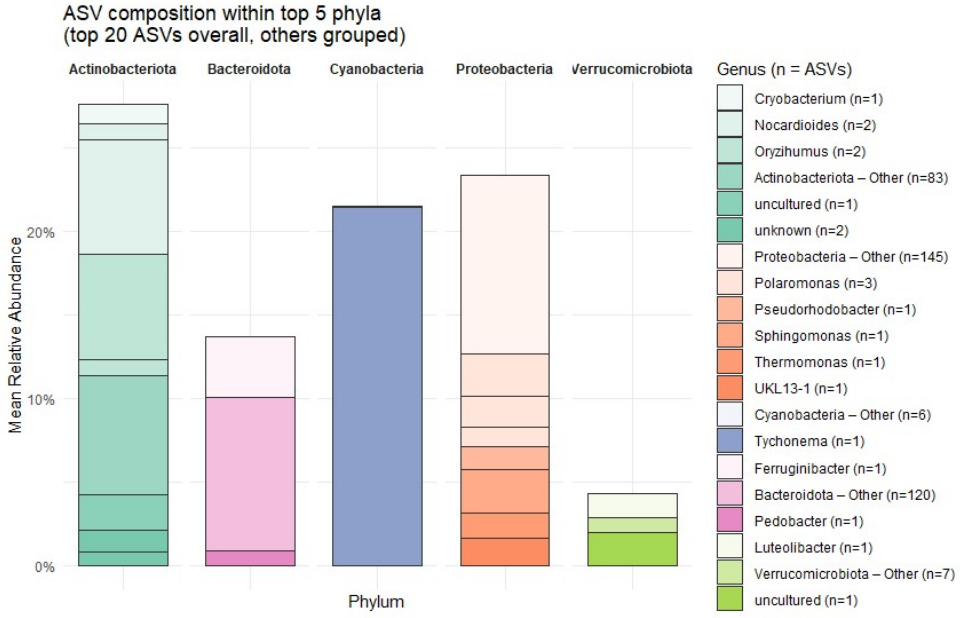

Figure 4: Composition of bacterial community based on 16S rDNA amplicon sequencing. Top 20 ASVs are included in top 5 phyla, the remaining AVSs in each phylum are grouped as "other".

3.5. Comparison of isolates *vs* 16S amplicons

16S rRNA gene of the cultured isolates obtained at two lowest temperatures (closest to the field conditions), 0.2 °C and 5 °C, were sequenced and compared to the 16S rRNA gene amplicon dataset to identify exact sequence matches. In total the isolates corresponded to only 21 of 511 ASVs (4.1%), indicating that cultured heterotrophic bacteria represent a small fraction of the overall amplicon diversity. *Cryobacterium*, the most diverse and frequent isolate genus, ranked among the top ten genera in the amplicon dataset but contributed only 1.86% relative abundance, far below the most abundant genus *Tychonema* (21.42%) (Supplementary Fig. 3). Out of 158 isolates, 150 matched at least one ASV in the amplicon dataset at ≥97% BLAST identity. The unmatched ASVs predominantly belong to *Arthrobacter* (*A. alpinus*, *A. bussei*, *A. psychrochitiniphilus*, *A. ruber*, *A. cheniae*), *Flavobacterium aquarium*, and *Cryobacterium aureum*.

3.6. Functional Potential from Metagenomes

In the metagenomic analysis, 111 MAGs were retrieved, spanning the range of phyla detected in 16S rRNA gene analysis. The majority belonged to Proteobacteria (34), followed by Actinobacteriota (32) and Bacteroidota (9). Cyanobacteria were represented by only one MAG, despite their high abundance in both amplicon and metagenomic reads.

MicroTrait analysis, which allows to characterise trade-offs between resource acquisition, energy yield, and stress tolerance, revealed a wide range of microbial life-history strategies: from MAGs favouring resource acquisition to the ones prioritising stress tolerance (Fig. 5.). Patescibacteria formed a distinct group, reflecting their limited genetic repertoire, whereas other phyla were widely dispersed across the strategies spectra and did not cluster strictly by phylum (Supplementary Fig. 4). Within Cyanobacteria, the MAG showed a markedly reduced (Supplementary Fig. 5) repertoire of substrate-transport genes compared to other MAGs, except for nitrogen-compound transport. No consistent differences were observed in substrate degradation or assimilation. Chloroflexota encoded genes indicative of methanotrophy, while Cyanobacteria and some Actinobacteriota harboured genes for CO_2 fixation. The Cyanobacteria MAG lacked nitrogen fixation capability but showed the capacity for assimilatory nitrate reduction. In terms of stress-tolerance genes, the cyanobacterial MAG showed a similar

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profile to other MAGs, encoding genes associated with general stress response (such as molecular chaperones and heat-shock proteins), oxidative stress, and osmotic stress. Some non-cyanobacterial phyla displayed chemotrophic traits, with Proteobacteria exhibiting the broadest range of chemolithotrophic capabilities. MicroTrait did not detect traits for phototrophy in cyanobacterial MAG; whereas two other phyla had traits for anoxygenic phototrophy. The cyanobacterial MAG also lacked traits for EPS production, a trait present in many other phyla to varying degrees. However, genes associated with EPS production and phototrophy were actively expressed as seen in the further metatranscriptomic analysis, indicating that they did not meet specific marker combinations and thresholds used by MicroTrait. Some non-cyanobacterial phyla displayed chemotrophic traits, with Proteobacteria exhibiting the broadest range of chemolithotrophic capabilities.

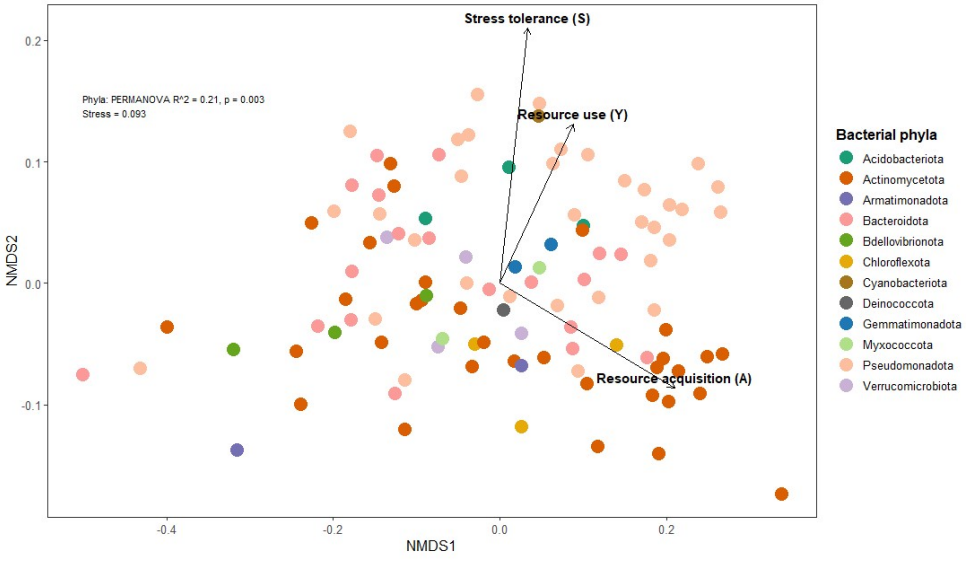

Figure 5. Life history strategies of MAGs based on MicroTrait analysis. Patescibacteria were omitted for clarity.

3.7. Active Community Signatures from Metatranscriptomes

Metatranscriptomic analysis quantified 18,379 genes expressed from 111 MAGs.

Gene expression distribution was dominated by the Cyanobacteria genes (93.8% of transcripts). MAG affiliated to Cyanobacteria phylum showed notably elevated transcriptional activity across multiple functional KEGG (Supplementary fig 6) and COG categories (Fig. 6), including photosynthesis, amino acid biosynthesis, cell envelope and carbon metabolism. The remaining MAGs showed more heterogeneous pathway expression patterns, with differential utilization of carbohydrate metabolism, energy conversion, and biosynthetic pathways, potentially reflecting niche partitioning and metabolic specialization within the microbial assemblage. A particular difference between Cyanobacteria and other MAGs was visible in the transporters, similar to the genes presence analysed from MAGs.

The metatranscriptomic analysis also showed that the cyanobacterial MAG actively expressed genes associated with both phototrophy and EPS/biofilm formation. For phototrophy, this MAG expressed high TPM for RuBisCO KOs (K00855/K01601/K01602) and photosystem markers (e.g. K02703 and K02689), consistent with CO_2 fixation and oxygenic photosynthesis. For EPS and biofilm traits, the same MAG showed high expression of type II/IV secretion and pilus genes such as pilB (K02652), pilC (K02653) and pilA-like adhesins (K02377), as well as polysaccharide export components annotated as ycf38-like transport permeases and Wzb/Wzc-like proteins (e.g. K01992, K01991).

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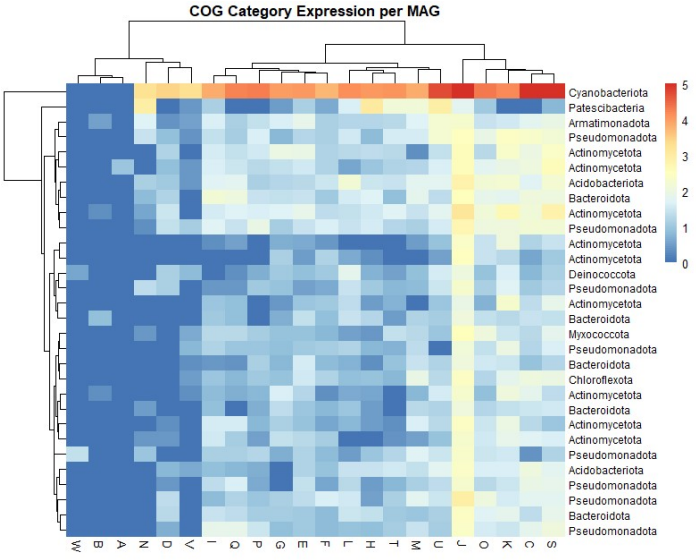

Figure 6. Transcriptional activity per MAG, grouped by COG categories. Top MAG belongs to Cyanobacteria. Colour indicates log10 of TPM. COG categories: A - RNA processing and modification, B - Chromatin structure and dynamics, C - Energy production and conversion, D - Cell cycle control, cell division, chromosome partitioning, E - Amino acid transport and metabolism, F - Nucleotide transport and metabolism, G - Carbohydrate transport and metabolism, H - Coenzyme transport and metabolism, I - Lipid transport and metabolism, J - Translation, ribosomal structure and biogenesis, K - Transcription, L - Replication, recombination and repair, M - Cell wall/membrane/envelope biogenesis, N - Cell motility, O - Posttranslational modification, protein turnover, chaperones, P - Inorganic ion transport and metabolism, Q - Secondary metabolites biosynthesis, transport and catabolism, R - General function prediction only, S - Function unknown, T - Signal transduction mechanisms, U - Intracellular trafficking, secretion, and vesicular transport, V - Defense mechanisms, W - Extracellular structures, X - Mobilome: prophages, transposons, Y - Nuclear structure, Z - Cytoskeleton

3.8. Eukaryotes

Targeted 18S rRNA gene analysis showed phylum-level relative abundances dominated by one phylum - Ciliophora (78.1%), followed by Chlorophyta (9.9%), Cercozoa (7.5%), Chytridiomycota (2.3%) and Basidiomycota (1%) (Fig. 7.). This was corroborated by rRNA reads from metatranscriptomics, where 42% of reads could not be taxonomically assigned, and amongst the rest Alveolata (represented by Ciliophora only) dominated (26%), followed by Fungi (13%) and Archaeplastida (10%). The protists seem to be the most abundant and active Eukarya in the holes, whereas invertebrates are absent in metatranscriptomic reads, which corroborates the microscopic observations of active protists and lack of invertebrates.

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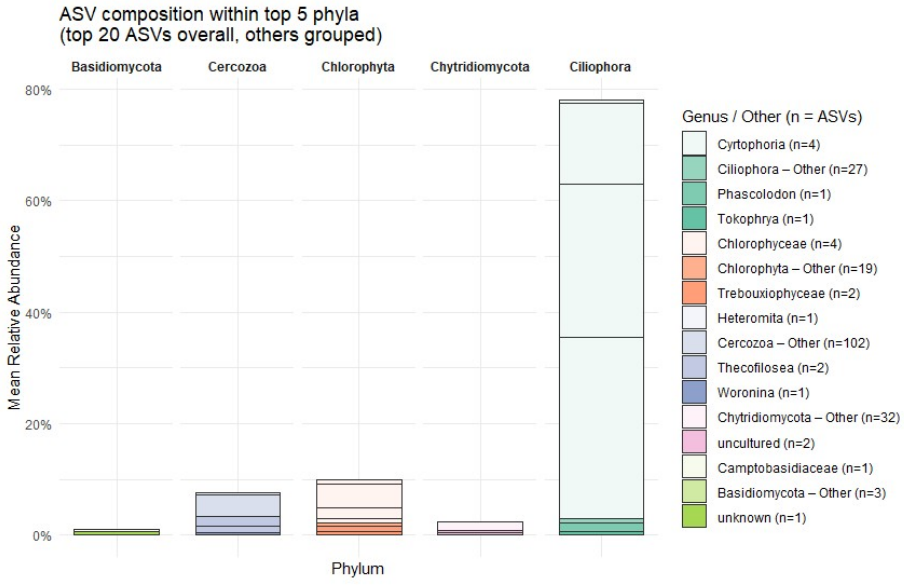

Figure 7. Composition of eukaryotic community based on 18S rDNA amplicon sequencing. Top 20 ASVs are included in top 5 phyla, the remaining AVSs in each phylum are grouped as "other".

4. Discussion

Community structure

Glaciers located in low latitude, but high-altitude regions, often isolated from other ice masses, are important for assessing biodiversity lost now and in the future due to glacier retreat and are frequently missing from biogeographical analyses of glacier-dwelling organisms. Cryoconite holes on Ağri Dağı Mountain are dominated by the phyla found in cryoconite worldwide, i.e. Actinobacteriota, Proteobacteria, Cyanobacteria and Bacteroidota (Ge et al., 2026; Millar et al., 2021). Environmental filtering in cryoconite holes selects these few phyla, suggesting that conditions such as low temperature, strong seasonality and fresh water support a common and relatively simple community structure. Isolation from other glacial environments (Fig. 1.) does not seem to limit the development of a stable community of phylogenetically connected bacteria. There are few biogeographically comparable studies against which to assess our snapshot of the community at Ağri Dağı Mountain. However, available data showed similarities; in the Caucasus, cryoconite is dominated by *Phormidesmis*, *Ferruginibacter*, *Chamaesiphon*, *Tychonema*, *Polaromonas*, *Hymenobacter*, *Sphingomonas*, and *Gemmatimonas* (Gladkov et al., 2024), from which *Tychonema*, *Polaromonas*, *Rhodoferax*, *Cryobacterium*, and *Hymenobacter* are also abundantly present in our Turkish samples. However, dominating bacterial genera are different than in other geographic regions, which likely reflects the uniqueness of environmental factors in subtropical area and can serve as a signature of local conditions (Ge et al., 2026). The samples of Turkish cryoconite were mostly dominated by few genera, with particularly high relative abundance of *Tychonema*. Whilst these genera are common to cryoconite, the high proportion of one cyanobacterial genus is unusual and has not previously been reported (Millar et al., 2021; Segawa et al., 2017). Surprisingly we did not detect the presence of genera belonging to the Oscilatoriales order, which are typical of cryoconite holes communities in the Alps or Arctic, such as *Phormidesmis*, *Phormidium* or *Microcoleus* (Segawa et al., 2017; Takeuchi et al., 2025; Wejnerowski et al., 2023)

Cultivability

Only a small subset of ASVs detected in the community were successfully cultured, and most of these ASVs were represented by relatively few isolates, highlighting a typical cultivation bias toward a limited set of taxa. Culture-dependent methods

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consistently recover a fraction of environmental diversity in oligotrophic, cold-adapted communities (Venkatachalam et al., 2015; Zhang et al., 2010). When sequences of isolated bacteria are clustered into broader OTU-like groups they match around one fifth of the total community from amplicon sequencing (Supplementary Fig. 3), indicating that many culturable bacteria fall within numerically important lineages rather than the rare biosphere. For example, *Cryobacterium* dominates cultures but contributes only ~2% of relative abundance in 16S amplicons, illustrating a classic cultivation artifact where fast-growing bacteria are over-represented in culture relative to the slower-growing majority or those that require syntrophic partners for growth such as Patescibacteria (Jaarsma et al., 2023; Lloyd et al., 2018; Ortiz et al., 2021).

Of the strains that grew in culture, lower temperatures (0.2 and 5 °C) produced similar results, though notably, *Knoellia* genus was only recovered at 0.2 °C. However, it is not typically found in cold environments and may therefore be evidence for cultivation artifact. Some strains did not grow above 5 °C, and multiple strains did not grow above 10 °C, therefore lower temperature give more representative cultivation of what inhabit and is active in cryoconite holes. Above 10 °C, some of the successful strains may belong to 'incidental' bacteria, where dormant cells preserved in low temperatures at the bottom of cryoconite holes became active in warmer laboratory conditions.

Despite the bias, culture techniques still provide valuable knowledge about functional capabilities and microdiversity driven by the environment. Cultures revealed different phenotypes of the same ASV, identified by 16S rRNA gene sequencing, yet exhibiting phenotypic differences, such as colony colors or growth at different temperatures (Supplementary Table 2). These phenotypically plastic effects have been observed elsewhere, with strains belonging to *C. breve*, *C. ruani*, *A. alpinus* and *A. ginsengisoli* were exhibiting different colony colours along temperature ranges (Ali et al., 2021; Liu et al., 2020; Rafiq et al., 2019; Singh et al., 2014b).

Functional analyses

On the genetic level, cryoconite bacteria tend to favour resource acquisition (e.g., via diverse transporters and exoenzymes for substrate uptake/degradation) and exhibit reduced stress tolerance (e.g., fewer osmolyte or chaperone investments), likely in response to oligotrophic glacial conditions. MAGs span the full gradient of opportunistic versus conservative strategies. Overall, diversification in cryoconite genomes is observed in MicrotTrait analysis, potentially indicating that individual MAGs adapt variably to fluctuating melt-derived resources amid high abiotic stresses.

Transcriptomic analysis showed that gene expression was disproportionately dominated by cyanobacterial transcripts. These transcripts were predominantly assigned to pathways involved in photosynthesis, primary carbon metabolism, amino acid biosynthesis, and cell envelope biosynthesis, consistent with *Tychonema* being a dominant photoautotroph. This pattern indicates extreme functional dominance where a single taxon appears to make the largest contribution to the core metabolic processes in the cryoconite ecosystem (Edwards et al., 2014; Jungblut and Vincent, 2017). Only a very limited set of transcripts was associated with non-cyanobacterial MAGs, further highlighting both the broad functional repertoire and the strong adaptation of cyanobacteria to the cryoconite environment. These non-cyanobacterial transcripts were mainly assigned to transport-related modules, including ABC transporters, PTS sugar transporters, response regulators/two-component systems, and conjugal transfer. The corresponding MAGs therefore likely specialise in nutrient scavenging, environmental sensing and horizontal gene transfers, consistent with their heterotrophic lifestyle alongside the dominant phototrophic cyanobacteria. In these conditions, the heterotrophic bacteria compete for dissolved organic matter released by *Tychonema* and respond to fluctuating conditions, while *Tychonema* maintains autotrophic primary production. In other cryoconite systems, Oscillatorialean cyanobacteria (such as *Tychonema*) function as ecosystem engineers (Wejnerowski et al., 2023). This function is likely present here, since there is strong and active expression of biomass production genes, but the key difference seems to be that one phototrophic organism controls core metabolism in the cryoconite system, in contrast to an Alpine glacier, where the distribution of expression was more even between heterotrophs and autotrophs (Pittino et al., 2018). The EPS produced by *Tychonema* likely binds mineral matter into granules. The size of granules was similar to cryoconite from Gulkana Glacier,

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Alaska (Rozwalak et al., 2022). Granule size seems to control biogeochemical processes (Langford et al., 2014), particularly controlling the spatial distribution of carbon and nitrogen through redox stratification (Segawa et al., 2020). Suprisingly, *Tychonema* does not possess the ability to fix nitrogen from the atmosphere, unlike for example some of the *Nostoc* species known from Antarctic cryoconite holes (Vonnahme et al., 2016; Zhu et al., 2025). Despite the lack of evidence for nitrogen fixation from sequencing, the C:N ratio of Ararat cryoconite is relatively low in comparison with studies from Greenland (Stibal et al., 2010) or the Tibetan Plateau (Liu et al., 2017) (Supplementary Table 3). This could be explained by either active fixation being missed with sequencing, or lack of fixation at the time of sampling, potentially because of the proximity of the ice cap to local sources of nitrogen, for example, anthropogenic activities such as sheep herding. The dominance of *Tychonema* and other non-nitrogen-fixing cyanobacteria has previously been associated with glaciers with higher nitrogen concentrations (Zhu et al., 2025).

Food webs

The absence of metazoan grazers — particularly tardigrades and rotifers – in our samples likely impacted the overall community structure. Studies of trophic webs in cryoconite holes and on glaciers in general are scarce (Crosta et al., 2025), yet studies on Forni Glacier in the Alps showed tardigrades form a central node in the trophic web in cryoconite holes, or tardigrades and rotifers co-dominate and feed on both algae and bacteria (Jaroměřská et al., 2026). Glacier tardigrades are known to also directly consume ciliates and cyanobacteria (Zawierucha et al., 2022). The low abundance of Chlorophyta in our samples may indicate limited prey for tardigrades, which largely depend on eukaryotic green algae on glaciers (Jaroměřská et al., 2025). It may also result in the success of cyanobacteria, directly through lack of grazing or indirectly by mediating ciliate communities (Jaroměřská et al., 2025; Vonnahme et al., 2016; Zawierucha et al., 2021). Ciliates are consistently detected at high relative abundances in 18S reads across a range of cryoconite ecosystems. Their abundance may be linked to metazoan presence: for example, Sommers et al. (2018) observed negative co-occurrence between metazoans versus ciliates and cyanobacteria on Taylor Glacier, Antarctica (Sommers et al., 2018). From other hand, this was not observed at Larsemann Hills and Amery Ice Shelf in East Antarctica (Antony et al., 2024). Neither tardigrades nor rotifers were detected in cryoconite holes on (relatively nearby) Georgian glaciers in the Caucasus (Zawierucha et al., 2021). In Svalbard, no links between metazoan densities and cyanobacterial abundances were observed, but rotifer densities were negatively associated with Oscillatoriales filament length (Vonnahme et al., 2016). This is mechanistically supported by tritrophic microcosm experiments using the filamentous, mat-forming *Phormidium* — a close relative of *Tychonema* — showing that filamentous cyanobacteria actively defend against specialist ciliate grazers by withdrawing trichomes. This defence collapses when rotifers are present, as they dismantle the mucilage mat and expose trichomes to ciliate ingestion, enabling near-complete removal of cyanobacterial biomass (Fiałkowska and Pajdak–stós, 1997; Pajdak-Stós et al., 2020).

These circumstantial links suggest deeper investigation is required into the spatiotemporal relationships between different trophic levels of the cryoconite ecosystem. Studies simultaneously resolving both eukaryotic and bacterial community structure remain sparse, and without quantitative abundance data across trophic levels and different regions, the full picture of the cryoconite hole food web — despite its apparent simplicity — remains incomplete.

5. Conclusion

Cryoconite holes on the ice cap of Ağrı Dağı, Türkiye, hosted 158 bacterial strains belonging to 11 genera and 41 species. Of these, nine species of *Cryobacterium*, *Flavobacterium* and *Knoellia* did not grow above 10 °C, indicating particular vulnerability to climate warming. The community was dominated by cyanobacteria, with *Tychonema* particularly abundant and active. The relatively low C:N ratio, combined with the absence of nitrogen fixation genes in the MAGs, suggests sufficient bioavailable nitrogen from meltwater or mineral substrate, aided by binding of mineral particles into cryoconite granules. No metazoans were observed, and smaller protists likely had reduced grazing pressure on the rest of the community and potentially

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contributed to the dominance of *Tychonema*. This snapshot of the community, observed at a single timepoint but assessed using an approach that integrated traditional, metagenomic and transcriptomic methods, demonstrated that even ice surfaces far isolated from other glaciers can host active cryoconite hole communities. Community structure is consistent with the global cryoconite signature at the phylum level, while exhibiting distinct genus-level composition and active metabolic function shaped by local conditions. These isolated glaciers are often the most vulnerable to rising temperatures, yet they remain the least studied. Their inclusion in future biogeographic surveys is therefore not only scientifically valuable but urgent — as each disappearing glacier represents an irreplaceable snapshot of cold-adapted biodiversity and ecosystem function.

6. Author contribution

Conceptualization: E.P., J.K., KZ; Formal analysis: E.P., G.M., J.K.; Funding acquisition: E.P., K.Z.; Investigation: E.P., J.K., N.M-Z., E.B.; Resources: All authors; Visualization: E.P., J.K.; Writing and editing: All authors.

7. Competing interests

Ewa poniecka are member of the editorial board of BG/TC inter-journal SI "Cryospheric ecosystems: climate feedback loops, threatened ecosystems, and consequences of climate change".

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