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Chronic environmental radioactivity suppresses microbial diversity and activity in threatened glacier surface ecosystems

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Abstract. Environmental radioactivity can act as a chronic stressor not only in nuclear accident zones but also in ecosystems that capture atmospheric pollutants, yet its effects on organisms remain understudied. Using biologically active glacier sediments that concentrate natural and legacy radionuclides, this study provides the ecosystem-scale test of how chronic radiation affects microbial life outside nuclear accident zones. Across a radioactivity gradient, higher levels corresponded to significant declines in microbial richness and phylogenetic diversity. Functional diversity decreased, with fewer KEGG Orthologs, indicating elevated radioactivity as a selection factor. However, showing strong redundancy in this ecosystem, as key functions remain in the higher radioactive habitat. Despite stable overall functional profiles, an essential DNA repair pathway (non-homologous end joining) was enriched. Metatranscriptomic revealed significant metabolisms and growth suppression, shown by reduced RNA transcripts per DNA copy for metabolisms pathways. These results show that chronic environmental radiation is a strong, underrecognized ecological factor that decreases the biodiversity of glaciers threatened by climate change.

1 Introduction

Ionising radiation (IR) occurs naturally on Earth, arising from cosmic radiation that penetrates the atmosphere and from the decay of primordial and secondary radioisotopes, primarily products of the decay of uranium and thorium. They are widely spread in the Earth's crust and the atmosphere, reaching all ecosystems (El-Daoushy and Garcia-Tenorio, 1988; Mihailović et al., 2014; Rastogi and Sarin, 2008; Trieloff and Kunz, 2005). During the last century, the Earth experienced a new source of radioisotopes linked to human activities, essentially related to nuclear weapons and nuclear energy, that resulted in the release of anthropogenic radioisotopes such as ^{90}Sr, ^{137}Cs, $^{238\text{-}241}$Pu or ^{241}Am (Bunzl and Kracke, 1988; Evrard et al., 2020; Ikeda-Ohno et al., 2016; Werner and Purvis-Roberts, 2007). More than 2000 nuclear tests have been conducted since 1945, of which more than 500 were blasted in the atmosphere, spreading radioisotopes over the globe (Mietelski, 2010). Moreover, Nuclear Power Plant (NPP) accidents, satellite accidents equipped with radioisotope thermoelectric generators, and other accidents

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related to nuclear weapons transport or the processing of nuclear fuel have contributed locally and globally to anthropogenic radioisotope contamination. These accidents released high amounts of radioisotopes, which do not occur naturally on Earth or only in extremely low concentrations (e.g., ^{240}Pu, ^{239}Pu, ^{241}Am), as well as those that occur naturally, whose amounts have increased since 1945, for instance, ^{3}H or ^{14}C (Hodge et al., 2000; Mietelski, 2010).

The effects of high doses of IRs on organisms are well studied, especially in an experimental approach (Bernhard et al., 1995; Kam and Banati, 2013; Paul et al., 2014; Sowa et al., 2006), however, studies demonstrating the effects of low-dose (exceeding background radiation) chronic exposure under natural conditions are limited (Geras'kin and Volkova, 2014; Kesäniemi et al., 2019; Videvall et al., 2023; Zeng et al., 2023), especially in the case of microscopic prokaryotes and eukaryotes, which are the most diverse and abundant organisms on Earth (Gu et al., 2014; Ogwu et al., 2019). In situ observations of chronic exposure to elevated IRs (relative to background levels) have shown null or negative effects on wildlife across distinct phylogenetic lineages. For instance, in the contaminated area close to the Chornobyl NPP accident, negative effects of chronic exposure to IR on people's health were observed, specifically the increased frequency of proliferative atypical cystitis (Romanenko et al., 2009), thyroid cancers or leukaemia (Moysich et al., 2002). In the same area, IRs affected small rodents' lipid metabolism, immune system, gut overall health, and their microbiota (Jernfors et al., 2024; Kesäniemi et al., 2019). Moreover, increased genetic diversity was observed in *Pinus sylvestris* along the exposure gradient near the Chornobyl NPP (Geras'kin and Volkova, 2014), but also cytogenetic and phytohormonal changes in *P. resinosa* in Fukushima Prefecture (Geras'kin et al., 2021). Despite this evidence, information on how elevated chronic exposure to radioactivity can influence wildlife far from test sites and areas affected by NPP disasters is limited (Cheng et al., 2023; Gu et al., 2014).

A relatively high concentration of anthropogenic and natural radionuclides has been found on glaciers worldwide (Buda et al., 2020; Clason et al., 2023; Łokas et al., 2018, 2022; Pinglot et al., 1994). Fallout radioisotopes, delivered by the atmosphere, are effectively captured on the ice through the dark sediment called cryoconite (Baccolo et al., 2017; Buda et al., 2024b; Clason et al., 2023; Łokas et al., 2022; Owens et al., 2023). This simple, but important ecosystem in the context of glaciers functioning, is a mixture of fine minerals and organics, often in the form of oval aggregates, including myriad microorganisms which mainly form biofilm on minerals and secrete extracellular polymeric substances (Rozwalak et al., 2022; Segawa et al., 2020; Takeuchi et al., 2010). During summer, cryoconite is warmed by solar radiation and melts the underlying ice, creating water-filled depressions known as cryoconite holes. On one side, this habitat is a hotspot of biological activity on glaciers, while on the other, the organisms themselves drive the accumulation of pollutants, including atmospheric-delivered radioisotopes (Buda et al., 2024b; Davidson et al., 2023). Consequently, the concentrations of fallout radioisotopes in the cryoconite are an order of magnitude higher than those in surrounding ecosystems (Baccolo et al., 2020b; Clason et al., 2021; Łokas et al., 2017; Owens et al., 2023). This raised two major questions: what are the consequences for biota living on glaciers and in downstream ecosystems, and how can this process be modulated by ongoing global warming and potential release of stored contaminants in ice, including radioisotopes? These issues are important because both climate change and glacier pollution are global phenomena, not local ones. Even though they have been raised many times, to date, no empirical evidence supports the effects of radioisotope pollution on glacier biota (Pittino et al., 2023). This is crucial, as we know that glaciers globally present unique evolutionary lineages, which are not found in other ecosystems, and are now threatened due to habitat loss caused by climate warming (Losapio et al., 2025; Stibal et al., 2020). In addition, over the past decade, it has been suggested that glacier biota may be under threat from atmospheric-delivered pollutants that accumulated on glaciers over the last century and are now being increasingly released as ice melts (Pittino et al., 2023).

To address the challenge of understanding the effects of chronic low-level radiation on microbial communities, we conducted a multilevel, large-scale survey of glacier surfaces. Glaciers provide a relatively simple model system, characterised by stable temperatures, simplified food webs compared to other terrestrial ecosystems, and lower yet unique biodiversity (Crosta et al., 2025). We focused on cryoconite, biologically active habitats on glacier surfaces, as a model for microbial community studies. We hypothesised that increasing IR would reduce bacterial and microeukaryotic diversity, as well as the

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abundance and diversity of top consumers. We further examined how biodiversity loss affected phylogenetic and functional diversity and assessed variation in microbial metabolic potential (DNA-based), with a focus on DNA-repair mechanisms, and metabolic pathways related to cellular growth and stress response. Finally, we quantified gene expressions relative to DNA abundance to control shifts in community composition and directly assess the functional activity of the above-mentioned crucial pathways. Elevated levels of fallout radioisotopes have been documented on glaciers worldwide (Baccolo et al., 2020b; Clason et al., 2023; Łokas et al., 2024), including the Alps, where sources and accumulation mechanisms are well-characterised (Baccolo et al., 2020a, b; Buda et al., 2024b, a; Sala et al., 2025; Wilflinger et al., 2018). Building on this knowledge, our study is the first to investigate how natural and anthropogenic radioactivity affects microbial communities across a broad geographic area of the Alps, providing strong insight into chronic IR effects on biota outside sites impacted by nuclear accidents or military activity.

2 Materials and methods

2.1 Sampling

Overall, 16 glaciers were sampled in the Alps from Dauphiné (France) to the Central Eastern Alps (Austria) during the summer season (July and August) of 2021. On each glacier, 10 cryoconite samples (bioactive glacial sediments) from cryoconite holes were collected using a clean stainless-steel spoon or sterile plastic pipettes and placed into sterile test tubes or bags. The material was frozen at -20°C or stored in 96% ethanol and then kept at -20°C as soon as possible. Knowing that the distribution of organisms is heterogeneous at the cryoconite hole's bottom (Zawierucha et al., 2019b), the sediment was sampled multiple times from the bottom of the holes. Before further analysis, the material was mixed and split into aliquots for separate analyses. Moreover, in 2024, we sampled Dosdè Glacier for metatranscriptomic analysis at a glacier scale. This glacier was chosen because cryoconite was found to contain high activity concentrations of natural and anthropogenic radioisotopes, with high variation within the glacier (Buda et al., 2024a). This material (16 distinct cryoconite samples) was collected while avoiding RNases, immediately mixed with PowerProtect solution (Qiagen) at a 9:1 ratio, then intimately frozen on dry ice and kept in the laboratory at -80 °C until further analysis.

2.2 Estimation of glacier surface area and altitude

The surface area of glaciers and their average altitude were estimated for 2015-2016, based on the Global Land Ice Measurements from Space (GLIMS) database (Raup et al., 2007), with glacier characteristics reused from our previous work (Buda et al., 2024a), including the use of 2022 data for Pers Glacier due to the lack of 2015-2016 data. The surface area of glaciers varied from 0.48 km^2 (Preda Rossa Glacier) to 83.38 km^2 (Aletsch Glacier), while the average altitude ranged from 2766 m a.s.l. (Forno Glacier) to 3278 m a.s.l. (Lang Glacier).

2.3 Radiometric analysis

As a proxy for overall radioactivity pressure, we used the activity concentrations of two major fallout radioisotopes on glaciers, one natural (^{210}Pb) and one anthropogenic (^{137}Cs) (Clason et al., 2023). Their activity concentrations for samples collected in 2021 were measured using a low-background, digital gamma-ray spectrometer equipped with a Broad Energy Germanium (BEGe) detector, BE5030, with a relative efficiency of about 48% and a multilayer passive shield surrounded by an active shield's detector (Gorzkiewicz et al., 2019). Efficiency calibration, including self-absorption correction, for the used measurement geometry was determined using LabSOCS calibration software (Mirion Technologies, USA). Samples collected in 2024 were analysed at the ISO/IEC 17025:2018-2-accredited laboratory of the Central Mining Institute – National Research Institute. Gamma counting was performed with a high-purity germanium (HPGe) detector, cooled by liquid nitrogen. A planar

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BEGe detector, BE5030 (Mirion Technologies, USA), with a relative efficiency of 50%, was used. The energy and efficiency calibrations were performed using a multi-gamma source containing isotopes that emit gamma lines over a wide energy range (46–2000 keV). This calibration source was prepared with reference materials containing natural radionuclides (RGU-1, RGTh-1, and RGK-1) provided by the International Atomic Energy Agency (IAEA). Calibration relationships were established using Canberra Genie 2000 software (Mirion Technologies, USA). The spectra were collected over approximately 24 h, or, for highly radioactive samples, over 12 h. The activity concentrations were corrected on the day of sampling. ^{137}Cs activity was determined by measuring the 661.6 keV emission peak of ^{137m}Ba, while for ^{210}Pb, the 46.5 keV peak was used as an analytical signal. Data quality was evaluated by measuring the IAEA Reference Material (IAEA-447) (IAEA, 2004) listed in Supplementary Table 1.

2.4 Amplicon sequencing microbial community assessment – library preparation and sequencing

Total DNA from cryoconite sediment was extracted using the FastDNA SPIN Kit for Soil from approximately 0.5 ml of wet cryoconite, following the manufacturer's instructions. Hypervariable V4 16S rDNA and V9 18S rDNA regions were used to assess bacterial and eukaryotic communities, respectively. Primers were tailed at 5′ ends with dual-indexed Ion Torrent adapters for sequencing using the Ion Torrent system (Life Technologies, USA). Primer pairs used in this study are presented in Supplementary Table 2, following previous studies (Lane, 1991; Makowska et al., 2020; Medlin et al., 1988; Therese et al., 1998). The target regions were amplified in two technical replications. Each PCR was carried out in a 10 µl reaction containing Hot FIREPol DNA Polymerase (Solis BioDyne, Tartu, Estonia), 0.25 µM of each indexed primer and 1 µl of DNA template. Amplification was initiated with a 95°C denaturation step for 12 min, followed by 30 cycles of 15 s at 95°C, 30 s at 50°C, and 30 s at 72°C, with a final extension at 72°C for 5 min. A blank sample was prepared for each DNA isolation kit and PCR. Amplicons of the corresponding length to the genetic markers were pooled and purified using a 2% E-Gel SizeSelect II Agarose Gel System (Invitrogen, Thermo Fisher Scientific, USA). The concentration and amplicon-length distribution of the libraries were assessed using the High Sensitivity D1000 Screen Tape assay on a 2200 Tape Station system (Agilent Technologies, USA). Clonal template amplifications were performed using the Ion Torrent One Touch System II and Ion Torrent OT2 Kit (Life Technologies, Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Sequencing was carried out using the Ion 540 Kit-OT2 and the Ion S5 system on an Ion 540 chip (Life Technologies, USA) according to the manufacturer's instructions.

2.5 Metatranscriptomic – library preparation and sequencing

Samples were thawed on ice and then vortexed vigorously. After that, the PowerProtect solution was washed with RNase-free Phosphate-Buffered Saline (1x) in a 4:1 ratio. The precipitate after centrifuging was split for RNA and DNA isolation. Total RNA was extracted using the RNeasy PowerSoil Total RNA kit (Qiagen) according to the manufacturer's standard protocol, with RNA precipitation extended overnight instead of 10 minutes to maximise RNA yield. The DNA was isolated using the DNeasy PowerSoil Pro Kit for Soil (Qiagen) following the manufacturer's standard protocol.

The residual DNA after total RNA extraction was degraded using RQ1 RNase-free DNase (Promega) according to the protocol. Afterwards, the RNA was purified again using the RNeasy PowerClean Pro CleanUp kit (Qiagen). The quality of isolated total RNA was assessed using the High Sensitivity RNA ScreenTape on the TapeStation (Agilent). One sample was rejected on this step due to low RINe (4.8). Total RNA was purified using SPRI bead–based cleanup with KAPA Pure Beads (KAPA Biosystems, Cat. No. 07983271001) at a sample-to-bead ratio of 1:2.2. rRNA depletion was performed using the QIAseq FastSelect –5S/16S/23S Kit (Qiagen, Cat. No. 335925). Sequencing libraries were prepared with the KAPA RNA HyperPrep Kit (KAPA Biosystems, Cat. No. 08098107702) according to the manufacturer's instructions, starting from 500 ng of input RNA or the maximum available amount. KAPA UDI Index adapters (KAPA Biosystems, Cat. No. 08098140702)

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were used for library indexing. RNA fragmentation was carried out at 89 °C for 3-4 minutes. Libraries were amplified using 8 or 14 PCR enrichment cycles. The last two steps were dependent on the initial RNA length and amount.

DNA samples were fragmented by sonication using a Covaris S220 instrument to obtain an average fragment size of approximately 500 bp (Duty Factor: 5%; Peak Incident Power: 175; Cycles per Burst: 200; Treatment Time: 35 s). Sequencing libraries were prepared from the fragmented DNA using the KAPA HyperPrep Kit (Roche, Cat. No. KK8504) and KAPA UDI Index adapters (KAPA Biosystems, Cat. No. 08098140702), following the manufacturer's recommendations with 100 ng of input DNA. Libraries were amplified with 7 cycles of PCR enrichment. Size selection for fragments of approximately 500 bp was performed using KAPA Pure Beads (Roche, Cat. No. 07983298001) with a two-step bead-based cleanup at the following sample-to-bead volume ratios: (i) 1:0.6 and (ii) 1:0.8.

The resulting libraries were assessed for fragment size distribution using the Agilent TapeStation 4150 system with High Sensitivity D1000 ScreenTape and Reagents (Agilent, Cat. No. 5067-5585). Library concentrations were then determined by quantitative PCR using the KAPA Library Quantification Kit (KAPA Biosystems, Cat. No. 07960298001), following the manufacturer's protocols.

Sequencing was performed on an Illumina NovaSeq 6000 instrument using the NovaSeq 6000 SP Reagent Kit v1.5 (300 cycles) (Illumina, Cat. No. 20028400) in paired-end mode (2 × 150 bp), according to the manufacturer's standard protocol, with the addition of 1% PhiX control library (Illumina, Cat. No. FC-110-3002). 15 RNA samples were first sequenced to assess the quality of rRNA depletion, using the most frequent reads and the proportion of duplicates. After this step, the 10 samples with the highest mRNA content were resequenced to achieve approximately 80 million RNA-seq reads per sample, compared to 30–50 million reads per sample for DNA sequencing. A higher RNA sequencing depth was chosen to ensure sufficient transcriptome coverage, as even after rRNA depletion, some rRNA and other non-coding RNAs remained, and environmental samples exhibit high transcriptomic complexity with many low-abundance mRNAs.

2.6 Tardigrades abundance estimation

Tardigrades were manually isolated from the sediment under a stereomicroscope, placed on Petri dishes, and counted. To facilitate scanning of Petri dishes, the bottom of each dish was covered with perpendicular lines. The volume of sediment was measured before tardigrade isolation and used as an offset in data modelling. During this procedure, the presence of other invertebrates was assessed to validate results from 18S rDNA V9 amplicon sequencing.

2.7 Amplicon-based microbial community assessment – data preprocessing

Raw sequences were pre-filtered by Ion Torrent Suite software version 5.10.1 (Life Technologies, USA) to remove polyclonal and low-quality sequences. The quality of sequences was assessed by FastQC and MultiQC software (Andrews, 2010; Ewels et al., 2016) after removing 5' and 3' primers. Leading and trailing nucleotides were trimmed based on the drop in quality, as visually inspected by MultiQC. If any leading bases with a quality score lower than or equal to 2 were present after preliminary trimming, they were also removed. Sequences were clustered in ASVs using DADA2 (Callahan et al., 2016), with no gaps allowed and a maximum EE equal to 1.5 in the case of V9 18S rDNA and 3 in the case of V4 16S rDNA (the maximum EE is length dependent, and the V4 amplicons were longer than the V9 ones). Chimaeras (11.66 % of 16S V4 filtered reads and 1.69% of 18S V9 filtered reads) were discarded from further analysis. Detailed procedure and error-rate model visualisations are presented in the repository linked at the end of this section. The total loss of reads after denoising is presented in Supplementary Table 3. Finally, the ASVs were taxonomically assigned using the SILVA databases (16S rDNA V4, release 138.1; 18S rDNA V9, release 128) with a Naïve Bayesian Classifier (Wang et al., 2007). For eukaryotes, all reads assigned to Fungi were excluded because the primer pair used was not validated for fungal diversity analysis. Moreover, reads belonging

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to Arthropoda and Nematoda were excluded, and observations under a stereoscopic microscope confirmed their absence in each sample.

The relative abundance of high-rank taxa in 16S V4 and 18S V9 rDNA was calculated to visualise variation between glaciers. Before abundance analysis, the data were normalised for differing sample depths by random sampling without replacement to 10,000 reads for 16S V4 rDNA (6 samples with less than 10,000 reads were discarded) and to 17,975 (the minimum sample size) for 18S V9 amplicons (see *Strengths and limitations of the study* section).

To understand whether biodiversity can be shaped by elevated radioactivity, the number of ASVs in the rarefied samples was used as a proxy of richness. Moreover, in the case of bacterial amplicons, we also used the abundance-based Shannon-Wiener Index for diversity measure and Pielou's Index for community evenness measurement, calculated using the Phyloseq package (McMurdie and Holmes, 2013) R 4.3.2 (R Core Team, 2024). Such an approach was not applied to eukaryotes, as the relationship between read count and population abundance can be highly biased for taxa that include both unicellular and multicellular organisms. Due to the relatively short length of 18S V9 rDNA amplicons (100-130 bp), most ASVs were not classified with the required confidence score (95) by the Naïve Bayesian Classifier, therefore, they were classified with a minimum bootstrap of 60. This approach allows avoiding the removal of numerous ASVs, while a threshold of 60 remains informative for short sequences, because the decrease in classification accuracy is less pronounced as sequence length decreases (Wang et al., 2007). We stress that our primary goal was to analyse changes in diversity across environmental radioactivity based on ASV richness, whereas the taxonomic classification was used solely for descriptive purposes.

The relationship between radioactivity and the abundance of DNA repair, metabolic, and stress-response pathways was assessed using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) analysis (Douglas et al., 2020), which, together with the KEGG Orthology database, enabled annotation of bacterial metabolic pathways in each sample. An ASV list with a raw abundance table for bacterial taxa was used as input. The raw dataset was reduced by removing extremely rare ASVs (i.e., those with < 5 reads across all samples). Following PICRUSt2 inference, 11 ASVs were removed because they did not align with the reference sequences. Moreover, 113 of 5322 ASVs exceeded the maximum NSTI cutoff of 2.0 and were removed from downstream analyses. The list of KEGG pathways analysed in the study, with codes, is in Supplementary Table 4.

2.8 Metatranscriptomic – data preprocessing

Raw paired-end reads were quality-filtered and trimmed using Trimmomatic v0.39 with the default Phred33 encoding. Adapter sequences were removed, bases with a quality score below 3 at the leading and trailing ends were trimmed, a sliding window of 4 bases was used to trim regions with an average quality below 15 and reads shorter than 36 bases were discarded.

High-quality DNA and RNA reads were processed with HUMAnN3 to profile microbial functional potential (DNA) and gene expression (RNA). For each sample, paired and unpaired reads remaining after Trimmomatic trimming were merged and used as input, since HUMAnN3 does not utilise pairing information. HUMAnN3 first aligned reads to the ChocoPhlAn pangenome database to quantify gene families, and unaligned reads were translated and aligned to the UniRef90 protein database. Initial gene family abundances were stratified by species; however, because taxonomic resolution was limited and many functions are shared across taxa, particularly core cellular processes, we generated unstratified tables to summarise total gene abundance independent of taxonomy. Gene abundances were then mapped from UniRef90 identifiers to KEGG Orthology (KO) terms to allow functional interpretation and comparison with PICRUSt2 analyses. During analysis, gene counts were normalised by gene length and further adjusted to copies per million (CPM) to account for differences in library size, producing values comparable across samples and DNA/RNA libraries. The resulting KEGG Ortholog abundance tables were used for downstream analyses and aggregated into KEGG pathways (Supplementary Table 4).

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2.9 Data analysis

Negative binomial (quadratic parameterisation) generalised linear models (GLMs) were built separately for bacterial and eukaryotic communities to test whether elevated environmental radioactivity was related to diversity indices and, in the case of bacteria, evenness. To avoid pseudoreplication, variables were averaged at the glacier level prior to analysis. Given the dynamic nature of supraglacial ecosystems, our focus was on differences between glaciers rather than within them. These models included the number of ASVs as the response variable, the total activity concentration (sum of ^{137}Cs and ^{210}Pb), the interaction between total activity concentration and the ^{210}Pb:^{137}Cs ratio, and glacier surface area and altitude as fixed effects. This structure enabled testing of the overall relationship between elevated radioactivity and biodiversity indices while accounting for potential differences between the effects of ^{210}Pb and ^{137}Cs through the interaction term.

Similarly, to test whether the abundance of tardigrades in cryoconite was related to elevated environmental radioactivity, a separate GLM was fitted with the number of individuals as the response variable and total activity concentration, along with the interaction between total activity concentration and the ^{210}Pb:^{137}Cs ratio, as fixed effects. In addition, the log-transformed sediment volume used to estimate tardigrade abundance was included as an offset.

To investigate functional responses using DNA-based pathway predictions from PICRUSt2, glaciers were ranked from least to most radioactive based on the average total activity concentration in cryoconite holes as a proxy. Mount Miné Glacier was excluded from this analysis because only three samples exceeded the minimum sequencing depth, which could reduce the precision of glacier-level estimates. From the remaining glaciers, we selected the seven with the lowest and the seven with the highest radioactivity levels and compared the abundance of metabolic pathways using Welch's t-test with a Benjamini-Hochberg correction for multiple testing. In the group with lower radioisotope activity concentrations, the average activity concentration was 2,439 Bq kg^{-1} for ^{210}Pb and 364 Bq kg^{-1} for ^{137}Cs. In contrast, in the group with higher radioactivity, the average activity concentration reached 5,187 Bq kg^{-1} for ^{210}Pb and 2,210 Bq kg^{-1} for ^{137}Cs.

Next, to examine RNA expression of selected pathways, log-normal GLMs were fitted with normalised RNA pathway abundance as the response variable, total activity concentration as the predictor, and normalised DNA pathway abundance as an offset (on the log scale). This approach accounted for variation in genomic potential across samples. Models were fitted separately for each pathway, and p-values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) procedure.

Model estimates were obtained using restricted maximum likelihood estimation. The significance of model terms was assessed using Wald χ^2 tests, evaluating each predictor's contribution while accounting for all other terms, including interactions (Fox et al., 2024). All statistical analyses were performed in R 4.3.2 (R Core Team, 2024) using built-in functions, the *glmmTMB* (Brooks et al., 2017) and ALDEx2 (Fernandes et al., 2013) packages. We assessed for any violations of assumptions using diagnostic plots generated with the *performance* package (Lüdecke et al., 2021).

3 Results

3.1 Gradient of environmental radioactivity across glaciers

The glacier surface habitat (cryoconite) in our study spanned a strong radioisotopes activity concentration gradient across glaciers, defined by previously published measurements of natural (^{210}Pb) and legacy anthropogenic (^{137}Cs) radioisotopes (Buda et al., 2024a). This study linked it to multilevel microbial community analyses of the same samples of 16 glaciers across the Alps, collected from the most glaciated areas in one year. Radioisotope activity concentrations varied strongly among glaciers (Fig. 1), spanning more than two orders of magnitude. Mean ^{137}Cs activity concentration ranged from 36 ± 35 Bq kg^{-1}

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(mean ± SD) on the Oberaar Glacier to 6,315 ± 2,803 Bq kg^{-1} on the Ventina Glacier, whereas ^{210}Pb ranged from 1,013 ± 576 Bq kg^{-1} on the Lang Glacier to 7,192 ± 2,210 Bq kg^{-1} on the Mont Miné Glacier. The observed activity concentrations are consistent with levels reported in cryoconite worldwide and represent a broad range of chronic environmental radioactivity capable of explaining variation in microbial richness, phylogenetic and functional diversity, the abundance of predicted stress-response genes and top-consumers population densities (Baccolo et al., 2020a; Clason et al., 2023; Miroshnikov et al., 2021; Owens et al., 2023; Tieber et al., 2009; Wilflinger et al., 2018; Wojciechowski et al., 2024).

Additionally, based on the results of the previous study (Buda et al., 2024a) we selected Dosdè Glacier as a suitable model glacier for studying the immediate effects of IR on glacier biota, given its relatively high radioisotope activity concentrations and strong variation within the glacier. Based on that knowledge, we did sampling in 2024, which showed a consistent perspective with previous work (Buda et al., 2024a). Activity concentrations of cryoconite samples, for which we analysed microbial metatranscripts, ranged from 3,824 Bq kg^{-1} to 9,113 Bq kg^{-1} for ^{210}Pb, while those of ^{137}Cs ranged from 1,100 Bq kg^{-1} to 8,900 Bq kg^{-1}. Such a gradient allowed us to assess the effects of radioisotopes on the activity of core microbial metabolic pathways while controlling for time-dependent and spatial processes.

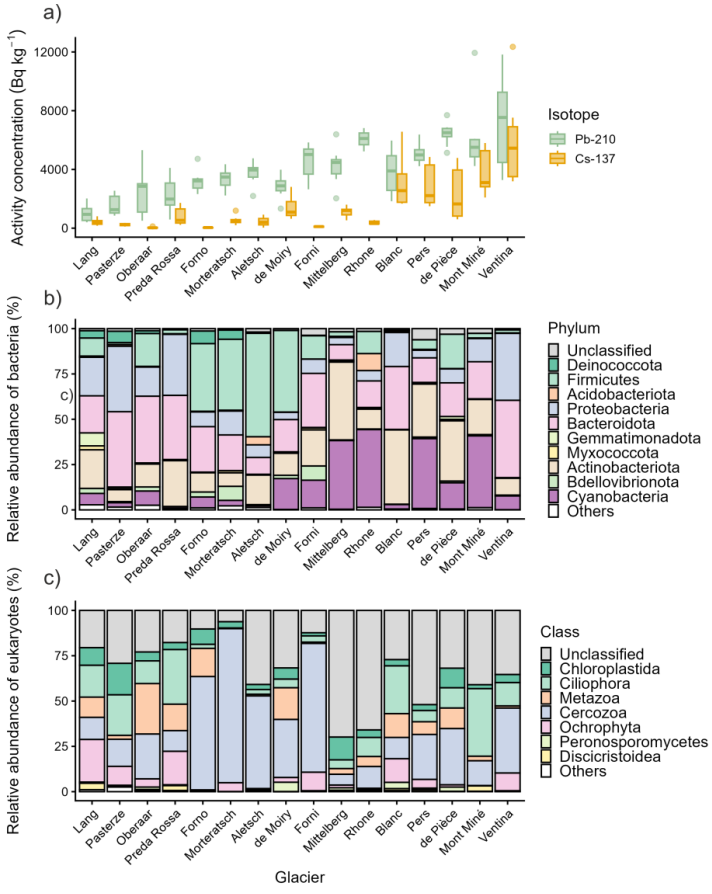

Figure 1. Radioactivity gradients across glaciers and associated variation in microbial community composition. (a) Variation in ^{137}Cs and ^{210}Pb activity concentrations in cryoconite samples, measured using gamma spectrometry with a BEGe detector. The horizontal line indicates the median, boxes represent the 25th–75th percentiles, whiskers show the minimum and maximum values, and dots represent outliers (1.5 × IQR). **(b)** Relative abundance of bacterial phyla

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across glaciers. **(c)** Relative abundance of eukaryotic classes across glaciers. "Others" (in phyla and classes) represents the sum of taxa with relative abundances below 0.1%.

3.2 High radioactivity is associated with lower taxonomic and phylogenetic diversity, but not top-consumer abundance

Microbial community structure was profiled using bacterial and eukaryotic ribosomal RNA gene amplicon sequencing, enabling an assessment of how chronic IR shapes taxonomic and phylogenetic diversity. After denoising and quality control, we identified 6,964 bacterial and 5,645 eukaryotic Amplicon Sequence Variants (ASVs) across all 157 cryoconite samples. Their relative abundances varied strongly among glaciers, however, with no clear pattern in relation to total radioactivity (Fig. 1).

Bacterial richness was negatively related to total radioactivity, expressed as the sum of ^{210}Pb and ^{137}Cs activity concentrations ($\chi^2 = 7.46$, df = 1, p = 0.006; Fig. 2a). This effect was independent of the ^{210}Pb:^{137}Cs activity ratio (interaction: $\chi^2 = 0.80$, df = 1, p = 0.37; Fig. 2c). Neither glacier size nor altitude influenced bacterial richness (size: $\chi^2 = 0.22$, df = 1, p = 0.637; altitude: $\chi^2 = 0.05$, df = 1, p = 0.815). Shannon diversity showed the same negative trend, although marginally non-significant ($\chi^2 = 3.49$, df = 1, p = 0.062) with no effects of other predictors (interaction: $\chi^2 = 0.01$, df = 1, p = 0.984; size: $\chi^2 = 0.76$, df = 1, p = 0.384; altitude: $\chi^2 = 0.05$, df = 1, p = 0.824), while evenness remained unaffected by total radioactivity ($\chi^2 = 1.49$, df = 1, p = 0.22), the radioisotope activity ratio (interaction term: $\chi^2 = 0.07$, df = 1, p = 0.78), or the control variables (size: $\chi^2 = 1.43$, df = 1, p = 0.232; altitude: $\chi^2 = 0.02$, df = 1, p = 0.876). Together with the lack of strong correlations between radioactivity and the relative abundance of specific bacterial phyla (Fig. 1; Supplementary Fig. 1), these results indicate that radioactivity is related to changes in bacterial diversity primarily by reducing the diversity of low-rank taxa rather than by shifts in dominance among high-rank taxa. Consistent with this pattern, phylogenetic diversity (Faith's Phylogenetic Diversity) also significantly declined along the radiation gradient ($\chi^2 = 7.58$, df = 1, p = 0.006; Fig. 2e), indicating a reduction of evolutionary history.

Microeukaryotic richness was also negatively related to total radioactivity ($\chi^2 = 3.97$, df = 1, p = 0.046; Fig. 2b). The effect showed a marginal dependence on the ^{210}Pb:^{137}Cs ratio ($\chi^2 = 3.63$, df = 1, p = 0.057; Fig. 2d), suggesting slightly stronger effects of ^{210}Pb. No effects of glacier size or altitude were detected (size: $\chi^2 = 0.26$, df = 1, p = 0.606; altitude: $\chi^2 = 1.91$, df = 1, p = 0.167). Microeukaryotes occupy diverse ecological niches in cryoconite, including mineral surfaces and organic matter. Previous work has shown that ^{137}C is more strongly associated with mineral particles, whereas ^{210}Pb is more closely linked to organic matter and developing communities (Buda et al., 2024b). These associations may contribute to differential impacts of radionuclides, however, given the low taxonomic resolution and ecological variability of detected ASVs, such interpretations remain tentative and require more detailed studies of specific eukaryotic groups on glaciers.

Across samples, 24 ASVs were classified as the glacier-dwelling tardigrade *C. klebelsbergi*, with two dominant ASVs comprising over 99% of sequences. Despite reductions in microbial diversity, we found no relationship between total radioactivity and the top consumer abundance, i.e., the tardigrade *Cryobiotus klebelsbergi* ($\chi^2 = 0.04$, df = 1, p = 0.839; Fig. 2f). Given the extremely low variation in ASV number per sample (1.45 ± 0.94 SD), separate diversity analyses were not performed for *C. klebelsbergi*.

These results indicate that chronic environmental radioactivity from elevated activity concentrations of fallout radioisotopes can selectively reduce microbial taxonomic and phylogenetic diversity while leaving higher trophic levels stable, as evidenced by no shifts in the dominant bacterial and eukaryotic groups and no effects on top-consumer abundance (Fig. 1 and Supplementary Fig.1).

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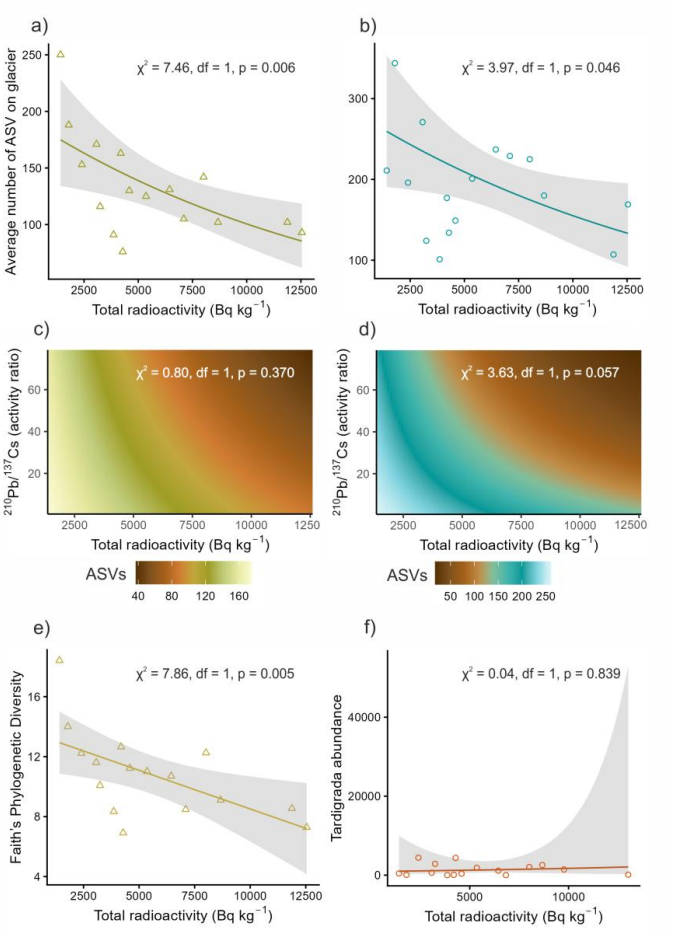

Figure 2. Relationships between environmental radioactivity, microbial diversity, and top-consumer abundance in cryoconite across glaciers. The upper panels **(a, b)** show relationships between total radioactivity and raw ASV richness: **(a)** bacteria, **(b)** microeukaryotes. The middle panels **(c, d)** show the interaction between the ^{210}Pb:^{137}Cs ratio and total activity concentration in relation to ASV richness: **(c)** bacteria, **(d)** microeukaryotes. The lower panels show relationships between total radioactivity and bacterial phylogenetic diversity **(e)** and top-consumer abundance **(f)**. Total radioactivity refers to the sum of ^{137}Cs and ^{210}Pb activity concentrations; ASVs denote amplicon sequence variants. Visualisation is based on estimation of generalised linear models, lines represent model estimates, grey shaded ribbons indicate 95% confidence intervals, and dots represent residuals. Panels **(a–e)** were generated from models based on amplicon sequencing data from 151 (bacteria) and 157 (microeukaryotes) cryoconite samples collected across 16 glaciers, while panel **(f)** was based on 160 cryoconite samples from the same glaciers. To avoid pseudoreplication, samples within each glacier were treated as replicates and averaged, and models were fitted using glacier-level means (n = 16).

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3.3 Functional simplification despite compositional stability

Whether radioactivity-associated diversity loss translates into changes in ecosystem functioning was examined using metabolic potential inferred from PICRUSt2-based KEGG Orthology annotations derived from bacterial community composition. Functional richness in cryoconite was significantly lower on glaciers where the sampled cryoconite had higher radioisotope activity concentrations compared to glaciers with lower cryoconite radioactivity (Wilcoxon rank-sum test, $W = 3$, $n = 14$, $p = 0.004$; Fig. 3a). However, when we examined the abundance of KEGG Orthologs lost in the higher-radioactivity glaciers, we found that most of these functions in low-radioactivity glaciers were of low abundance (Fig. 3). These results, together with the lack of significant Bray–Curtis functional dissimilarity between glaciers differing in cryoconite radioactivity (PERMANOVA: $R^2 = 0.048$, $F = 0.606$, $n = 14$, $p = 0.588$), indicate that the overall functional structure remained similar across glaciers. Altogether, these findings suggest that increasing environmental radioactivity is associated with functional simplification but does not induce major shifts in dominant metabolic pathways. The reduction in functional richness appears to reflect the loss of rare functions rather than a restructuring of the overall metabolic profile, implying functional redundancy within cryoconite communities.

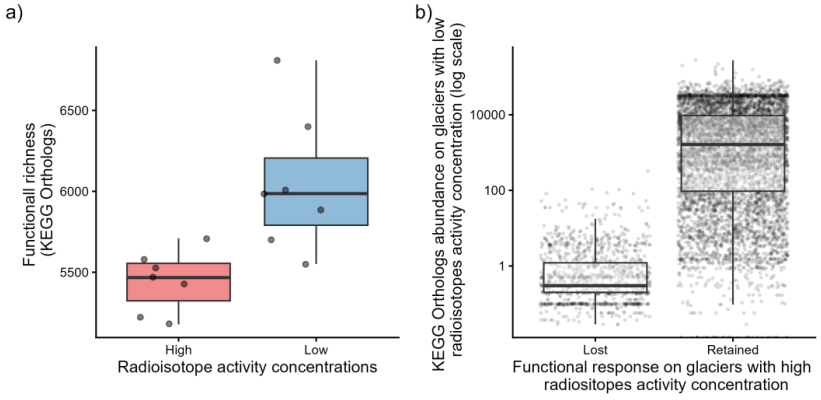

Figure 3. Functional diversity of cryoconite on glaciers with low and high radioisotope activity concentrations. (a) Functional richness in cryoconite on glaciers with low versus high activity concentrations of ^{137}Cs and ^{210}Pb. **(b)** KEGG Orthologs abundance lost or retained on glaciers with higher radioisotope activity concentrations in cryoconite compared to those with lower activity concentrations. The horizontal line indicates the median; boxes represent the 25th–75th percentiles; whiskers extend to the most extreme values within 1.5 × IQR; and points represent observations including values beyond 1.5 × IQR. In panel **(a)**, functional richness is shown as glacier-level averages for glaciers classified as having low or high radioisotope activity concentrations (n = 14; 7 glaciers per group). These averages were derived from 137 separate cryoconite samples and used to estimate glacier-level means. The same glacier-level dataset was used to generate panel **(b)**, but focusing not on overall functional richness, but on the abundance of KEGG Orthologs classified as lost or retained in glaciers with higher radioactivity compared to those with lower radioactivity.

3.4 Selective enrichment of DNA-repair capacity under chronic radiation

To assess whether chronic IR exposure selectively enriches key cellular pathways, including DNA repair and stress-related mechanisms, we compared the predicted relative abundance of 15 targeted KEGG pathways between glaciers with cryoconite classified as low- versus high-radioactivity based on total radioisotope activity concentration. Among all analysed pathways,

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non-homologous end joining (NHEJ; ko03450) was the only pathway showing a significant difference after correction for multiple testing (Welch's t-test, BH-adjusted p = 0.027; Fig. 4). NHEJ was more abundant in highly contaminated glaciers (mean ± SD: 0.0066 ± 0.0011) than in low-radioactivity sites (0.0043 ± 0.0009), corresponding to an approximately 52% higher predicted relative abundance. Other DNA repair pathways, including base excision repair, nucleotide excision repair, mismatch repair, and homologous recombination, as well as stress-related mechanisms, did not show consistent differences across radiation levels (Fig. 4).

These results indicate that, among the analysed pathways, enrichment under chronic radiation is specific to NHEJ, suggesting a selective increase of genes involved in template-independent double-strand break repair in bacterial communities exposed to elevated environmental radioactivity.

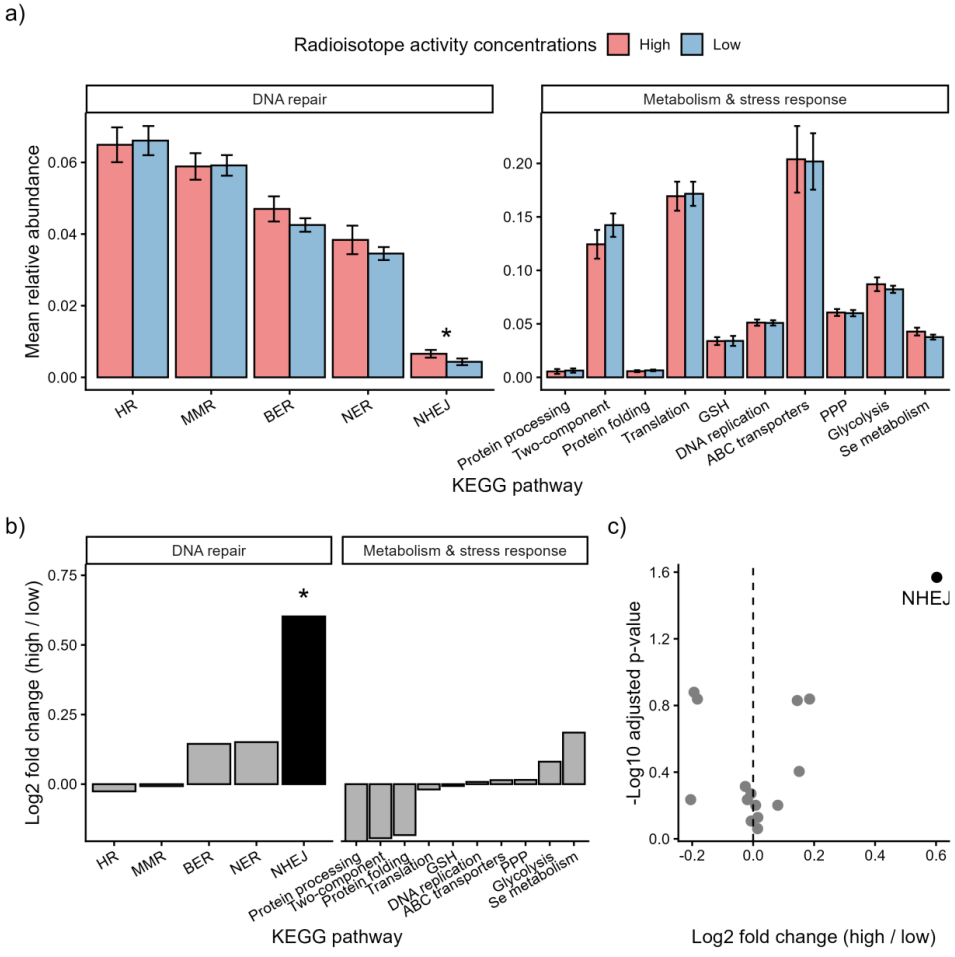

Figure 4. Changes in DNA repair, metabolic, and stress response pathway predictions in cryoconite on glaciers with low and high radioisotope activity. (a) Differences in mean relative abundance between groups. **(b)** Log2 fold change (High/Low). **(c)** Log2 fold change versus BH-adjusted p-value. Differential abundance was tested using ALDEx2 (Welch's t-test with Benjamini–Hochberg correction). Pathways shown in bold and marked with an asterisk differ significantly between groups (adjusted $p < 0.05$). Pathway abbreviations: HR – Homologous Recombination; MMR – Mismatch Repair; BER – Base Excision Repair; NER – Nucleotide Excision Repair; NHEJ – Non-

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Homologous End Joining; Two-component – Two-component system; GSH – Glutathione metabolism; PPP – Pentose Phosphate Pathway (NADPH); Se metabolisms – Selenocompound metabolism. All panels are based on KEGG pathway abundance (PICRUSt2 inferred) expressed as glacier-level averages for glaciers classified as having low or high radioisotope activity concentrations (n = 14; 7 glaciers per group). These averages were derived from 137 separate cryoconite samples and used to estimate glacier-level means.

3.5 Metabolic pathway expression declines with increasing radioisotope concentrations

Dosdè Glacier, selected for its high and heterogeneous levels of radioisotope activity concentrations, was analysed using metatranscriptomic to quantify transcriptional responses of DNA repair and core metabolic pathways across the radiation gradient. Across the 15 targeted pathways, expression (RNA transcripts per DNA copy) showed an overall declining trend as radioactivity increased, with five remaining significant after FDR correction ($p < 0.05$; Fig. 5).

Among the DNA-repair pathways, homologous recombination showed a significant negative linear relationship with total radioactivity ($\beta = -0.0754$, SE = 0.0264, $z = -2.86$, pFDR = 0.022; Fig. 5). Four of the ten metabolism- and stress-related pathways – glycolysis ($\beta = -0.0688$, SE = 0.0245, $z = -2.81$, pFDR = 0.022), selenocompound metabolism ($\beta = -0.0858$, SE = 0.0311, $z = -2.75$, pFDR = 0.022), the two-component system ($\beta = -0.0654$, SE = 0.0260, $z = -2.51$, pFDR = 0.036), and the pentose phosphate pathway (NADPH branch) ($\beta = -0.0897$, SE = 0.0299, $z = -3.01$, pFDR = 0.022) – also showed significant negative relationships with radioactivity (all FDR-corrected $p < 0.05$; Fig. 5). Effect sizes represent the change in pathway abundance per 1 kBq kg^{-1} increase in the combined activity concentration of ^{137}Cs and ^{210}Pb.

Notably, pathways enriched at the DNA level did not exhibit increased transcription under higher radiation, indicating that genomic enrichment does not translate into elevated functional activity. Together, these negative relationships over a variety of cellular processes indicate that chronic radiation can be associated with broad suppression of transcriptional activity, particularly affecting central metabolic functions related to growth and reproduction.

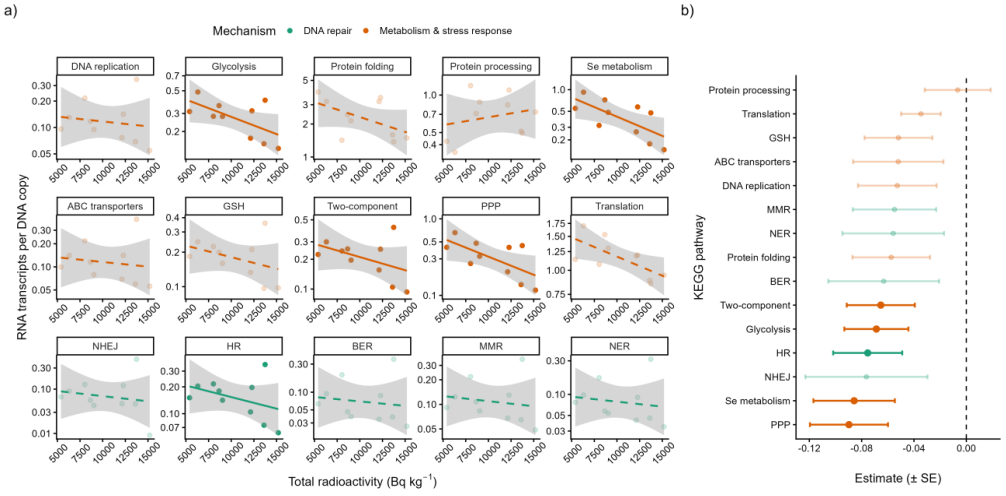

Figure 5. Relationship between total radioactivity and the expression of DNA repair, metabolic, and stress-response pathways, quantified as RNA transcripts per DNA copy. (a) Linear model fits. **(b)** Model estimates. Pathways shown in bold with solid lines are significantly related to total radioactivity after false discovery rate correction (adjusted $P < 0.05$), effect sizes represent the change in pathway abundance per 1 kBq kg^{-1} increase in the combined activity concentration of ^{137}Cs and ^{210}Pb. Pathway abbreviations: HR – Homologous Recombination; MMR –

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Mismatch Repair; BER – Base Excision Repair; NER – Nucleotide Excision Repair; NHEJ – Non-Homologous End Joining; Two-component – Two-component system; GSH – Glutathione metabolism; PPP – Pentose Phosphate Pathway (NADPH); Se metabolisms – Selenocompound metabolism. Plots were generated from models based on metatranscriptomic data from 10 distinct cryoconite samples collected on a single glacier. For each sample, DNA and RNA were sequenced separately and included in the models.

4 Discussion

4.1 Significance of this study in understanding the effects of IR on microbial communities

In the 20th century, anthropogenic activities released radionuclides across the globe, leading to their long-term accumulation in the environment and contributing significantly to natural radioactivity (Mietelski, 2010). Most research on the biological consequences of ionising radiation has focused on human health and wildlife in areas associated with NPP accidents, as well as in highly contaminated areas such as Karachay Lake (Atamanyuk et al., 2012; Geras'kin et al., 2021; Moysich et al., 2002; Nybakken et al., 2023; Steinhauser et al., 2014). By contrast, ecosystems distant from nuclear accident sites – and particularly microbial communities within them – have received far less attention, despite microbes' central role in ecosystem functioning, including nutrient cycling, soil formation, and primary productivity (Videvall et al., 2023).

A correlative study in a contaminated area in North-West China has shown a decrease in bacterial diversity and abundance along a gradient of radioisotope activity concentrations, from 100–200 Bq kg^{-1} up to over 10,000 Bq kg^{-1} (Gu et al., 2014). The contamination gradient from nuclear activities in the above study matches the range observed on Alpine glaciers in our study. Another study using mesocosm experiments showed that soil bacterial diversity decreases as the IR dose increases, however, due to reduced competition, fungal and algal species take over their functions and increase in diversity (Ogwu et al., 2019). On the other hand, in situ observations in an area near the Chornobyl NPP (Videvall et al., 2023) have shown that elevated IR does not influence the diversity of microbial communities in aquatic habitats but can shape their distribution, as shown by variation in beta-diversity. Collectively, these studies demonstrate that environmental radioactivity can influence microbial diversity, providing a broader context for understanding the effects of IR on microbial communities.

Our research goes beyond these examples by demonstrating that elevated environmental radioactivity can act as a selective pressure on microbial communities inhabiting glaciers, a remote ecosystem in which such effects have not previously been documented. Using extensive sampling across 16 Alpine glaciers, we show consistent relationships between radionuclide activity concentrations and both taxonomic and phylogenetic diversity, as well as functional signatures indicating selective enrichment of key metabolic pathways under elevated radioactivity with microbial activity suppression at the local (within-glacier) scale. These findings provide the first field-based evidence that chronic exposure to accumulated radionuclides is associated with both the structure and functional traits of microbial communities in supraglacial ecosystems. These results are essential, as on Alpine glaciers, an important fraction of radioactivity is attributed to anthropogenic legacy, both from nuclear military activities and the Chornobyl NPP accident (Sala et al., 2025). Our findings demonstrate that anthropogenic radionuclides, along with natural sources, affect microbial communities even in remote glacier ecosystems, highlighting the far-reaching ecological consequences of nuclear activities.

4.2 Ecosystems implication for glaciers

Understanding the processes that affect organisms is crucial for assessing their ability to adapt to changing environmental conditions. On glaciers, atmospheric pollution has been proposed as an important factor shaping microbial communities, particularly those on glacier surfaces that interact directly with the atmosphere, precipitation, and meltwater (Pittino et al.,

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2023). Our results show that elevated radioisotope activity concentrations can act as important selective pressure on glacial organisms living in the cryoconite. At the glacier scale, transcriptomic activity relative to genomic potential of core pathways related to cell growth and division decreased with increasing radioisotope activity concentrations (Fig. 5), indicating suppressed microbial activity under elevated radioisotope exposure. Such a shift from growth to survival under chronic stress is a well-known phenomenon in bacteria, where stationary-phase cells are generally less sensitive to environmental stress (Gray et al., 2019; Hengge, 2014). On glaciers, this effect may be particularly pronounced because cryoconite is already characterised by persistently low temperatures, frequent freeze–thaw cycles and high UV levels, which can impose additional physiological stress on biota (Poniecka et al., 2020; Zawierucha et al., 2019a).

Across glaciers, higher radioactivity was associated with reduced microbial diversity, yet the overall functional structure remained stable, suggesting loss of rare functions rather than major shifts in dominant metabolic pathways. At the same time, the increased relative abundance of genes related to non-homologous end joining (NHEJ), a key DNA repair pathway in dormant or slow-growing cells (Pitcher et al., 2007), suggests that microbial communities may adapt to chronic exposure by increasing their DNA repair capacity. Together with the lack of association between radioisotope activity concentrations and densities of top consumers (tardigrades), these results suggest that the overall functioning of cryoconite ecosystems may remain relatively stable despite taxonomic shifts in microbial communities. Given that tardigrades are among the most radiation-tolerant animals known and can survive ionising radiation doses far exceeding those observed on glaciers (Ghosh et al., 2025; Jönsson, 2019), changes in their abundance would more likely result from indirect ecological effects rather than direct radiation damage. Efficient DNA repair mechanisms likely support their persistence in cryoconite environments.

In summary, elevated levels of natural and anthropogenic radioisotopes in glaciers can act as an additional selective pressure on organisms, favouring those capable of coping with chronic stress, as reflected in increased NHEJ abundance and the maintenance of core functions (Figs. 3 & 4). Considering that, functionally, cryoconite ecosystems as key supraglacial ecosystems worldwide, do not appear to be limited by elevated radioisotopes, and key biochemical processes on glaciers seem largely maintained (Fig. 3). However, this selective filtering is accompanied by the loss of rare taxa and a suppression of microbial activity at the glacier scale (Figs. 2 & 5), which may have negative ecological consequences despite the preservation of major functions. Our conclusion is based on a single pollutant type, whereas glaciers efficiently accumulate diverse atmospheric contaminants that can also negatively impact supraglacial communities, including plastics, persistent organic pollutants (POPs), and heavy metals (Pittino et al., 2023). This highlights the growing importance of understanding pollutants on glaciers, as they serve as long-term reservoirs (Beard et al., 2022). With accelerating glacier melt due to climate warming, stored contaminants – including radionuclides – can be progressively released, potentially affecting glacier biota directly or being transported into downstream ecosystems via meltwater (Bogdal et al., 2010; Li et al., 2017; Xue et al., 2025). Understanding how glacial organisms respond to these multiple stressors and the broader ecological consequences of contaminant release is essential for predicting the resilience of supraglacial and downstream ecosystems under ongoing global change.

4.3 Strengths and limitations of the study

This study provides a large-scale field assessment of the relationship between environmental radioactivity and microbial communities inhabiting glacier surfaces. Intensive sampling across 16 Alpine glaciers, combined with analyses of microbial diversity, functional potential, and transcriptomic activity, enabled us to examine ecosystem-level responses to elevated fallout radionuclide activity concentrations. The results show that glacial microbial communities can be influenced by environmental radioactivity, with shifts in diversity patterns and an increased abundance of genes involved in major bacterial DNA repair systems, suggesting potential adaptation to chronic exposure, but reduced activity at a smaller scale.

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Several limitations should be considered. First, the study is correlative and therefore does not allow direct inference of causal mechanisms linking IR to observed biological patterns. Second, microbial diversity was assessed using rDNA, which may include DNA from inactive or dead organisms that can persist in cryoconite sediments under cold, organic-rich and often anaerobic conditions (Buda et al., 2022; Poniecka et al., 2018), thereby potentially masking the active fraction of microbial communities (Pittino et al., 2023). Third, environmental radioactivity was represented by radionuclide activity concentrations rather than radiation dose, but a reliable dose model for complex cryoconite systems is currently lacking. So far, only two studies have attempted to link cryoconite features to the spatial variation of radioisotopes or their mobility (Buda et al., 2024b; Davidson et al., 2023). All the above-mentioned issues are not easy to resolve due to logistical difficulties of studying glacial communities, which increase with the scale of the study. On the other hand, experimental setups that mimic specific glacial conditions in the laboratory are extremely difficult to prepare, especially at the community level and with open sources of radioactivity, while single-species short-term experiments do not reflect ecosystem-scale processes. In ecological studies, a trade-off between precision and spatial scale is often unavoidable. In this case, we chose the latter, but we also took a closer look at one glacier using RNA-based analysis, which complements our understanding of the problem.

Code and data availability

All data used in this study are publicly available. Raw amplicon sequencing and metatranscriptomic data are deposited in the Sequence Read Archive (NCBI) under accession numbers PRJNA1091145 and PRJNA1438628, respectively. Corresponding metadata and the full data-analysis pipeline are available in a public GitHub repository (https://github.com/jakbud1/Radioactivity-suppresses-glacier-microbes.git). All analyses can be reproduced by downloading the input data and running the provided scripts in the prepared environment. This study did not generate new biological materials.

Supplement link

The online version contains supplementary material Supplementary Figure 1 – 2 and Supplementary Table 1 - 4.
The link to the supplement will be included by Copernicus, if applicable.

Author contributions

Conceptualisation: JB; Methodology: JB; Formal analysis: JB, RA; Investigation: JB, RA, SB, KG, ON, MB, KS, KB, RS, RL, FP, AC; Resources: JB, EŁ, RA, KZ; Validation: JB; Visualisation: JB; Funding acquisition: JB, EŁ, KZ; Writing – original draft: JB; Writing – review & editing: All authors.

Competing interests

The authors have the following competing interests: Prof. Roberto Ambrosini serves as associate editor for the special issue to which this paper belongs.

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Disclaimer

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Review statement

The review statement will be added by Copernicus Publications listing the handling editor as well as all contributing referees according to their status anonymous or identified.

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