A TALE OF BACTERIA, VIRUSES AND SNOW: SOIL MICROBIAL DIVERSITY IN THE PYRENEAN CRYOSPHERE

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INTRODUCTION

Microbial communities are key to how newly exposed **cryospheric** and **subalpine soils** develop and function in the Central Pyrenees. **The Aneto Glacier** is experiencing a rapid shrinkage generating new proglacial enviroments, while subalpine catchments such as **Izas Catchment** experience marked changes in **snow cover duration** and seasonality. Across these connected environments, **bacteria**, **archaea** and **viruses** act as early **ecosystem engineers**, driving **carbon, nitrogen and sulfur cycling**, **initiating mineral weathering** and influencing **pathogenicity** and **antibiotic resistance** dynamics long before plants establish. Because snow cover duration modulates temperature, moisture and resource availability, it likely acts as a key filter on the composition and **functional potential** of these microbiomes.

Here, as part of the **MERIDIAN** project, we present ongoing **microbial shotgun metagenomic data** to investigate how **snow cover** structure **microbial diversity** and **functional potential** in Aneto proglacial soils, and to characterize how **microbial communities** are structured under **contrasting moisture regimes** in wetter and drier subalpine soils at the Izas Experimental Catchment in Huesca, northeast Spain.

MATERIALS AND METHODS

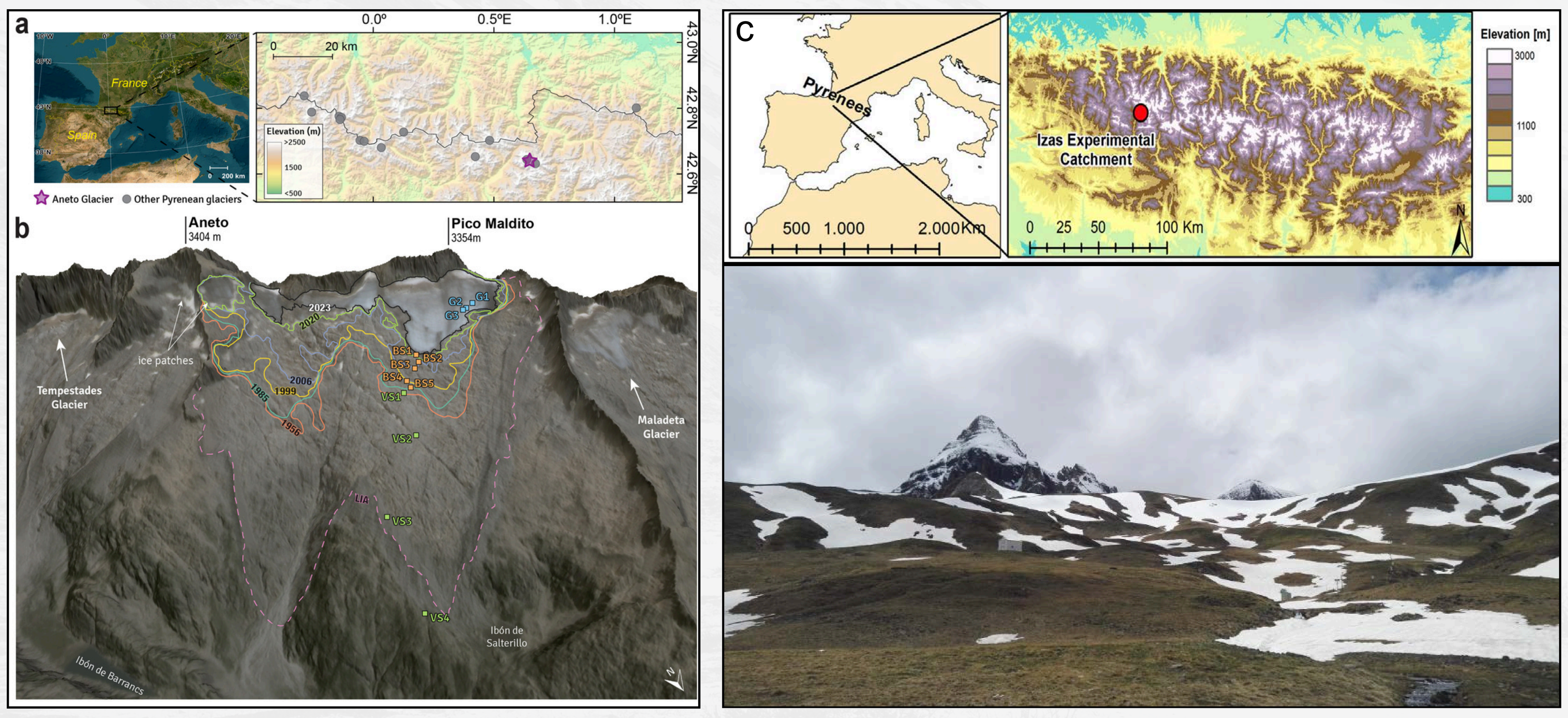


Fig. 1. Location of study sites presented in this study. Modified from 1Revuelto et al., 2021

Soil samples were collected along **ecological and environmental gradients** at Aneto proglacial soils (Fig 1. a, b) and Izas subalpine sites (Fig 1. c). **DNA** was extracted using standard soil DNA extraction protocols. Metagenomic libraries were prepared for shotgun sequencing and sequenced on Illumina platforms, followed by **quality control**, **assembly**, **gene prediction** and **taxonomic/functional** annotation to profile **microbial diversity**, **biogeochemical pathways** and **antibiotic resistance genes**.

Sample collection
Aneto Glacier, Izas Experimental Catchment

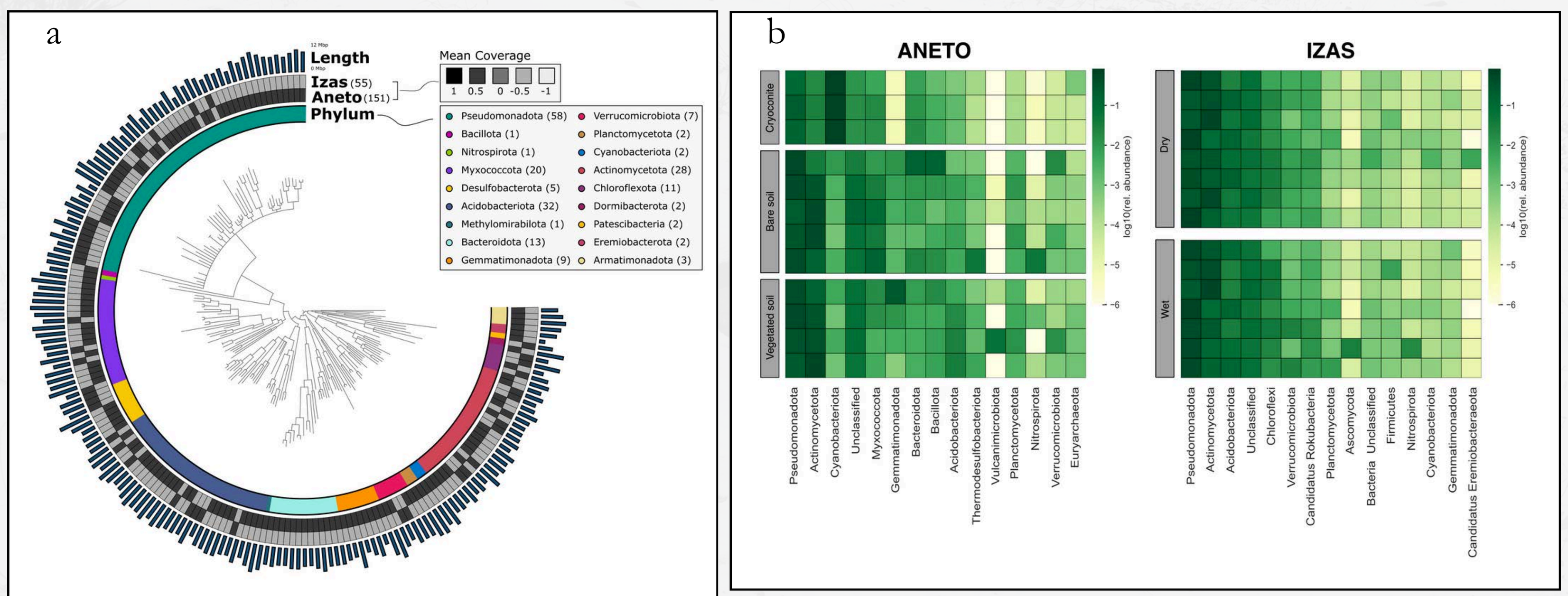
DNA Extraction
Library preparation and Sequencing

Shotgun Metagenomics
Assembly, Metagenome Assembled Genomes, Gene Prediction and Annotation

Microbiome, DNA virus analys
Diversity and functional analyses

RESULTS

1. MICROBIOME



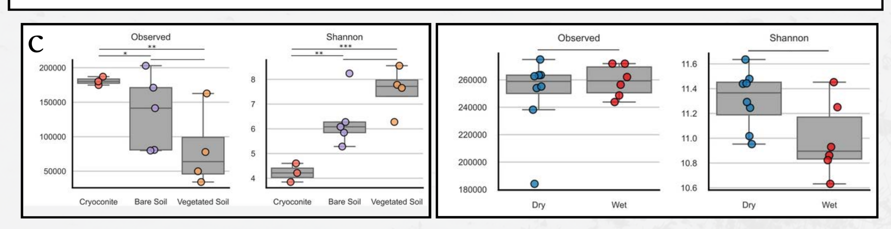
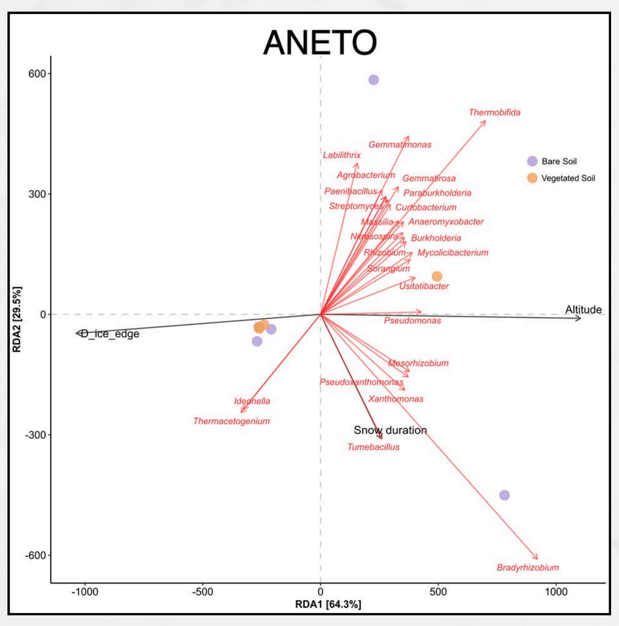


Fig. 2. Phylogenomic tree of MAGs recovered from Aneto and Izas, showing (from inside to outside) phylum affiliation (colored ring, numbers in parenthesis indicate MAG number), mean coverage normalized by MAG (grayscale ring), and genome length (outer bars) (a). Relative abundance (log10) of the 15 most abundant phyla at the contig level across study sites (b). Alpha diversity of Aneto and Izas (asterisks indicate the level of statistical significance between sites (c). From the 206 recovered MAGs, 55 originated from Izas and 151 from Aneto. In Aneto, MAGs affiliated to **Myxococcota, Bacteroidota and Cyanobacteriota** were particularly abundant, whereas no clear phylum predominance was observed in Izas. At the contig level, **Pseudomonadota and Actinomycetota** were the two most abundant phyla in both sites, although Cyanobacteriota showed a markedly higher relative abundance in Aneto **cryoconites**. For alpha diversity, Aneto cryoconites showed a **higher** number of **observed taxa** (17587 ± 121) but **lower Shannon** diversity (4.5 ± 0.8) compared with the surrounding soils, indicating lower evenness; in Izas, no significant differences were detected between dry and wet soils.

3. MICROBIAL COMMUNITY ORDINATIONS



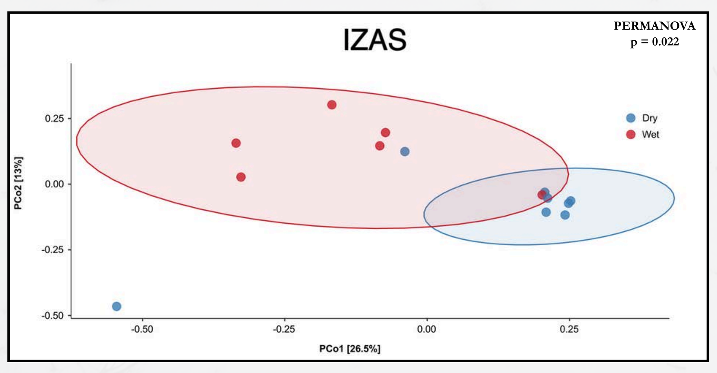


Fig 4. RDA of bacterial genera in Aneto constrained by environmental variables (a), and PCoA of Bray–Curtis dissimilarities for Dry and Wet soils in Izas with 95% confidence ellipses (b).

In Aneto, the RDA indicates that bacterial **genus composition** is strongly structured along gradients of **altitude and snow duration**. In Izas, the PCoA shows a slight separation between Dry and Wet soils, but PERMANOVA still detects a **significant effect of moisture** regime on community composition (p = 0.022), indicating statistically supported differences between groups.

2. DNA VIRUS COMMUNITY

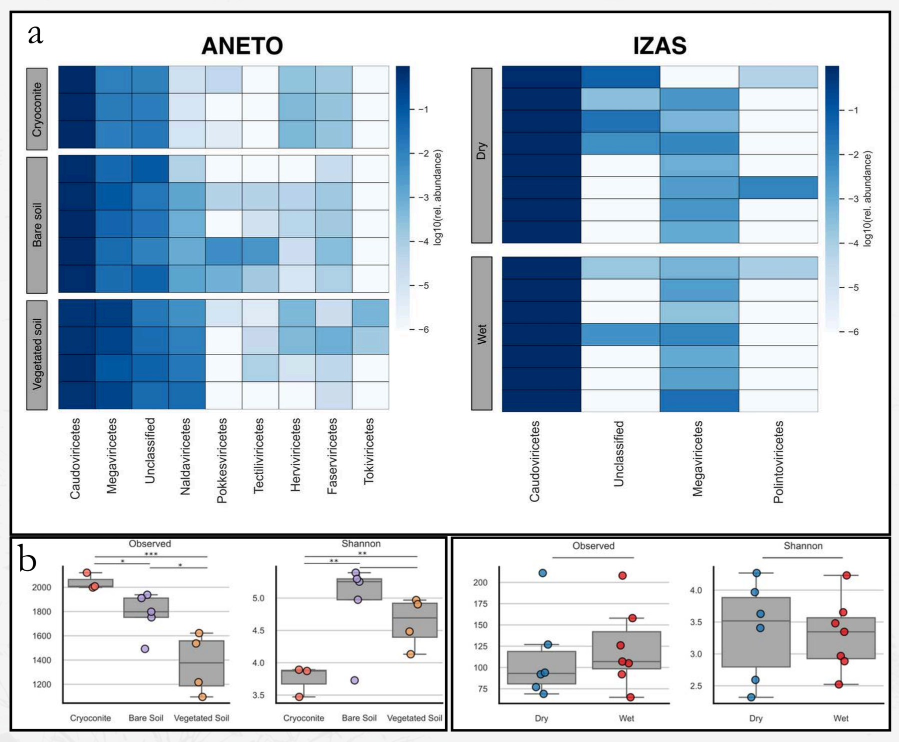


Fig. 3. Relative abundance (log10) of the most abundant virus classes across study sites (a). Alpha diversity of viral communities from Aneto and Izas (observed ASVs and Shannon index); asterisks indicate the level of statistical significance between sites (b).

In Aneto, we found similar dominant viral classes across sample groups, although only 3 viral classes were recovered from Izas. **Caudoviricetes dominated** in both study sites. Although **Megaviricetes** was the second most abundant class in Aneto, specially in vegetated soils.

Alpha diversity analysis shows that **cryoconites** show the **highest richness but the lowest Shannon diversity**, while bare and vegetated soils have lower richness but higher evenness, suggesting strong dominance of a few viral populations in cryoconites versus **more balanced communities in soils**.

In Izas, a more restricted set of viral classes and very **similar patterns** between dry and wet soils are shown. The alpha-diversity analysis confirm the absence of significant differences in richness or Shannon between conditions, indicating a relatively **homogeneous viral community** across moisture regimes.

4. FUNCTIONAL PROFILES

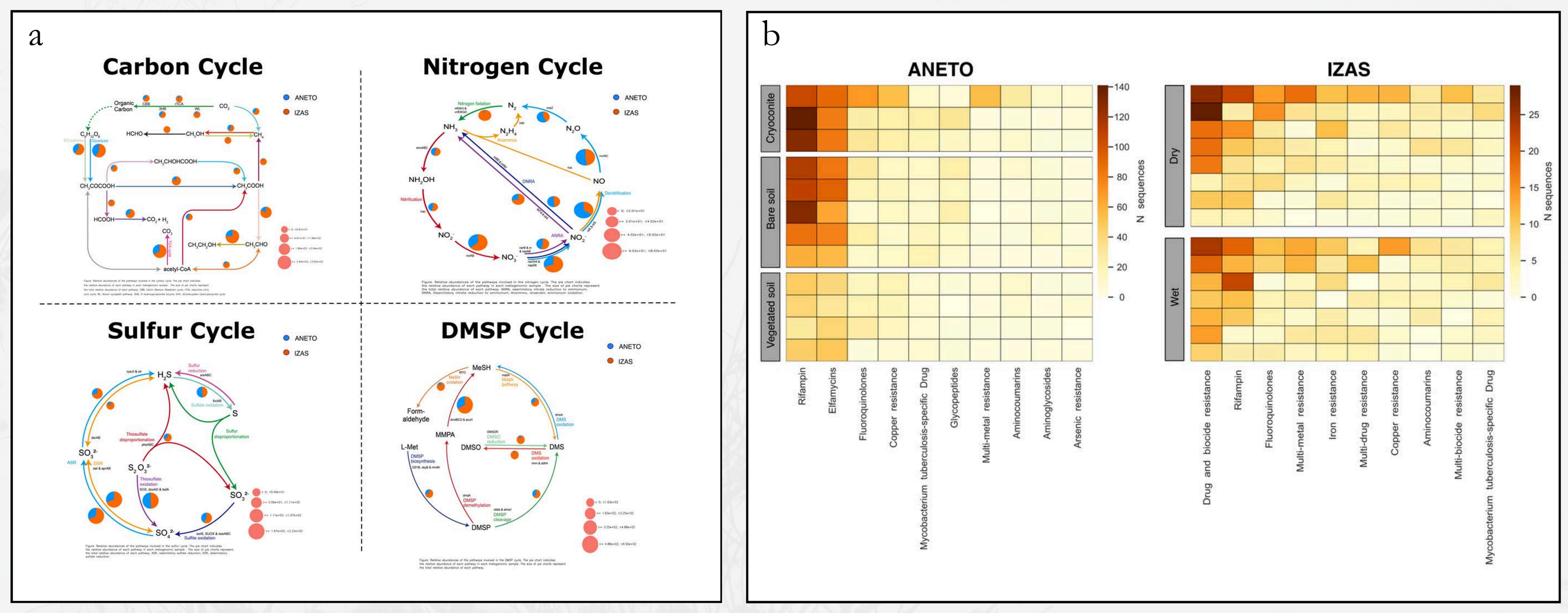


Fig 5. Abundance of pathways from the biogeochemical cycles (carbon, nitrogen, sulfur and dmsp) included in this study (a). Number of sequences assigned to each antibiotic, metal and biocide resistance gene across study sites (b).

Most pathways in the carbon, sulfur and DMSP cycles are **more abundant in the subalpine Izas soils** than in the proglacial Aneto site, indicating a more metabolically **versatile** community in Izas. In contrast, the relative **enrichment of denitrification** pathways in Aneto indicates that bacterial communities are specialized in converting **fixed nitrogen into gaseous forms** (NO, N_2O, N_2). In **cryoconites** and **bare soils** from Aneto, resistance genes are dominated by **antibiotic** related categories (e.g. rifampicin, elfamycins, fluoroquinolones). In Izas, dry and wet soils show higher representation of resistance genes linked to **biocides and metals** (multi-metal, iron, iron, copper), while specific antibiotic classes are less prominent.

CONCLUSION

This study shows that proglacial Aneto and subalpine Izas areas host **contrasting bacterial communities and functionality**. In Aneto, community composition is linked to **altitude and snow duration**, cryoconites contain many taxa but with strong dominance of a few lineages, functional profiles point to an overrepresentation of **denitrification and antibiotic resistance genes**. In Izas, dry and wet soils differ more subtly in composition, alpha diversity is similar between groups, and a **wider range of pathways** for carbon, sulfur and DMSP cycling, as well as **resistance genes related to metals and biocides**, consistent with developed subalpine soils that support diverse metabolic niches. Overall, our results indicate that along the Pyrenean study sites, **deglaciation and soil development** do not simply **increase bacterial diversity**, but **reorganize how communities are assembled and which biogeochemical and resistance processes they support**, with proglacial Aneto communities emphasizing nitrogen transformation and antibiotic resistance and Izas communities expressing a **broader set of metabolic functions** in developed soils.

Grant PID2022-136691NA-I00 funded by MICIU/AEI/ 10.13039/501100011033 and by the ERDF/EU.

Acknowledgements: Pablo Domínguez Aguilar for his help sampling at Aneto glacier

Bibliography: 1. Revuelto, J., Alonso-Gonzalez, E., Vidaller-Gayan, I., Lacroix, E., Izagirre, E., Rodríguez-López, G., & López-Moreno, J. I. (2021). Intercomparison of UAV platforms for mapping snow depth distribution in complex alpine terrain. Cold Regions Science and Technology, 190(103344), 103344. https://doi.org/10.1016/j.coldregions.2021.103344