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MICROBE
the Microbiome Biobanking (RI) Enabler

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY
TOMORROW TODAY

TOWARDS MICROBIOME PRESERVATION: THE FIRST INSIGHTS FROM SOIL MICROBIOME PRESERVATION EXPERIMENT

The EU-funded MICROBE (Microbiome Biobanking (RI) Enabler) project brings together leading research institutions, biological resource centers and European infrastructures to tackle the critical challenges in microbiome preservation and metadata management. In order to support future scientific research and industrial applications, MICROBE's approach involves the development of innovative and validated technological solutions that allow the long-term preservation of the maximum taxonomic and functional biodiversity in microbiome samples, along with comprehensive metadata storage.

To support the project's objectives, we developed a comprehensive experimental framework focused on the soil microbiome, a key target due to its complexity, the wide range of ecosystem services it provides, and its crucial role in human health through its impact on the food system[1,2,3].

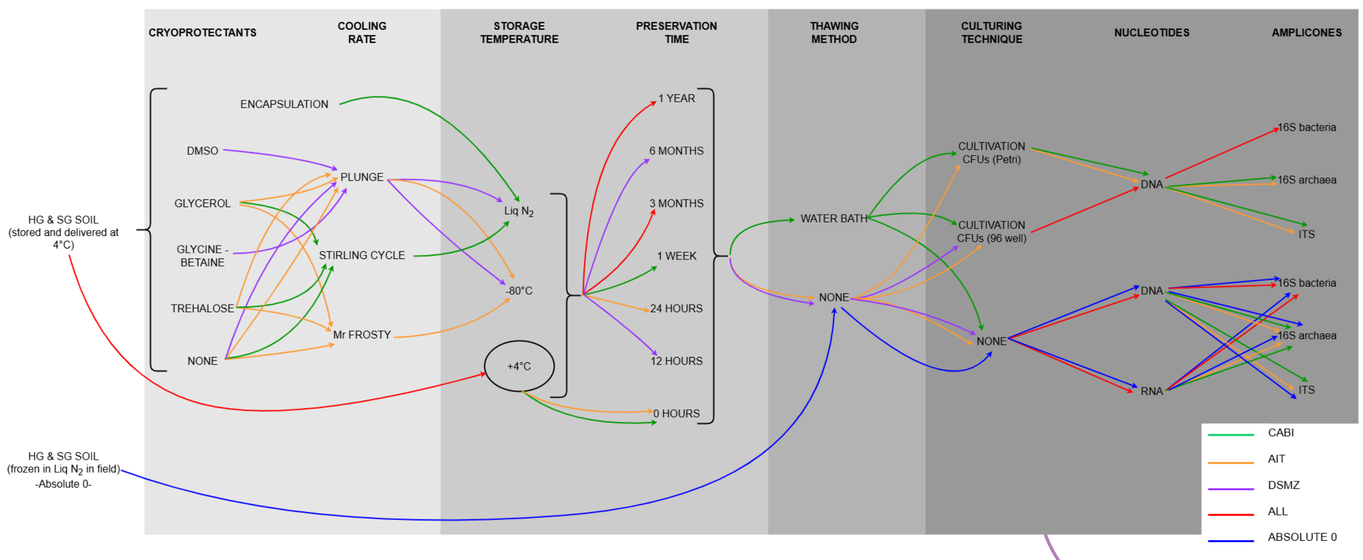
TWO SOIL SAMPLES

biodiversity exploratories – functional biodiversity research

THREE PARTNER INSTITUTES INVOLVED

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY · CABI · DSMZ Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH

FIRST ROUND



The three partner institutes tested:

- 16 preservation methods generated by different combinations of three variables (cryoprotectant, cooling rate, and storage temperature).
- Six different storage periods.
- Two thawing methods.

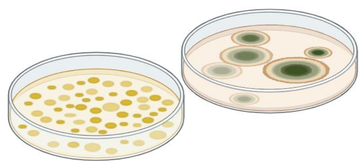
The samples were analyzed employing two complementary approaches:

- conventional or high throughput culture methods, followed by DNA extraction, amplification, and sequencing of the ITS region for fungi and 16S rRNA gene for bacteria.
- direct DNA and RNA extraction from preserved soil samples, followed by amplification and sequencing of the ITS region for fungi and 16S rRNA gene for bacteria.

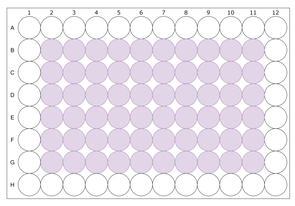
BioSamples — Each soil sample was entered into the BioSample database (EMBL-EBI) and linked to its associated metadata and experimental data.

CULTIVATION ASSAYS

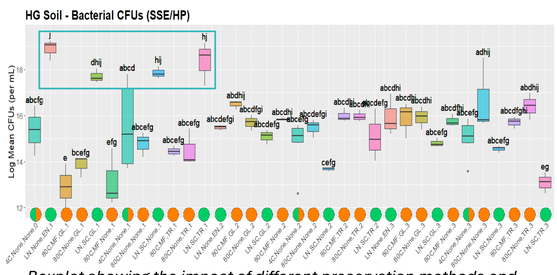
CONVENTIONAL - ON SOLID MEDIA -



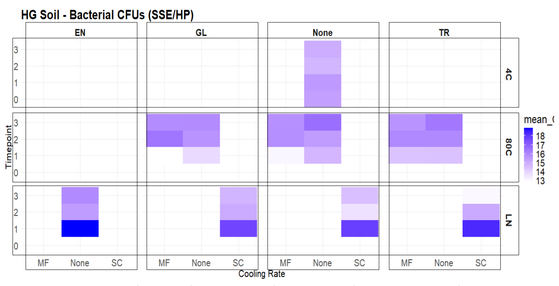
HIGH THROUGHPUT - IN LIQID MEDIA -



CFUs count



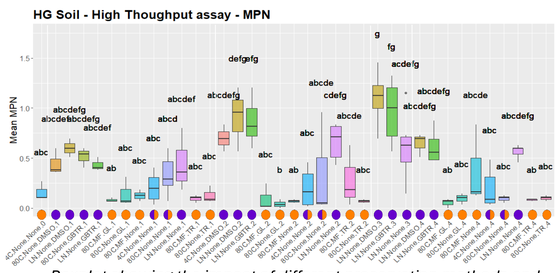
Boxplot showing the impact of different preservation methods and of the duration of storage on CFUs count.



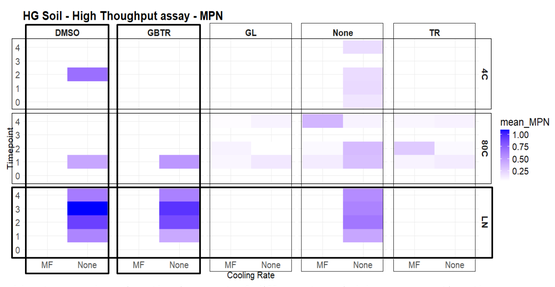
Heatmap showing the impact of different variables generating the preservation methods and of the duration of storage on CFUs count.

Preliminary results suggest that none of the tested preservation methods had a strong detrimental effect on bacterial and fungal culturability over time.

MPN – Most Probable Number



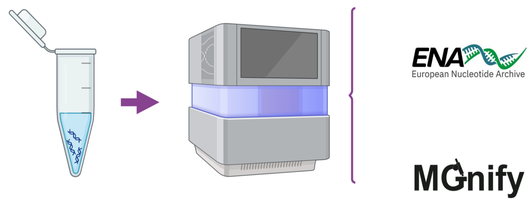
Boxplot showing the impact of different preservation methods and of the duration of storage on MPN.

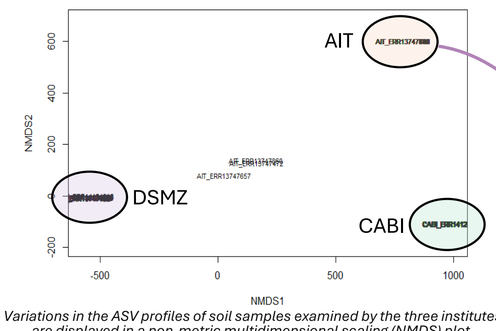


Heatmap showing the impact of different variables generating the preservation methods and of the duration of storage on MPN.

The effect of storage duration appears to be stronger than the effect of the different preservation methods. This trend is consistent across the different amplified regions (results not shown).

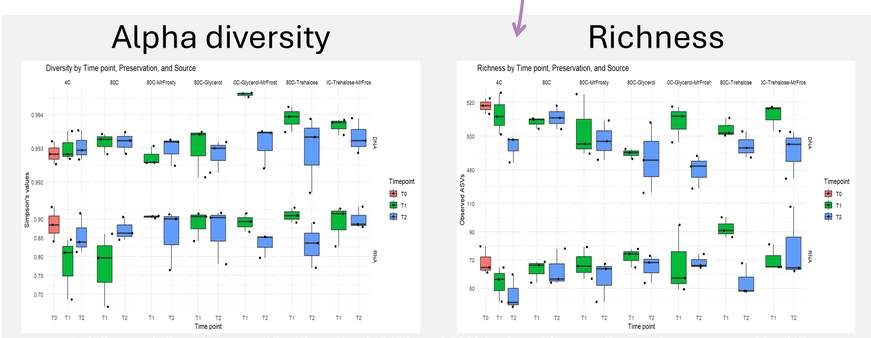
DNA- & RNA-BASED COMMUNITY ANALYSIS





Variations in the ASV profiles of soil samples examined by the three institutes are displayed in a non-metric multidimensional scaling (NMDS) plot.

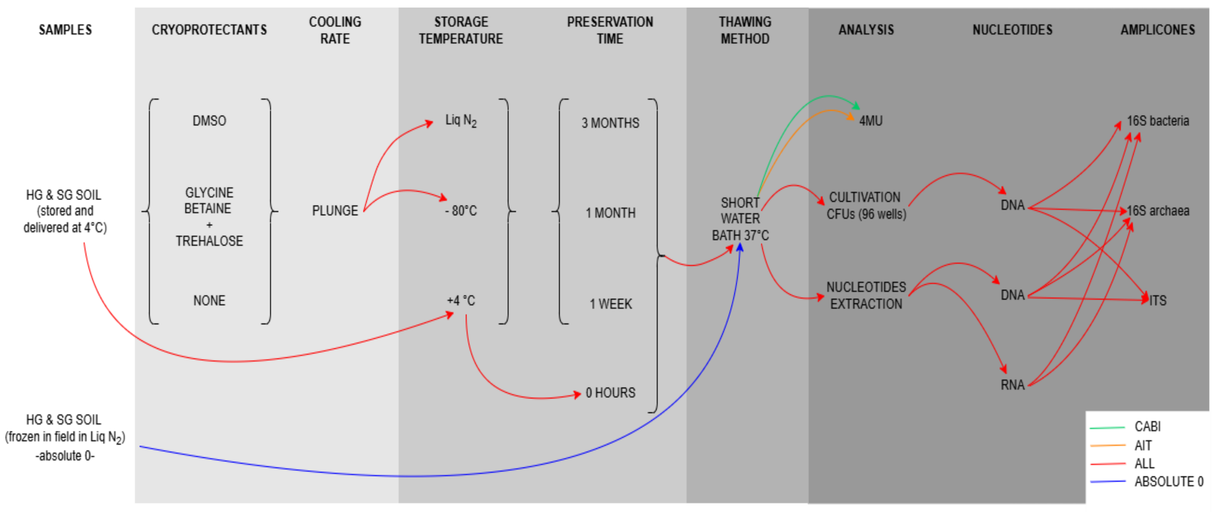
Different partners and procedures have a significant impact on the overall results and affect comparability.



16S (bacterial) region amplified from both DNA and RNA isolated from soil samples preserved by AIT.

Preliminary results suggest that all preservation methods successfully maintained the integrity of DNA and RNA in the stored samples.

SECOND ROUND



Following a new sampling campaign, with pivotal attention given to the preanalytical handling of samples, the second round of the experiment has begun and is currently ongoing.

The number of preservation methods was reduced, and protocols were meticulously harmonized across the partners.

The refinements applied were necessary to address the issue of variability between partners and to obtain a stronger and validated indication of the most effective preservation method(s).

1 Banerjee, S., van der Heijden, M.G.A. Soil microbiomes and one health. Nat Rev Microbiol 21, 6–20 (2023). https://doi.org/10.1038/s41579-022-00779-w
2 European Commission, 2023 https://data.europa.eu/doi/10.2777/507125
3 FAO, 2019 http://www.fao.org/3/ca6767en/CA6767EN.pdf

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