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MICROBE the Microbiome Biobanking (RI) Enabler

AUSTRIAN INSTITUTE OF TECHNOLOGY

TOMORROW TODAY

IMPACT OF PRE-ANALYTICAL STORAGE CONDITIONS ON MICROBIOME-CONTAINING SAMPLES: FOCUS ON SOIL USE CASE.

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

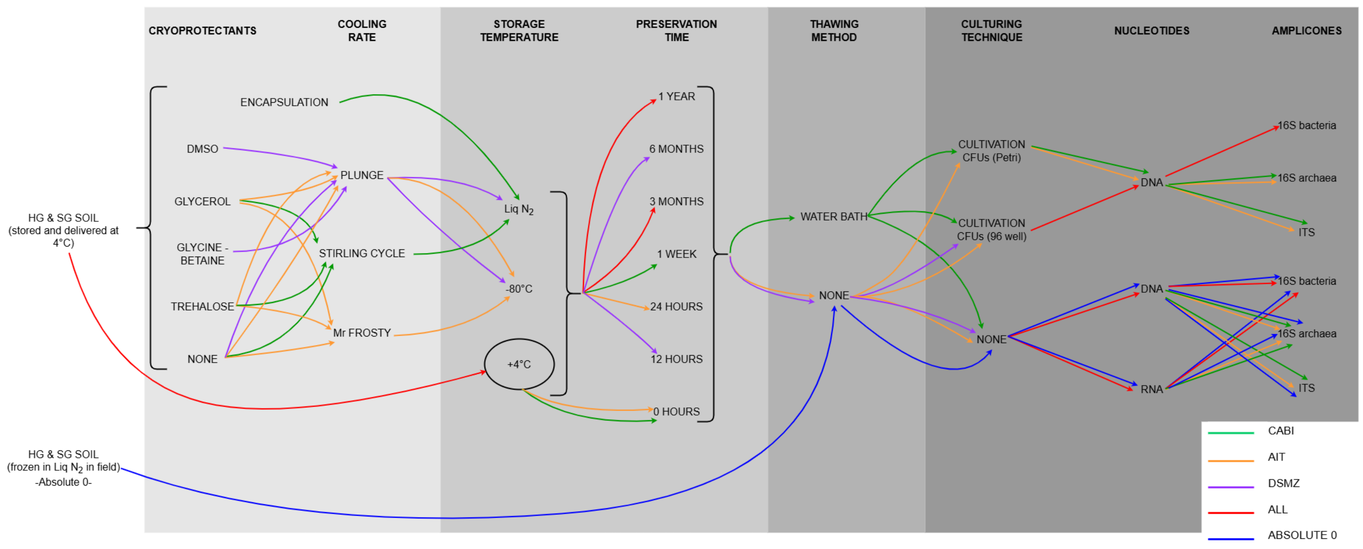


biodiversity exploratories – functional biodiversity research

THREE PARTNER INSTITUTES INVOLVED

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

EXPERIMENTAL DESIGN

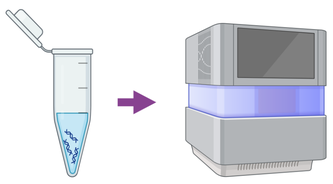


- 16 preservation methods generated by different combinations of three variables (cryoprotectant, cooling rate, and storage temperature).
- Six different storage periods.
- Two thawing methods.

BioSamples

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

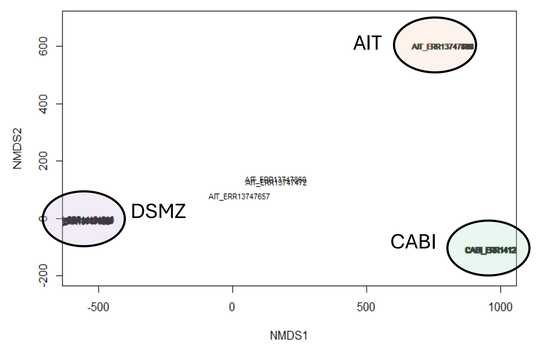
DNA- & RNA-BASED COMMUNITY ANALYSIS



ENA European Nucleotide Archive

Sequencing data were uploaded to the European Nucleotide Archive (ENA) (EMBL-EBI).

Variations in the ASV profiles of soil samples



The Institute-related factor had a stronger impact on community composition than the preservation method, possibly because the institutes differed in:

- Shipment process duration.
- Storage time at 4°C prior to preservation.
- Storage time at 4°C prior to preservation.





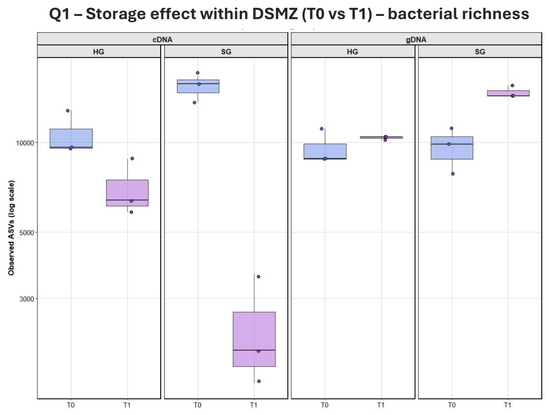
A DEEPER DIG

Bacterial community profiling of soils based on DNA- and RNA-derived 16S rRNA (V3-V4)

- T0 → plunged in liquid nitrogen in the field and analysed by DSMZ.
- T1 → shipped at 4°C to the three partner institutes and analysed by each institute after different storage period at 4°C (**DSMZ** ~ 1.5 months, **AIT** ~ 2.5 months and **CABI** ~ 4.5 months).

Alpha diversity

Q1 – Storage effect within DSMZ (T0 vs T1) – bacterial richness

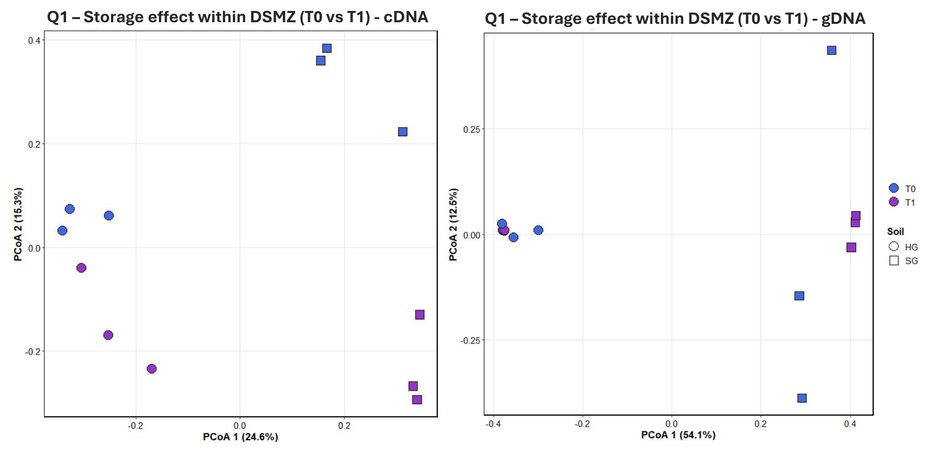


T0 *vs* T1(DSMZ)

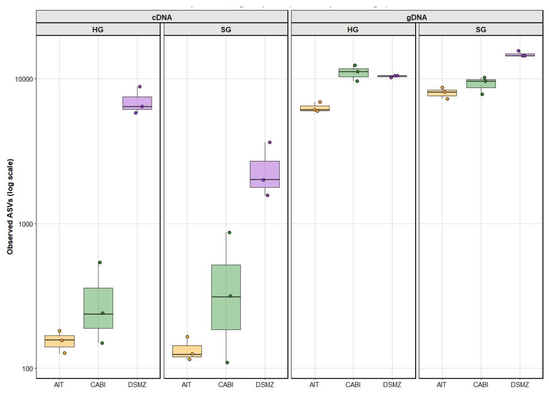
- Same institute performs the analyses.
- Evaluates the effect of transfer from the field to the institute and of ~1.5-month storage time at 4°C → *STORAGE EFFECT*.

Storage at 4°C for 1.5 months reduces alpha diversity in the active (cDNA-based) community, while total community diversity (gDNA) remains largely stable.

Beta diversity



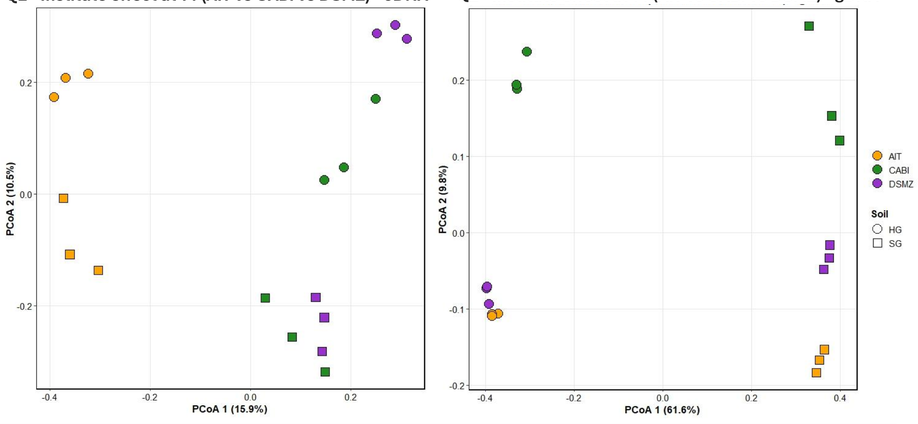
Q2 - Institute effect at T1 (AIT vs CABI vs DSMZ) – bacterial richness



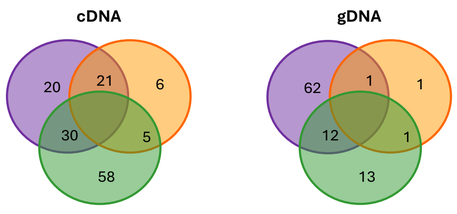
T1 (AIT) *vs* T1 (CABI) *vs* T1(DSMZ)

- Three different institutes perform the analyses.
- Evaluates the effect of different storage times at 4°C and different handling procedures due to internal laboratory practices → *INSTITUTE EFFECT*.

A longer storage period further reduces alpha diversity in the active (cDNA-based) community, but some handling practices (e.g., a longer water bath for thawing) could partially counteract this while modifying the composition of the microbial community.



Q2 - Institute effect at T1 (AIT vs CABI vs DSMZ) – indicator ASVs





Particular attention should be given to the preanalytical handling of samples including sampling procedures, shipment, and storage. It is essential to meticulously harmonize protocols using tools such as SOPs.

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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
European Commission, 2023 https://data.europa.eu/doi/10.2777/507125
FAO, 2019 http://www.fao.org/3/ca6767en/CA6767EN.pdf