Developing methods for long-term preservation of complex microbial communities: Insights from soil microbiomes

MICROBE
the Microbiome Biobanking (RI) Enabler

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Background

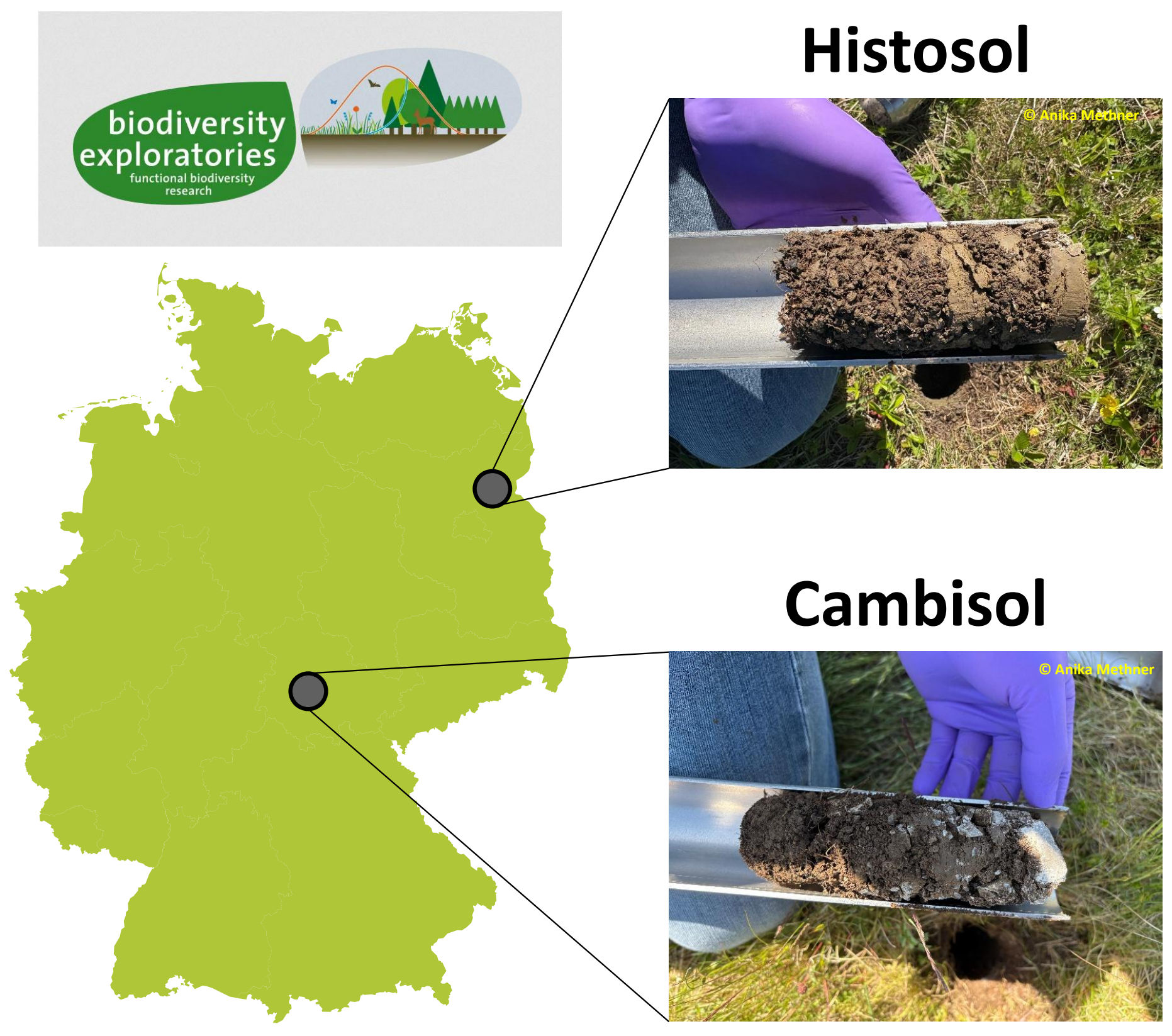
The rapid development of microbiome research marks a paradigm shift in the field of microbiology, from traditional focus on individual strains to that on complex communities.

While methods for preservation of axenic strains are highly refined, and the necessary infrastructure is well developed, many techniques cannot be directly applied to microbiomes, and studies on long-term preservation of microbiomes are scarce.

This underlines the importance of developing supporting infrastructure and methods for long-term microbiome preservation.

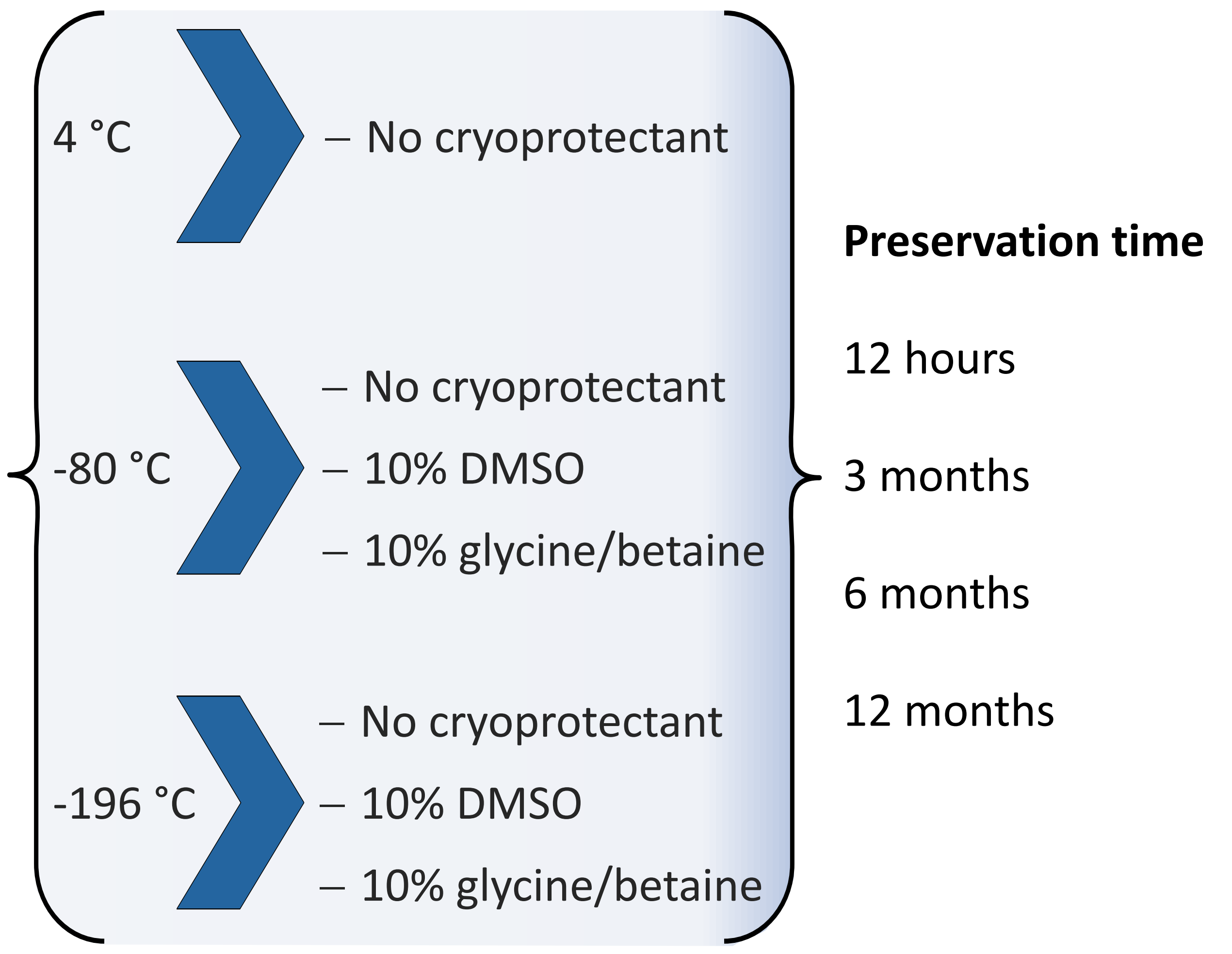
Preservation of functional microbial communities

Sampling



Sampling regions and sediments cores; Biodiversity Exploratories

Preservation conditions



4 °C
– No cryoprotectant

-80 °C
– No cryoprotectant
– 10% DMSO
– 10% glycine/betaine

-196 °C
– No cryoprotectant
– 10% DMSO
– 10% glycine/betaine

Preservation time

12 hours

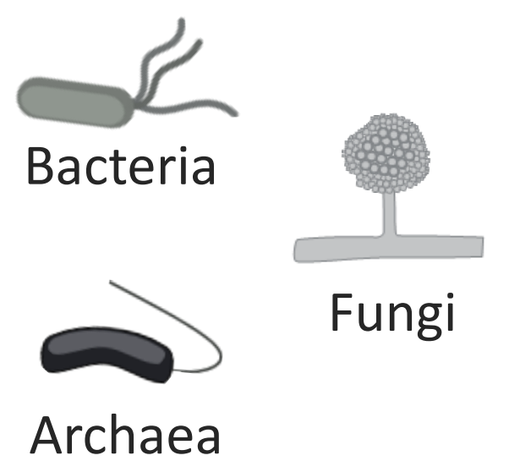
3 months

6 months

12 months

Preservation conditions and preservation times

Analysis

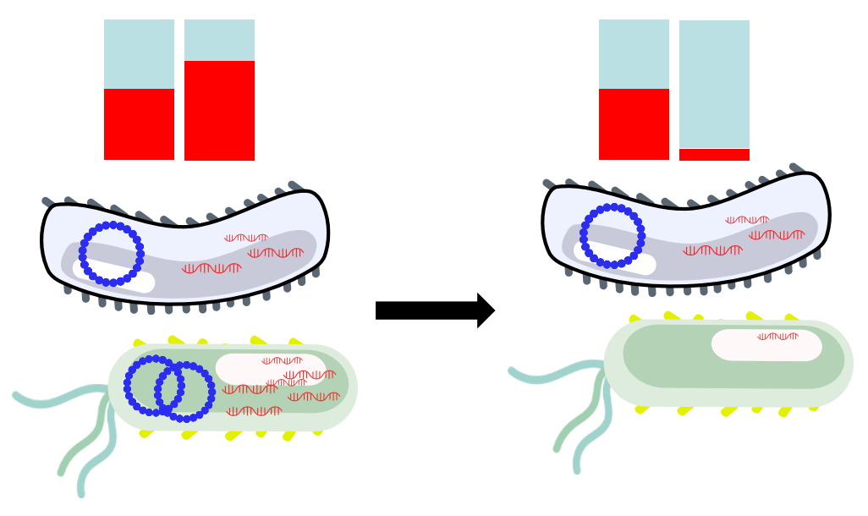


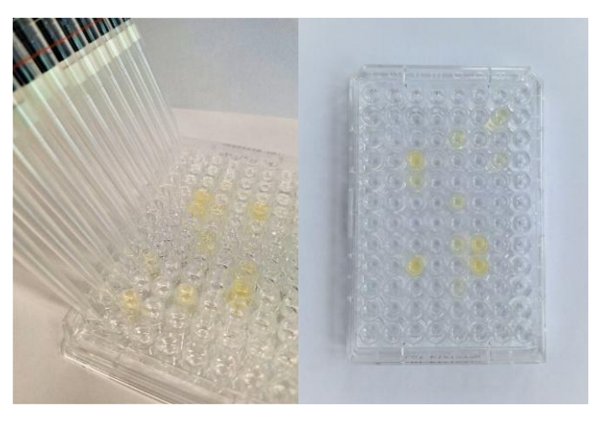
Community stability
- Amplicon sequencing
- 16S rRNA gene
- ITS Region

Ribosomal content
- Ratio of rRNA to rDNA

High ratio → ribosome rich

Low ratio → ribosome low

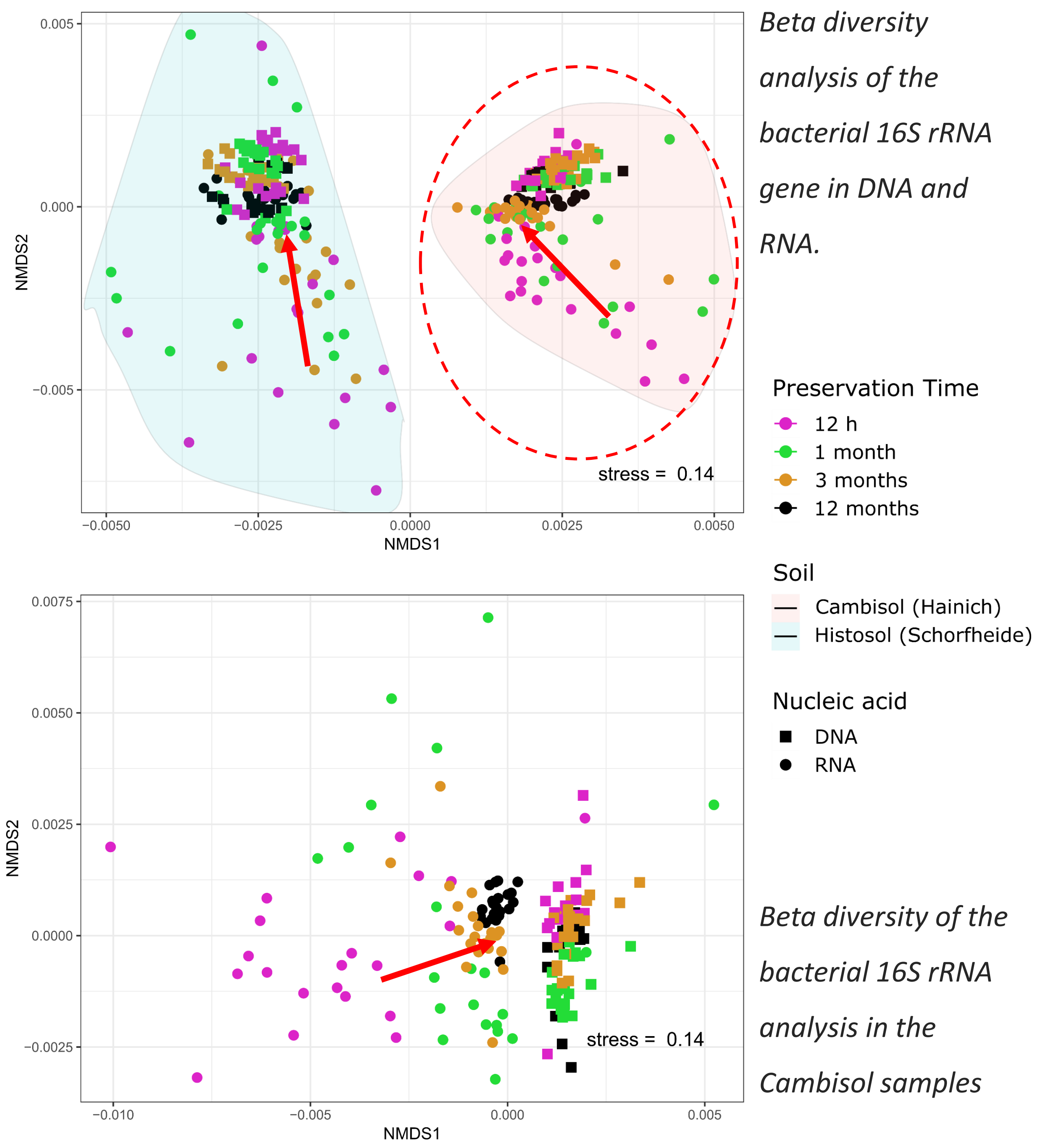




Resuming growth
- Most probable number (MPN)
- 16S rRNA gene sequencing

Evaluation of preservation success

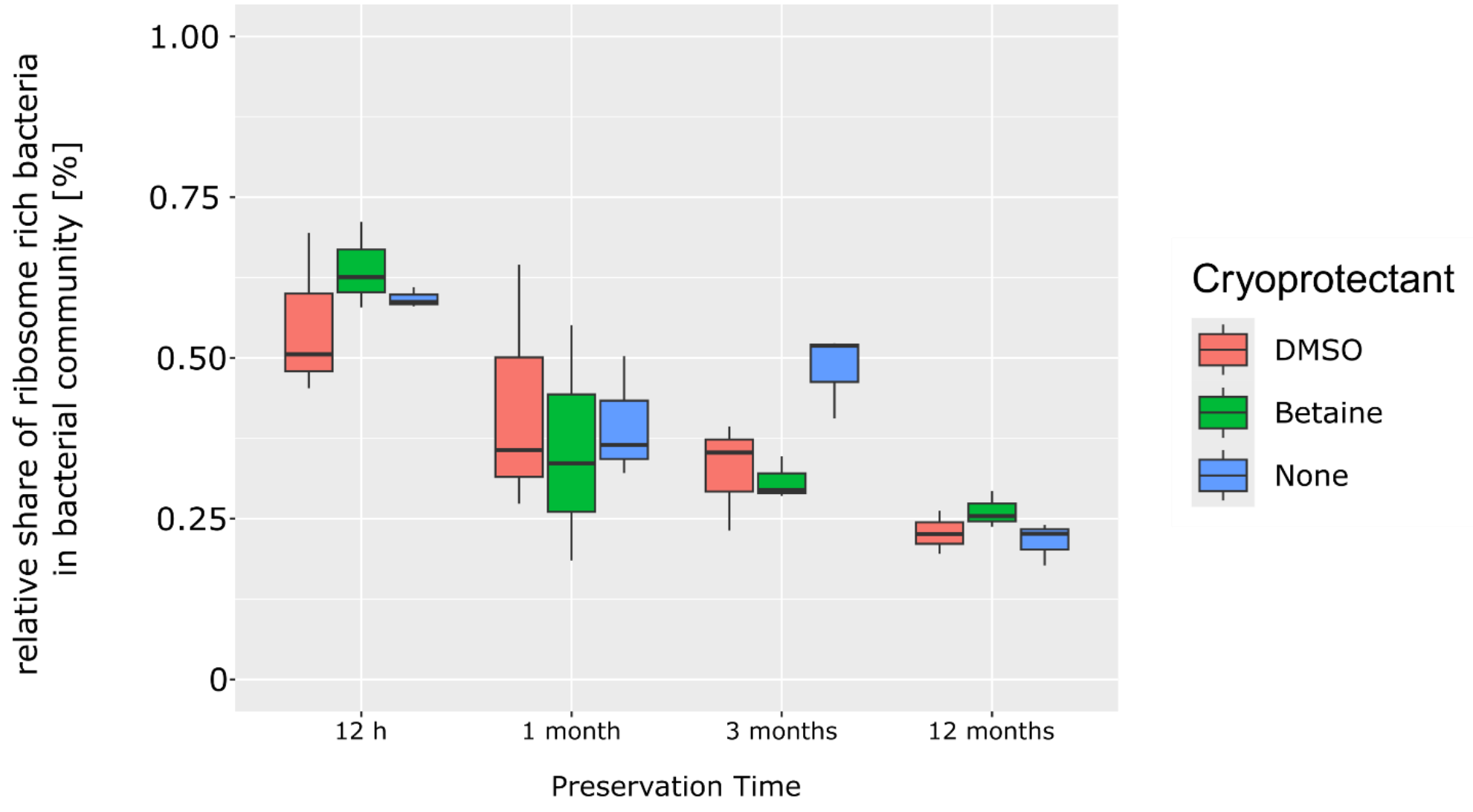
Community stability



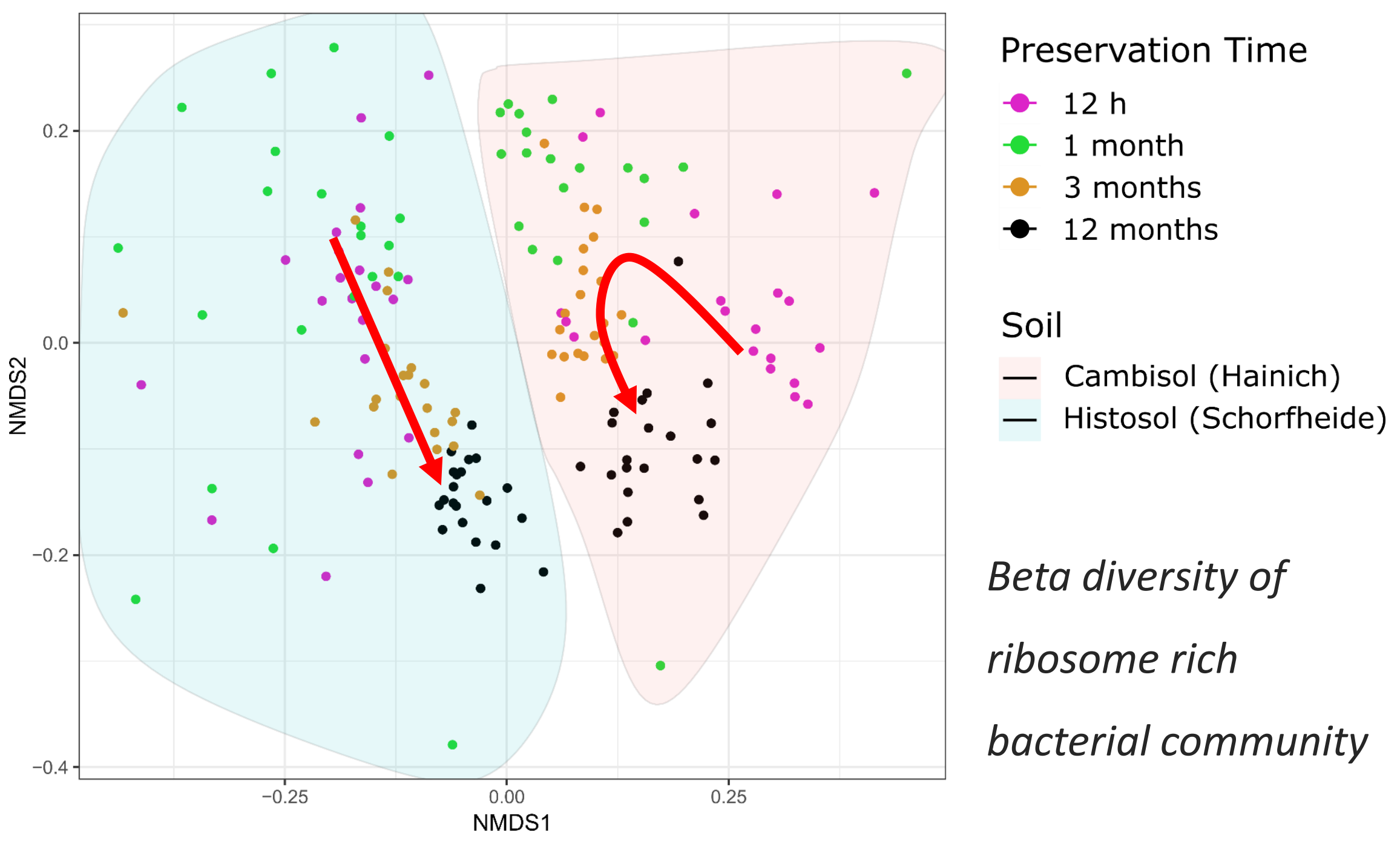
Beta diversity analysis of the bacterial 16S rRNA gene in DNA and RNA.

Beta diversity of the bacterial 16S rRNA analysis in the Cambisol samples

Ribosomal content

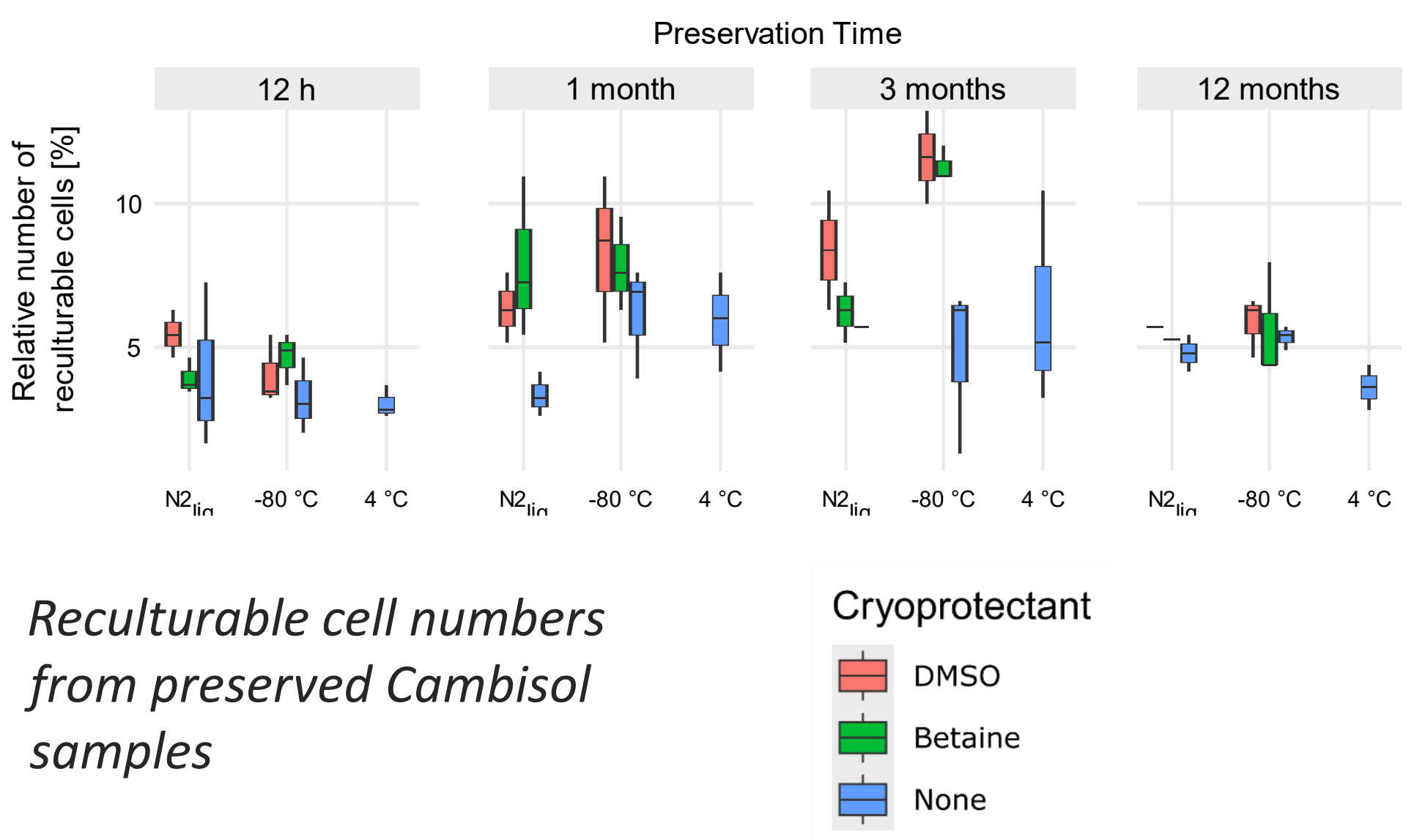


Relative share of ribosome rich members in soil in the Cambisol samples

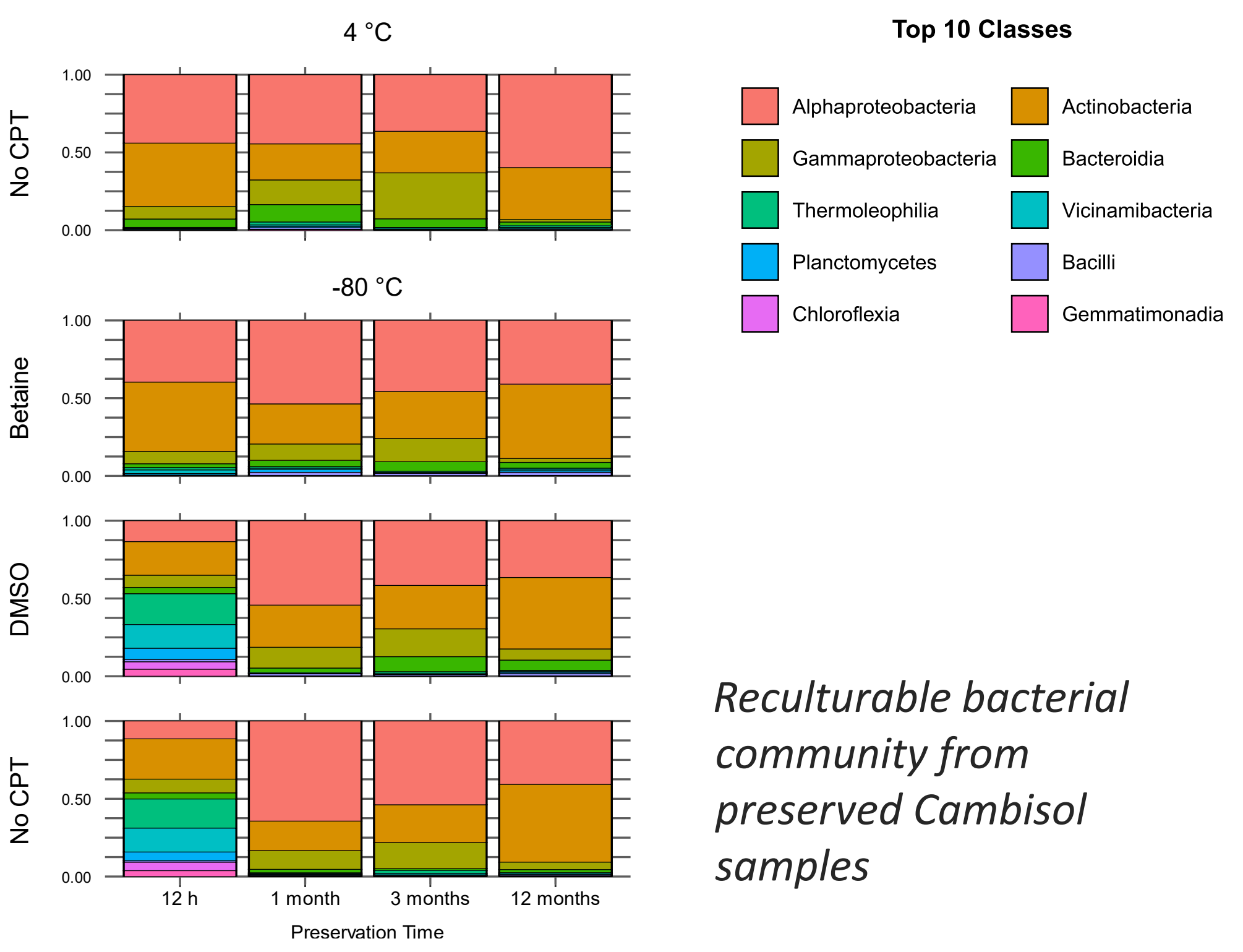


Beta diversity of ribosome rich bacterial community

Resuming growth



Reculturable cell numbers from preserved Cambisol samples



Reculturable bacterial community from preserved Cambisol samples

Community stability

- DNA more stable than RNA
- RNA shows stronger change in composition
- RNA compositions becomes similar over time

Ribosomal content

- Decrease over time, independent of used method
- Shift in ribosome rich community similar between used methods

Resuming growth

- Higher success of resuming growth when cryoprotectants are used
- Strong community shift shortly after preservation