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RECYCLES WORKSHOP

Metagenomics and metabarcoding approaches to describe ecological systems and infer their development

5th, 6th & 7th of July 2022

Metabarcoding analysis of storage conditions for long term experiments

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European
Commission



GA: 872053 — H2020 - MSCA - RISE-2019



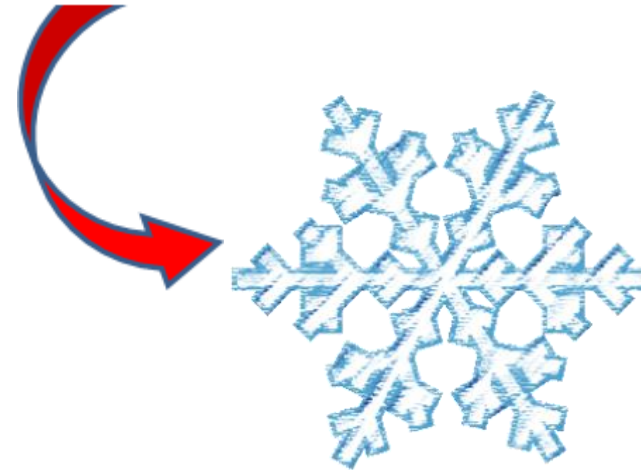
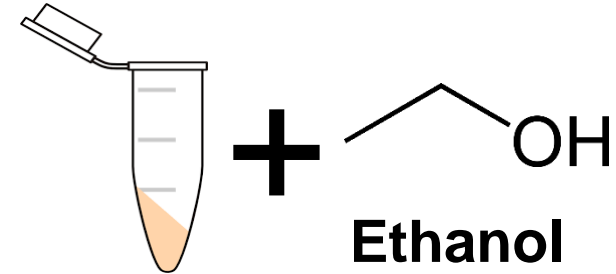
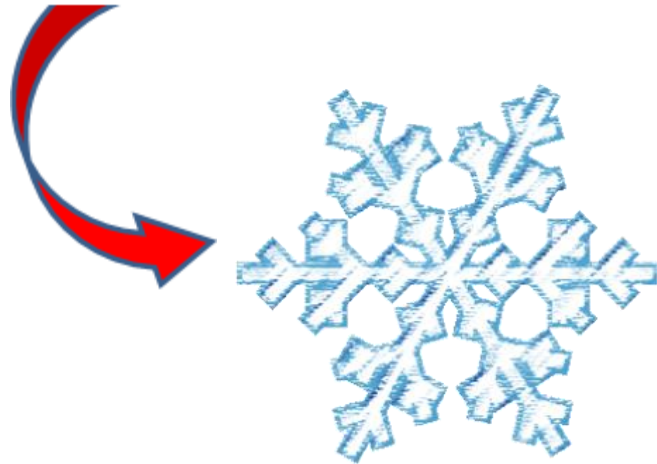
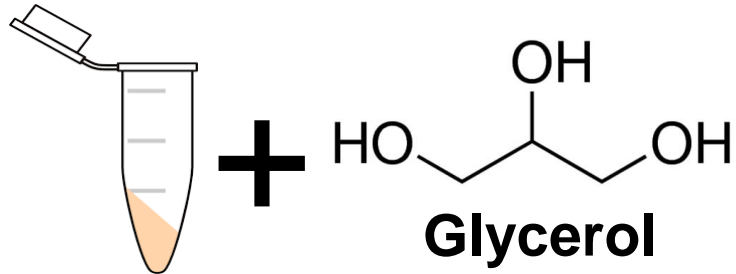
Why to store samples?

- 1) to process and analyze all the samples at end point;
- 2) to create starter batches to reproduce growth.

What may happen?

- 1) variations due to the storage method;
- 2) variations during the storage.

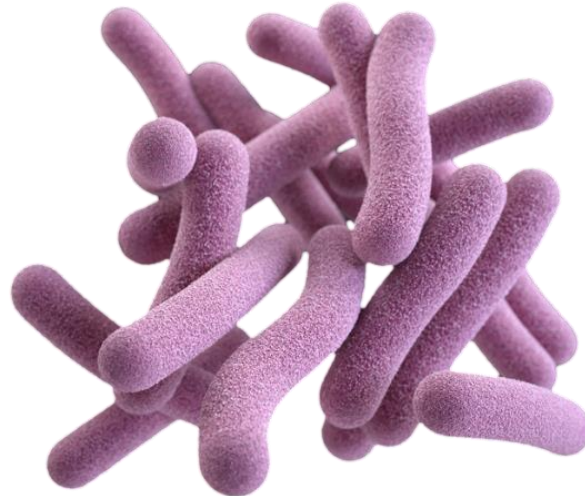
Common storage methods



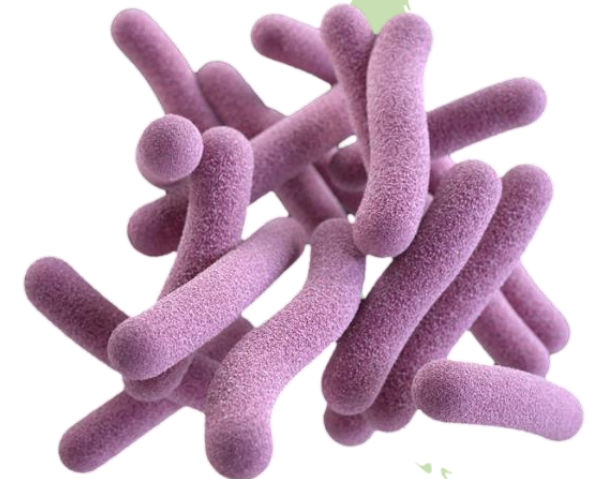
“Contamination” from DNA of dead microbes



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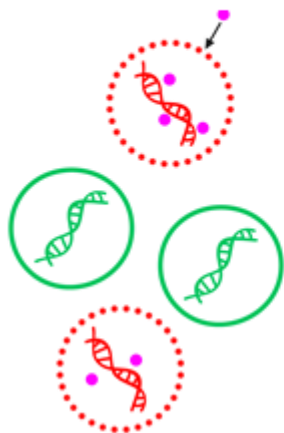


Environmental DNA
from dead cells

“Contamination” from DNA of dead microbes



PMAxx enters in the dead cells and binds to DNA

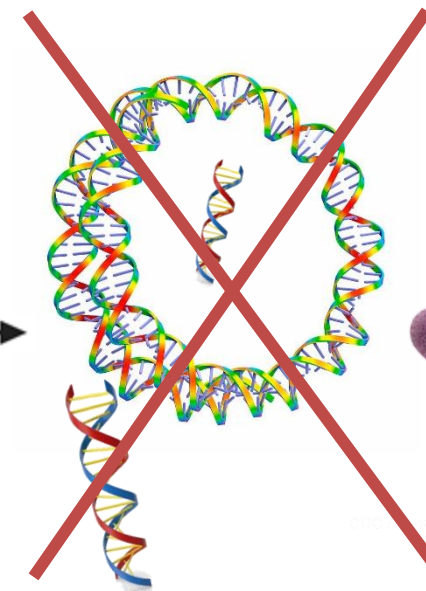
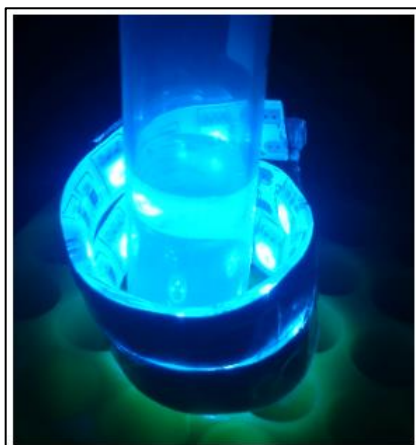


10 min
100 μ M
4° C



1 min

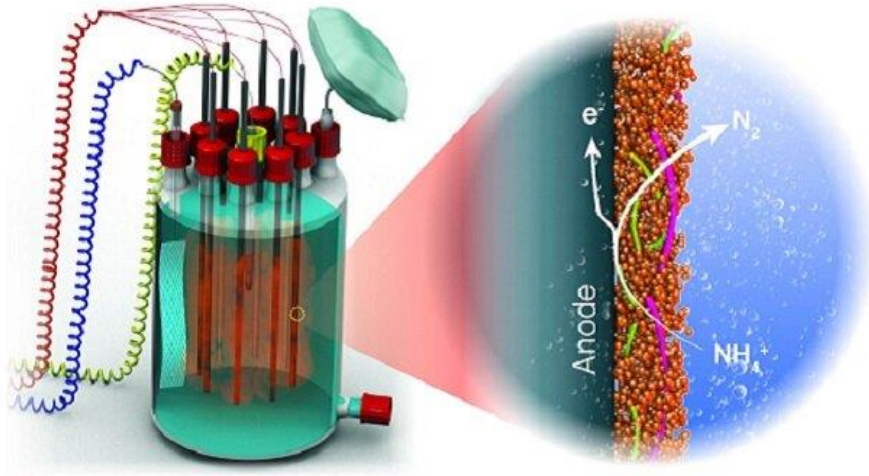
Intense light exposure causes the PMAxx to become reactive and covalently bind the DNA



The PMAxx blocks the DNA extension by Taq polymerase



Study design



Ethanol

Glycerol

Et_IL

Gl_IL

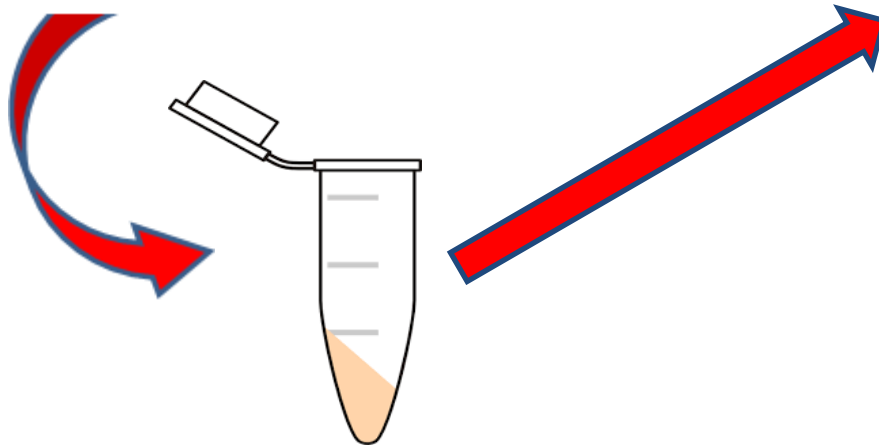
Illuminated



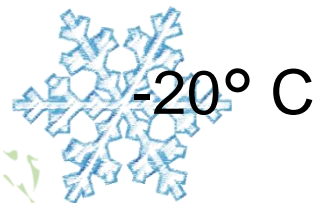
Et_NI

Gl_NI

Not Illuminated

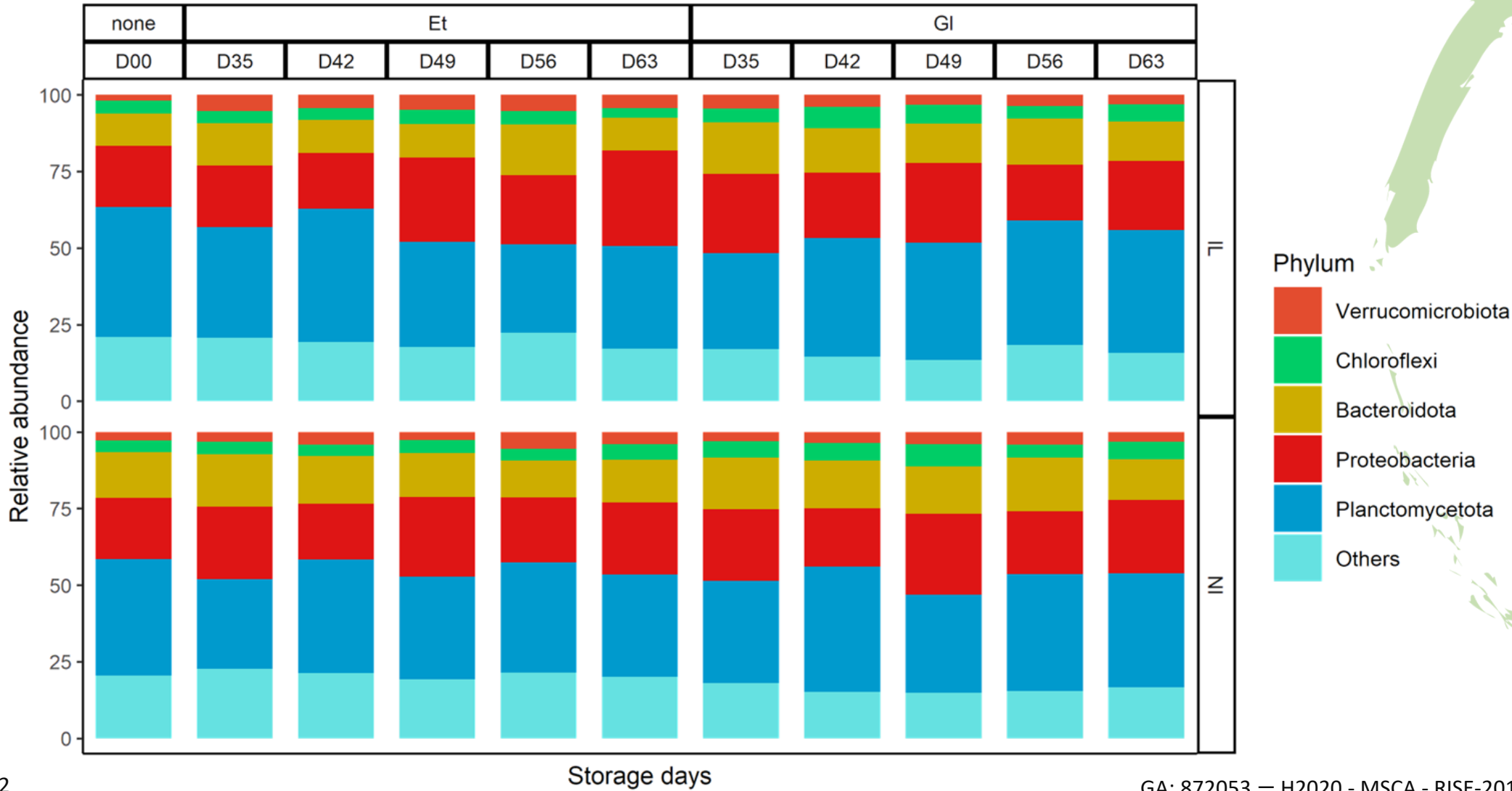


0 35 42 49 56 63 days

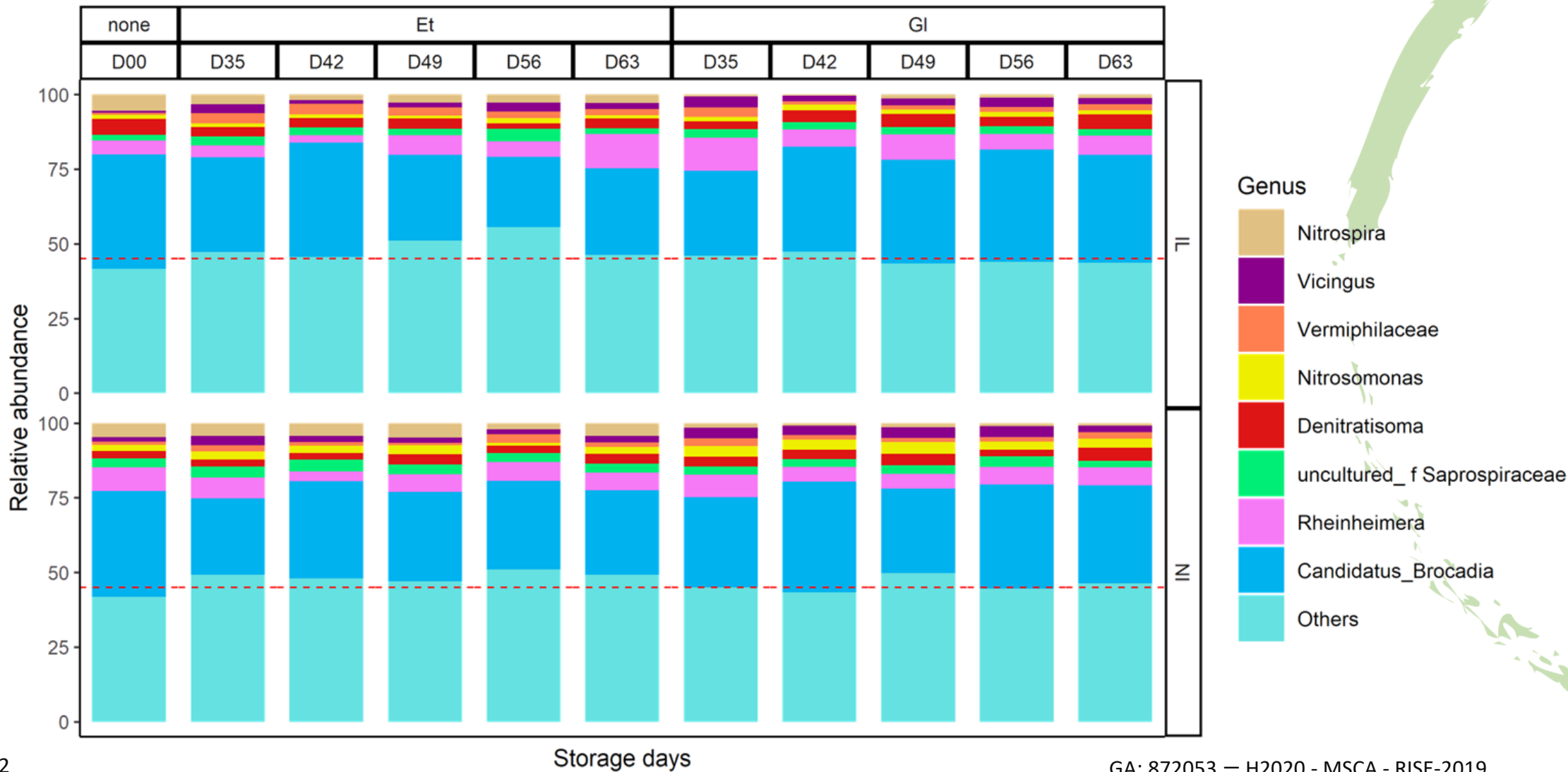


PRO341FB
805R

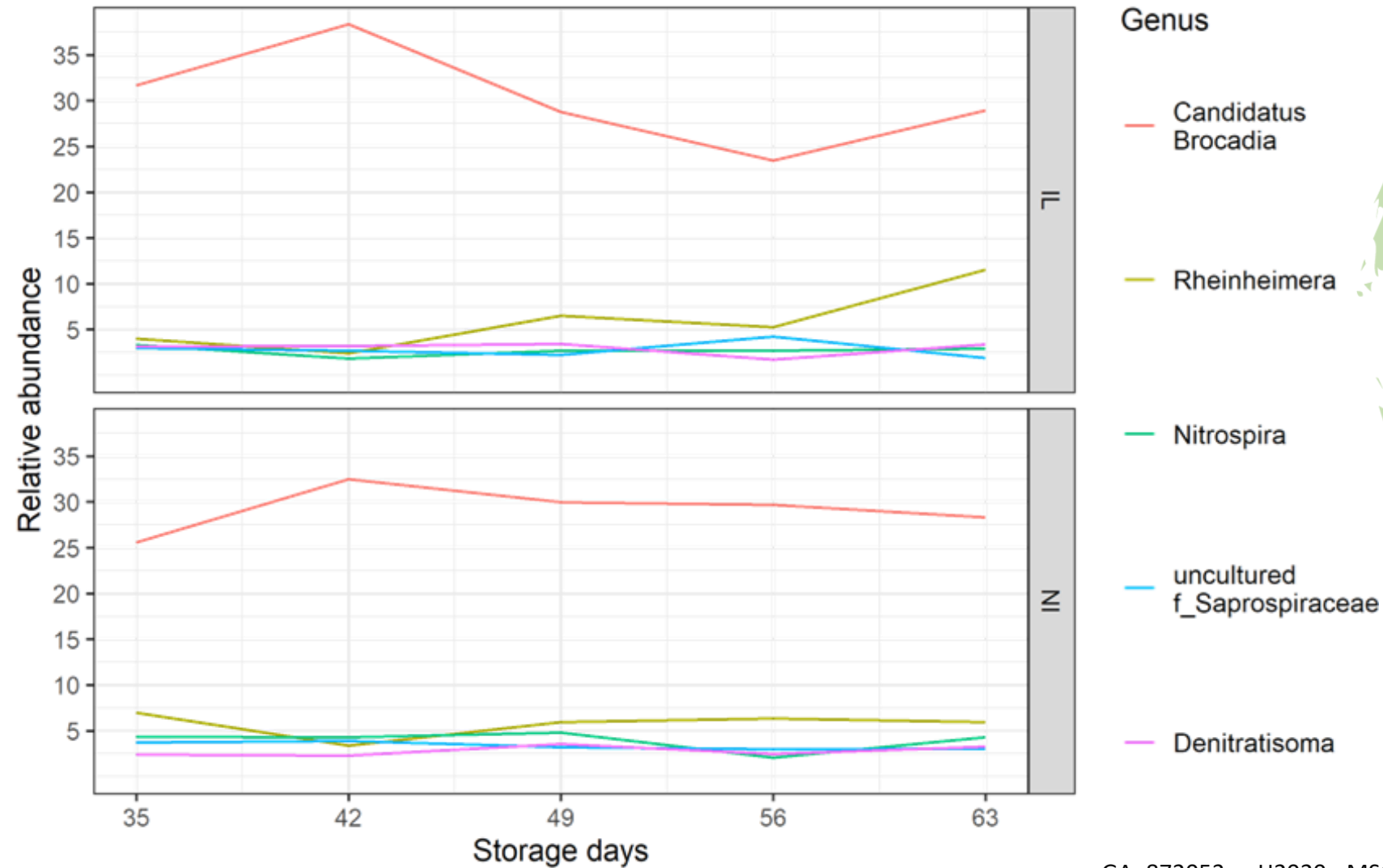
Results: Five most abundant phyla



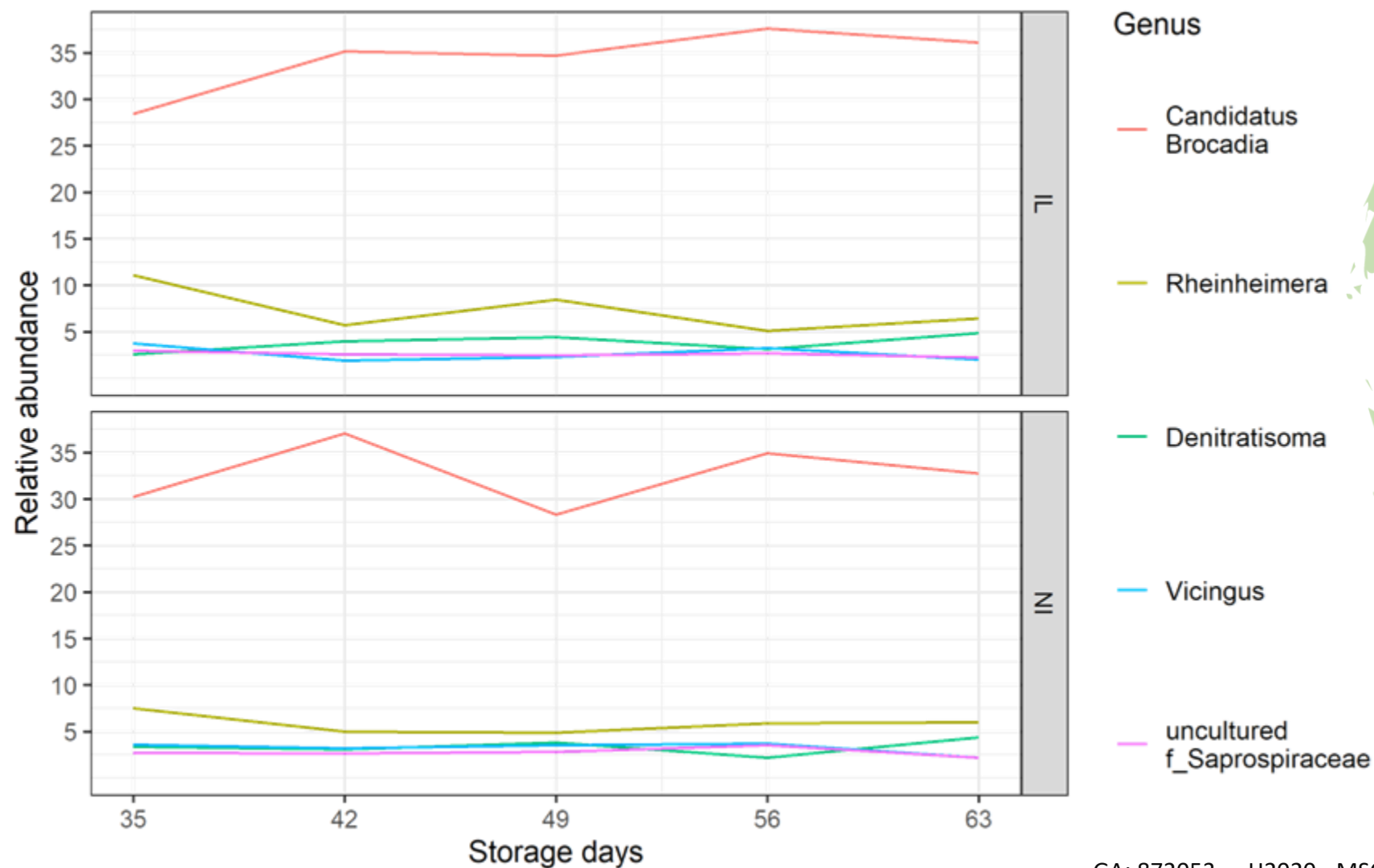
Results: Eight most abundant genera



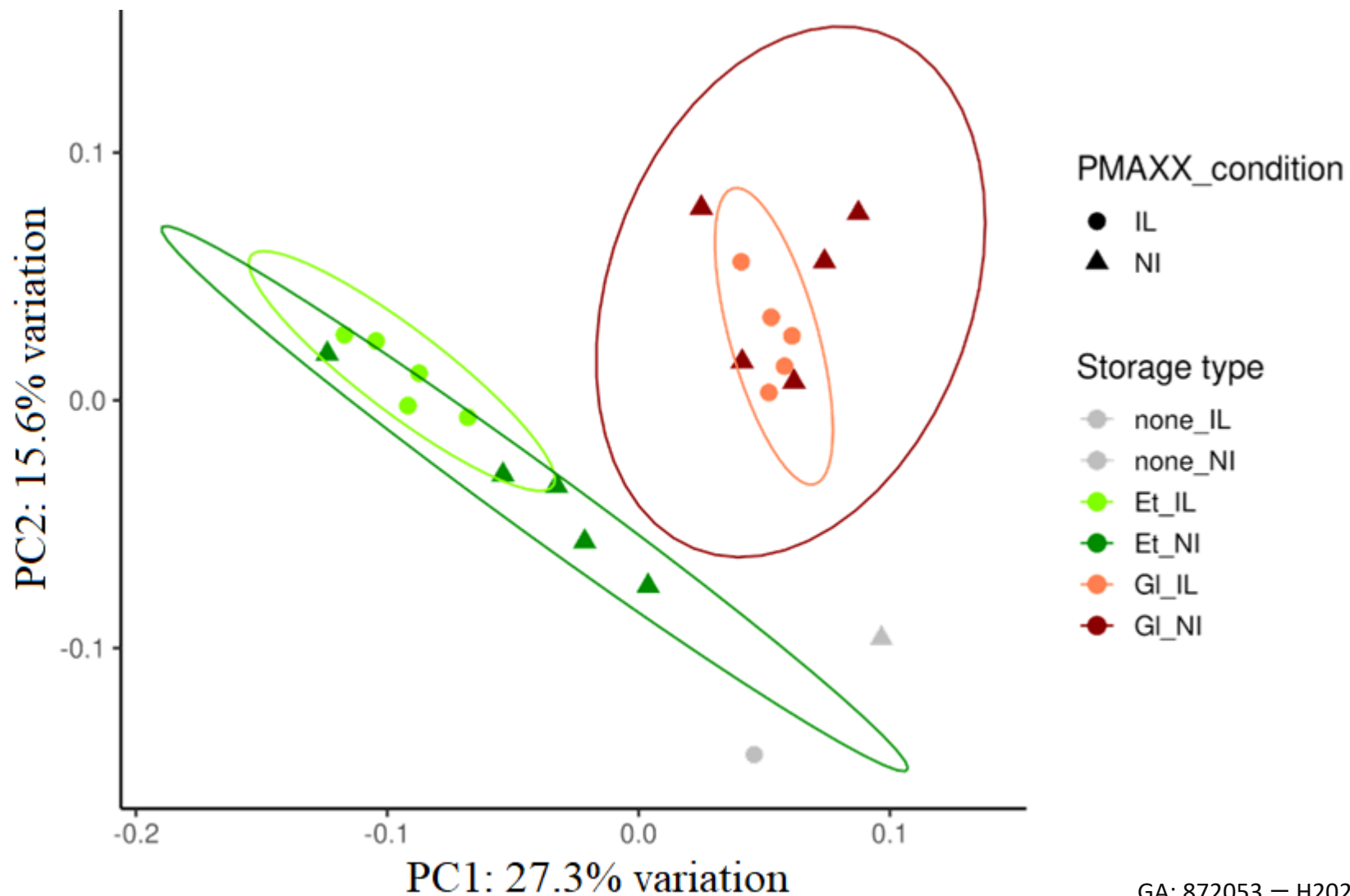
Results: Line chart of TOP5 genera (Et sample)



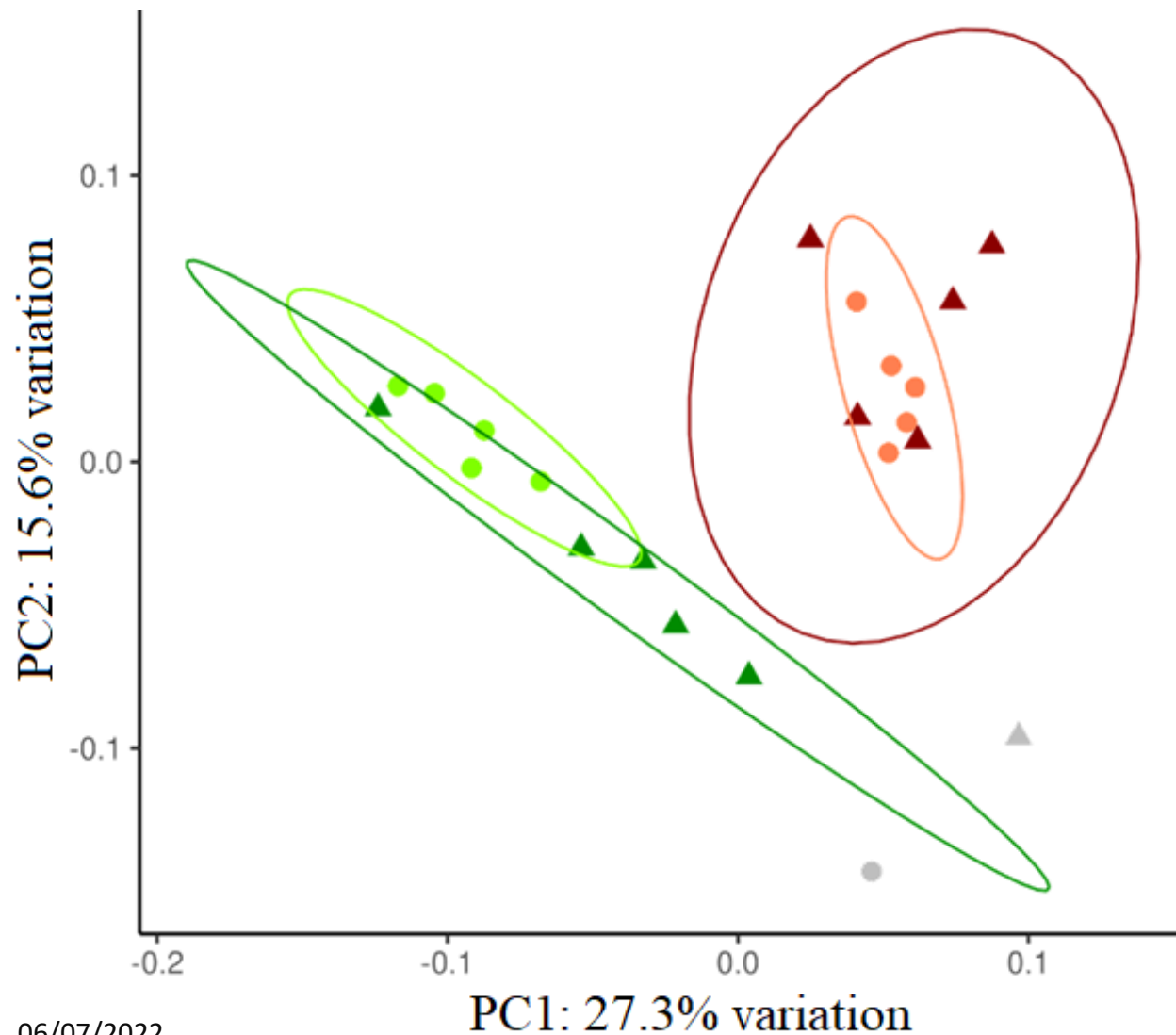
Results: Line chart of TOP5 genera (GI sample)



Results: PCoA (Bray-Curtis) on genera



Results: PCoA (Bray-Curtis) on genera



Comparison	Significance
Et vs GI diversity	*
Et vs GI dispersion	
IL vs NI dispersion	

NB: only two months

Conclusions



- The freezing slightly changes the inferred composition of the samples.
- There are strong and significant differences between the inferred composition of samples preserved with ethanol or glycerol.
- The freezing with glycerol seems to have a slightly better efficiency in preserving the samples compared to the preservation with ethanol but this difference is *not* significant *at least* by the time of two months.
- The presence of dead microbial DNA (IL vs NI samples) can led to biases which still are not significant at least by the time of two months.