Microbial community structure and activity in trace element-contaminated soils (phyto)managed by Gentle Remediation Options (GRO)

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Introduction

Europe (EEA 2007**)**

3 million potentially contaminated sites (0,5 m

Local and diffuse contamination: industry, agr

activity…

- **Trace metals (TE)**
- **Essential vs. non-essential**
- Over a threshold can be **toxic**
- **Persistent** : bioaccumulation and biomagnification

- **Phytoextraction: plants remove contaminants from the soil and accumulate them in their shoots.**
- **Phytostabilisation: plants to establish a vegetation cover to immobilize or accumulate the contaminant into the roots without translocation to the aerial part.**
- **Phytoexclusion: metal-excluding crop cultivars are used to reduce transfer of metals to animals and humans**

potential to restore soil quality and functions

Soil **microorganisms** play an **important role :**

- Biogeochemical TE cycling
- Ecosystem functionality

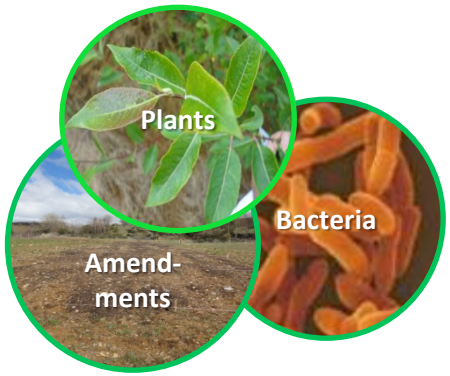
Microbial properties are considered ideal **indicators of soil quality**

Effects of heavy metals on the bacterial community

- ↓ **Microbial biomass**
- ↓ **Enzymatic activity**
- ↓ **Growth, survival, microbial diversity and structure**

Remediation techniques

- ↑ **Microbial biomass**
- ↑ **Key soil enzyme activity**
- ↓ **Microbial stress**



Microorganism response will depend on the plant combination, changes in soil properties, use of amendments ...

Objectives

- Study the effect of phytomanagement on microbial community structure and activity in six field experiments around Europe where different GRO's were used for over seven years.

For this purpose we measured:

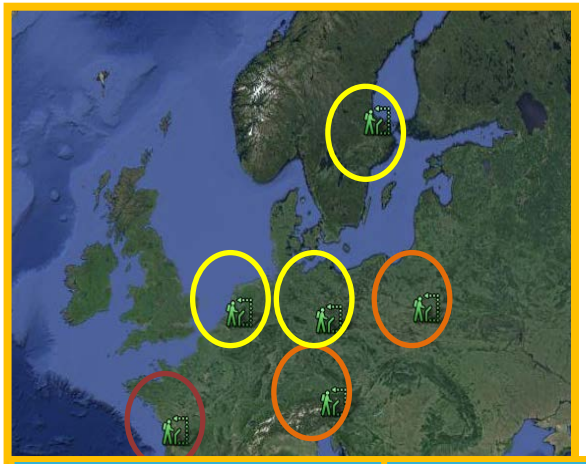
- Hydrolase enzymes involved in the biogeochemical cycles of C, N, P, and S in soil.

- The denaturing gradient gel electrophoresis (DGGE) technique.

- qPCR to study genes involved in the nitrogen cycle (nirK, nirS, nosZ, AOB and AOA).

Studied sites

Six European field studies of the EU GREENLAND project (FP7-KBBE-266124)



Piekary (PL)

Site/management strategy	Country	Main contaminants	Description	Abbreviations
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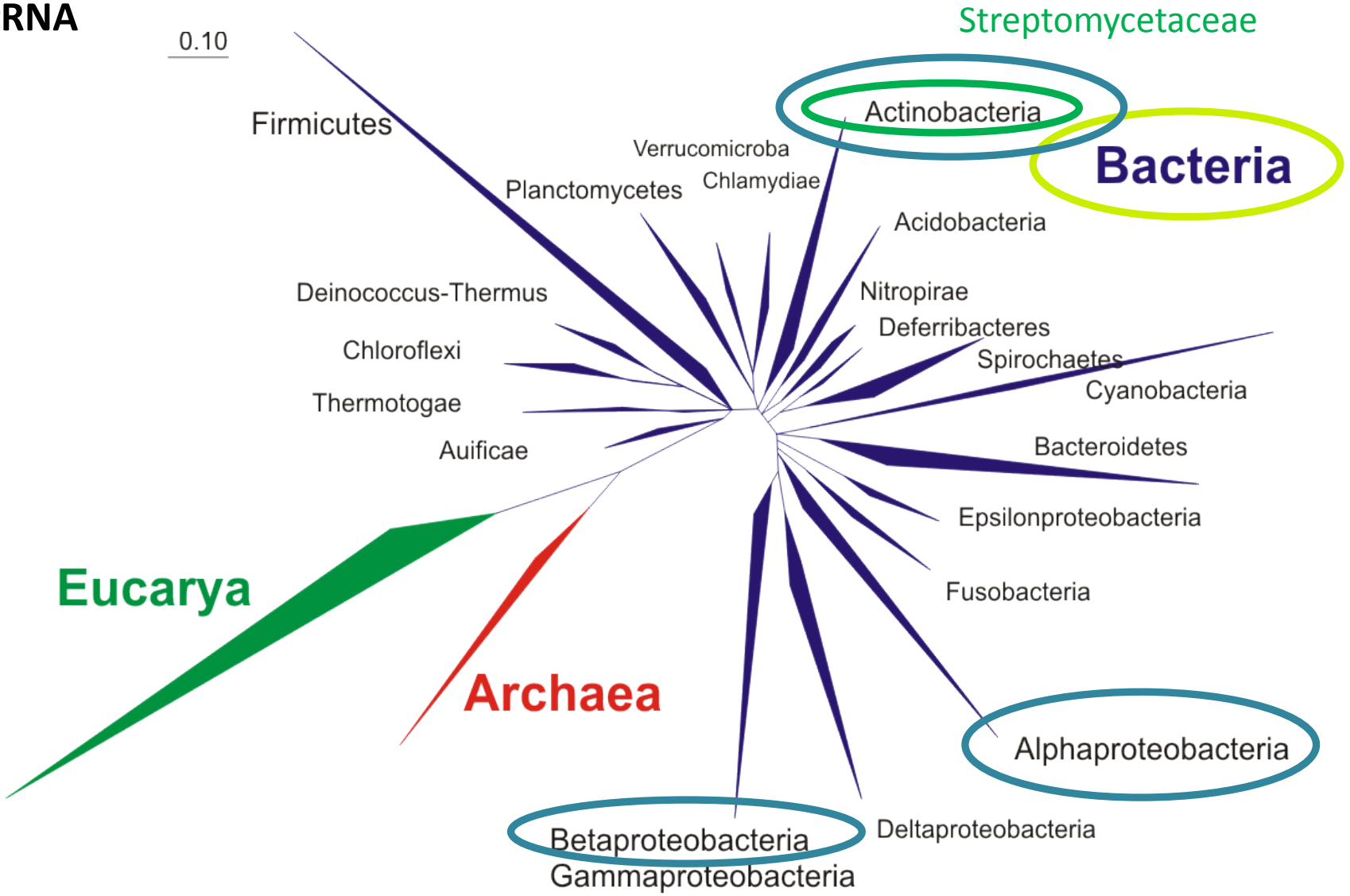
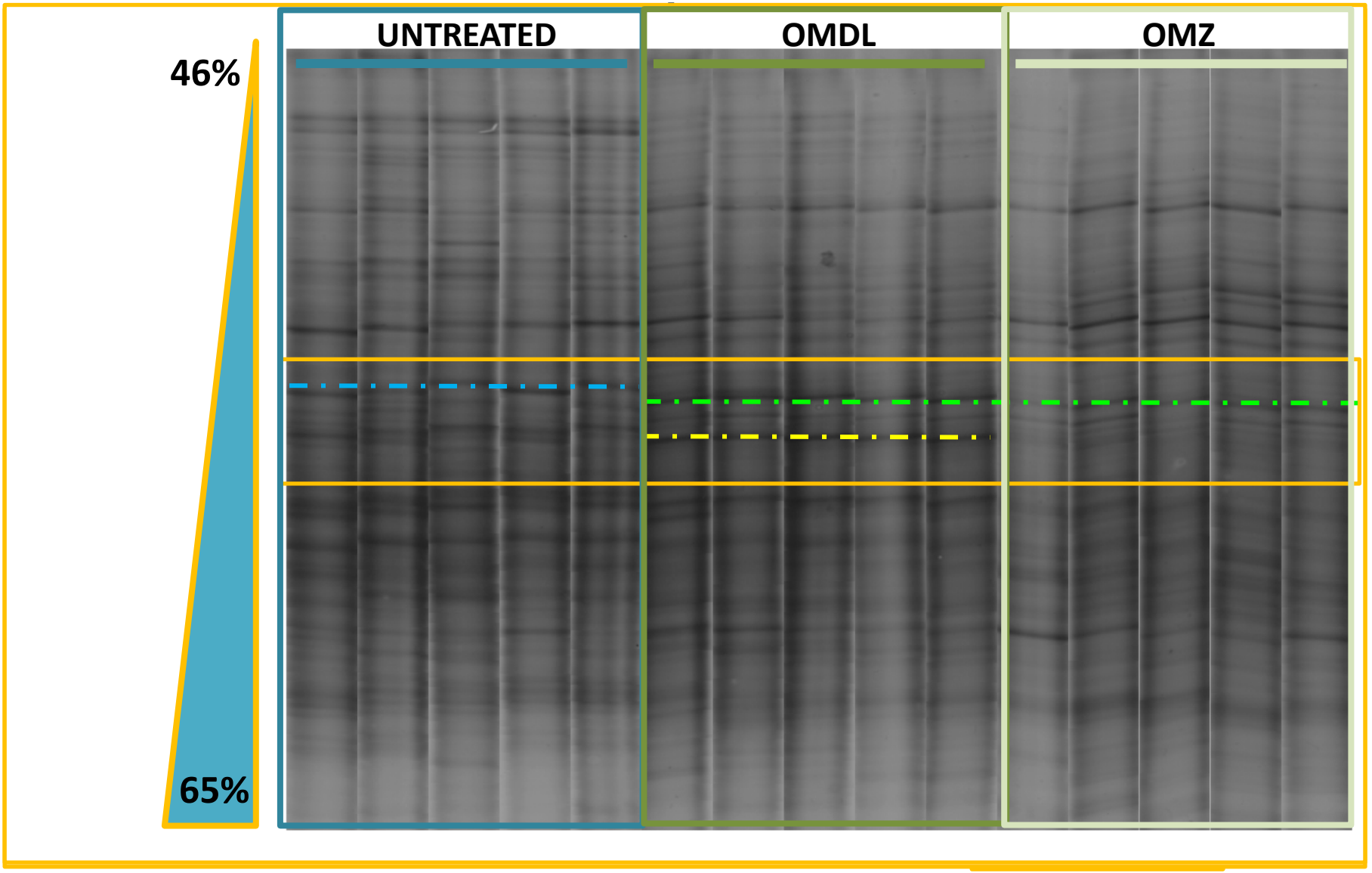
In situ stabilisation / phytoexclusion				
Piekary (PAS)	Poland	Zn, Cd, Pb	- Two amendments: By-product limestone (L)/ municipal biosolids (B) - Two rates: low -L and high -H Vegetation cover: grassland	PAS_UNT PAS_LB PAS_LB+LL PAS_HB+HL


SOIL

16S rRNA


Streptomycetaceae
0.10
Actinobacteria
Bacteria
Firmicutes
Verrucomicroba
Chlamydiae
Planctomycetes
Acidobacteria
Deinococcus-Thermus
Nitropirae
Deferribacteres
Chloroflexi
Spirochaetes
Cyanobacteria
Thermotogae
Auificae
Bacteroidetes
Epsilonproteobacteria
Eucarya
Fusobacteria
Archaea
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria

Material and methods



SOIL

DENITRIFICATION

N_2

nosZ

Nitrous oxide reductase

N_2O

Nitric oxide reduction

norB,norZ

NO

Nitrite reductase

nirK,nirS

NITRIFICATION

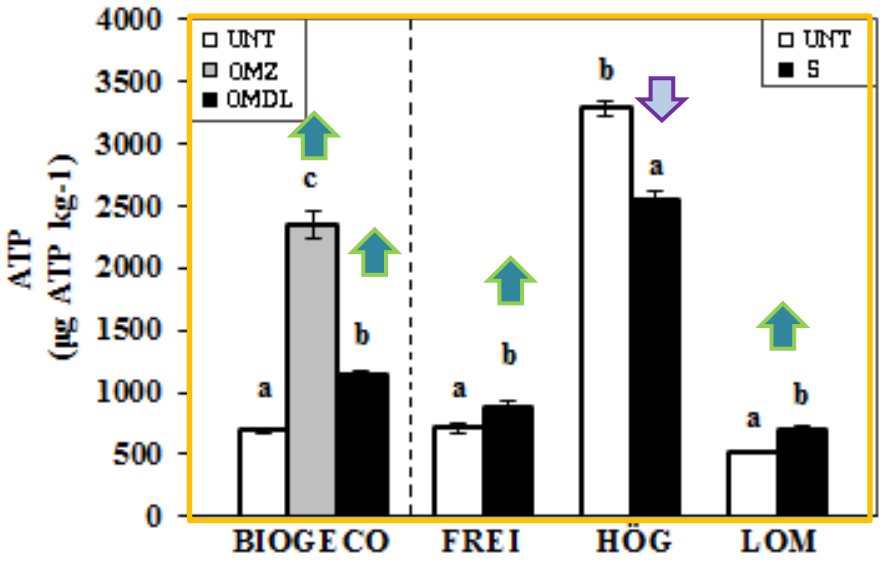
NO_2^-

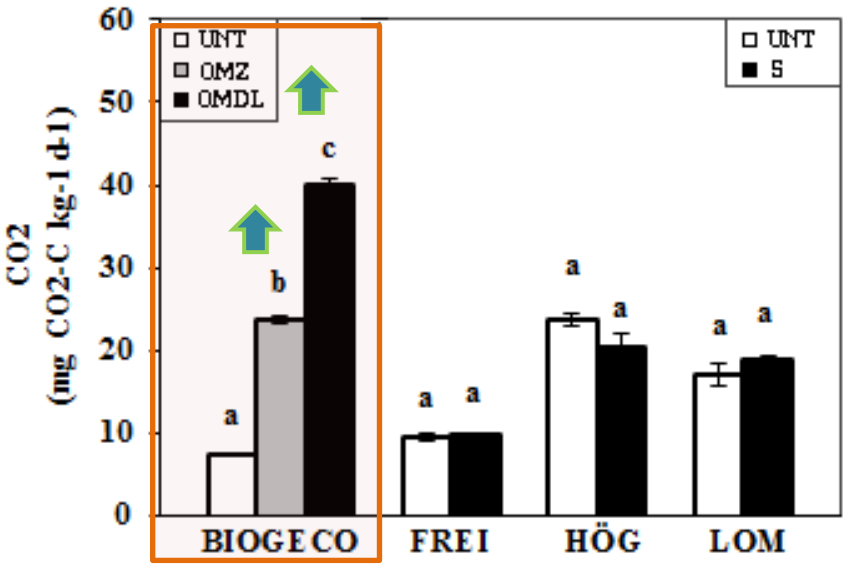
amoA

Ammonia monooxygenase

NH_4^+

MICROBIAL BIOMASS AND RESPIRATION





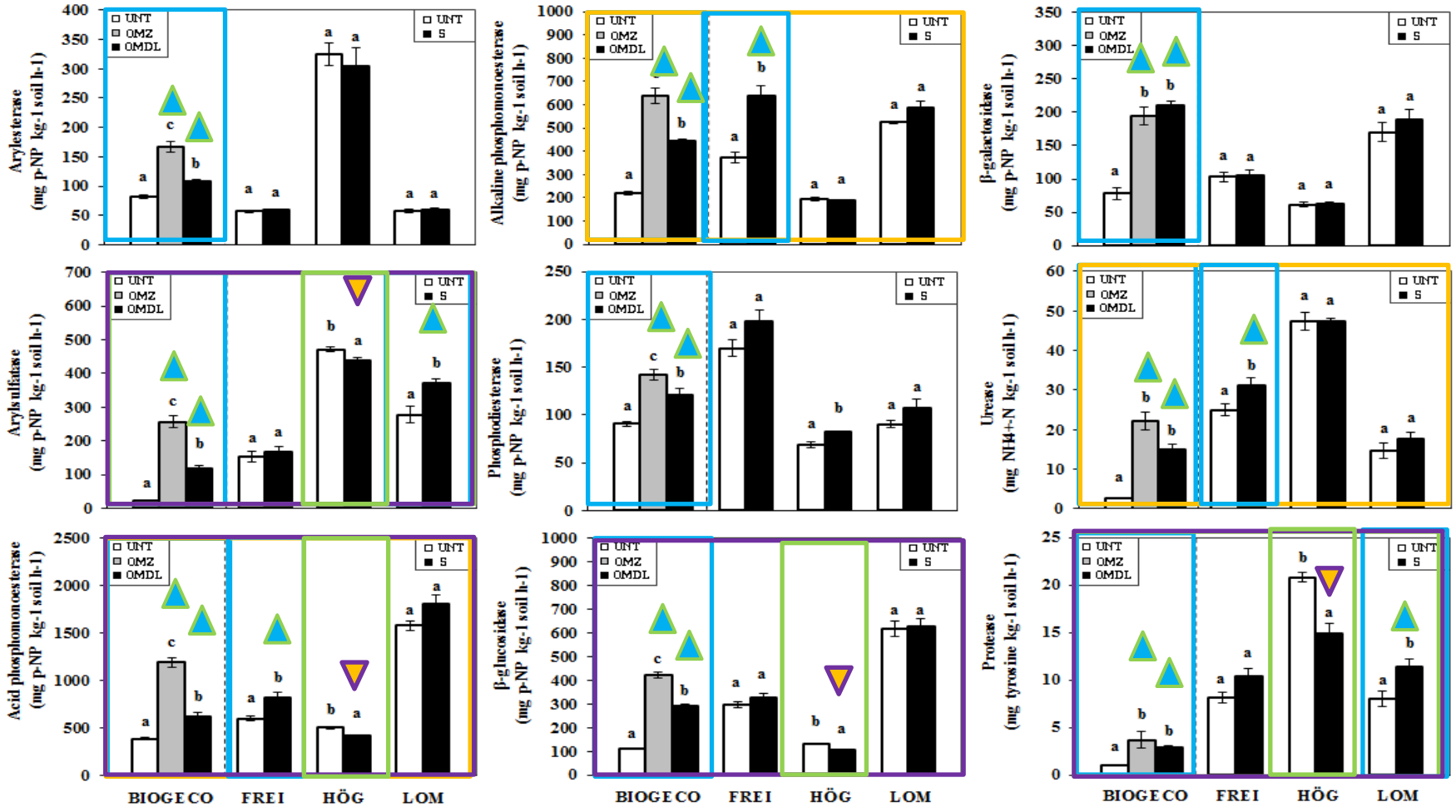
- The ATP content in Biogeco, Lommel and Freiburg suffered a significant increase in treated soils.

- In HÖG the content was significantly lower in the phytomanaged soils.

- Significant differences in basal respiration between treated and untreated soils were only found in the Biogeco.

Results and discussion

MICROBIAL ACTIVITY



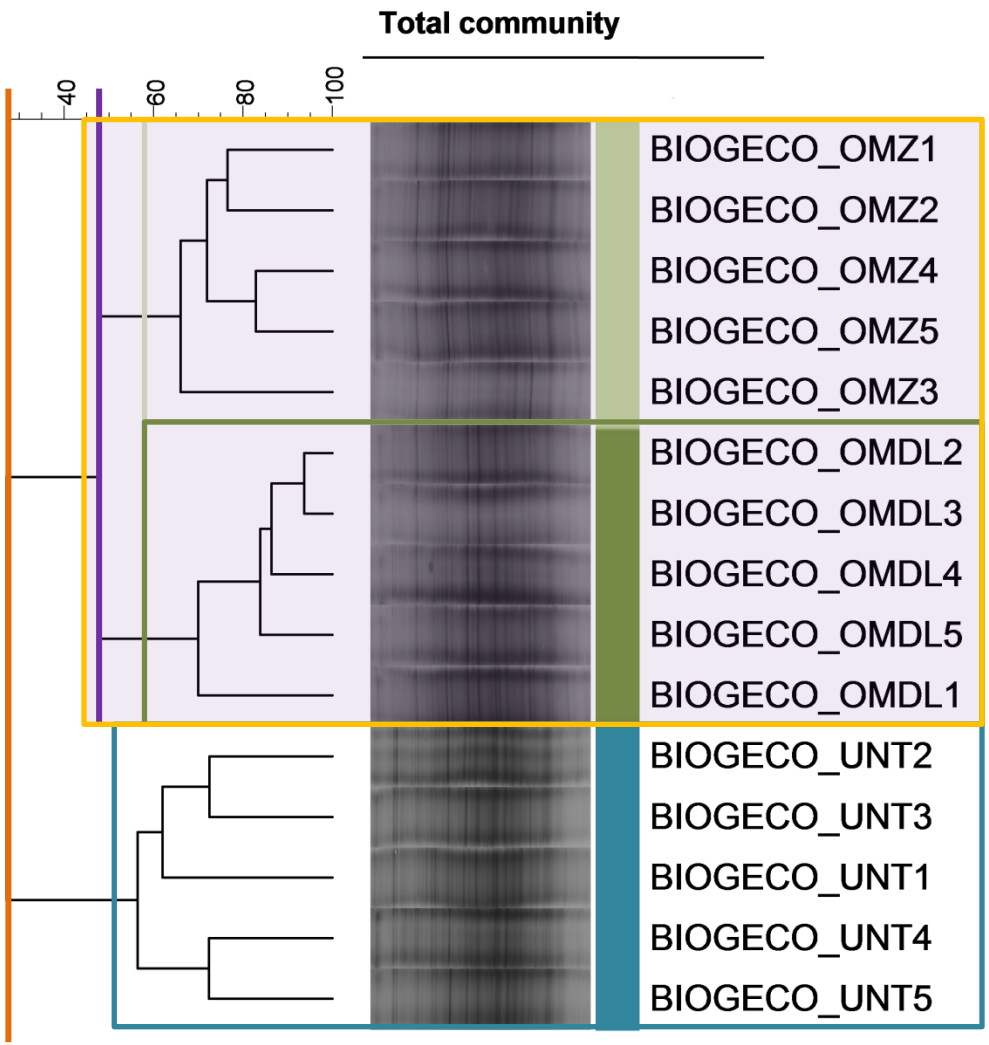
- Soil treatment (OMZ and OMDL) of the Biogeco site caused a significant increase in all enzyme activities. In general the activity is higher in OMDL treatment.

- In Freiburg soil, the activities of acid phosphomonoesterase, alkaline phosphomonoesterase, b-glucosidase and protease.

- In HÖG a lower activity was found in the treated soils in arylsulfatase, acid phosphomonoesterase and urease were significantly higher in treated soils than untreated soils

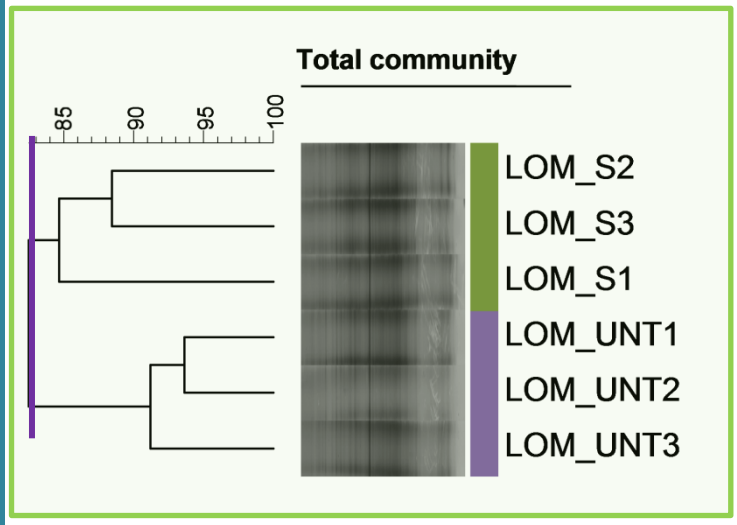
BACTERIAL COMMUNITY STRUCTURE

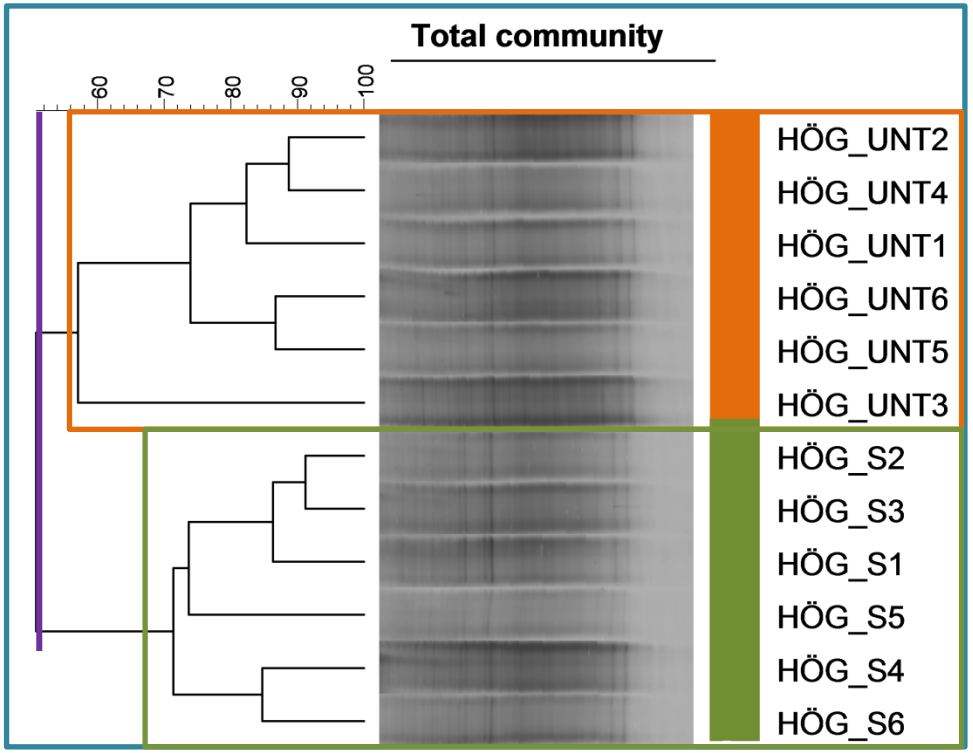
OMZ- (Aided) phytostabilization
OMDL- (Aided) phytoextraction

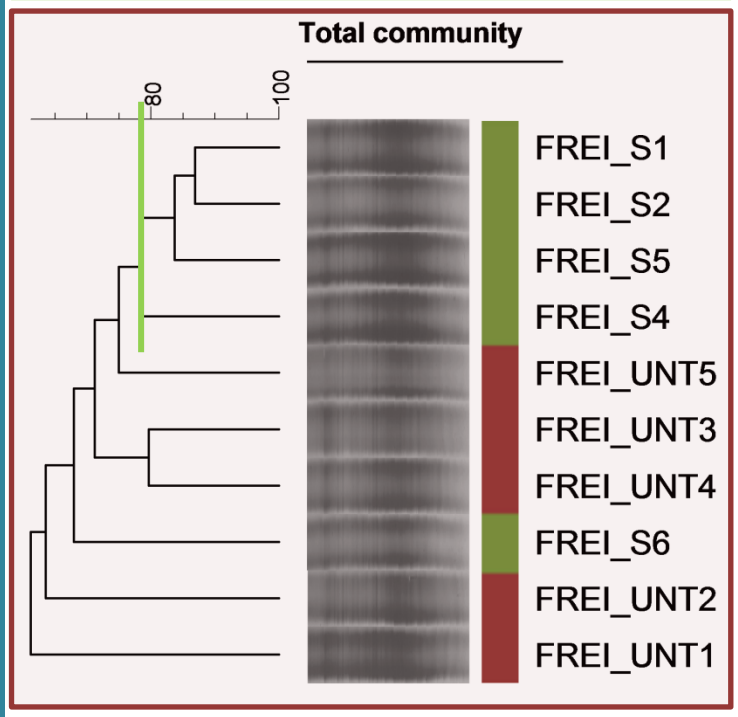


- Untreated & Treated
- The different treatments cluster separately.
- OMZ and OMDL patterns are more similar between each other than to the untreated one.
- The similarity value between OMZ and OMDL is 48%
- The similarity value between untreated and treated is 30%

BACTERIAL COMMUNITY STRUCTURE : Phytoextraction



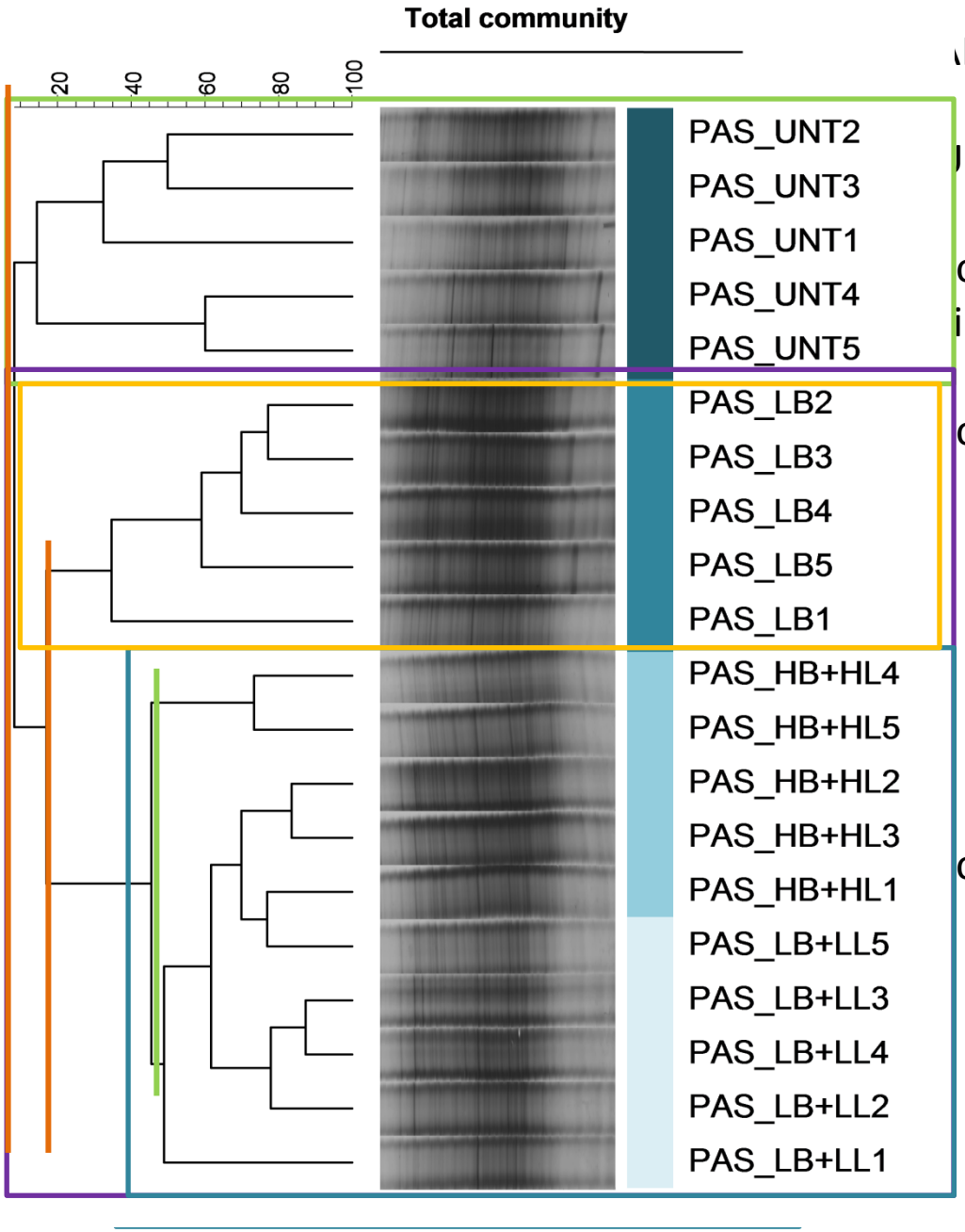




- Untreated & Salix
- Lommel, Freiberg: no clear differences between treatments.
- The similarity values in this cases are close to 80% or greater.
- In HÖG the untreated and the treated cluster differently with a similarity value of 53%

Results and discussion

BACTERIAL COMMUNITY STRUCTURE : In situ stabilization & phytoexclusion



ARN_B & ARN_D

Untreated & Treated

oil B and D cluster separately (40% similarity).

oilB:

- LB+LL and HB+HL cluster together with a similarity of 50%
- LB cluster separately from the combined treatments (biosolid + lime) at 20% similarity.

oil D:

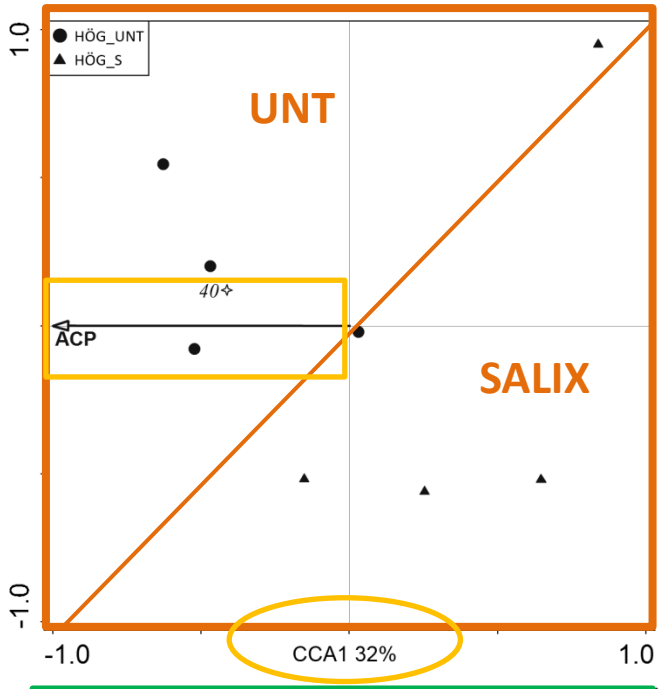
- UNT and GS+RM cluster separately (77% similarity)

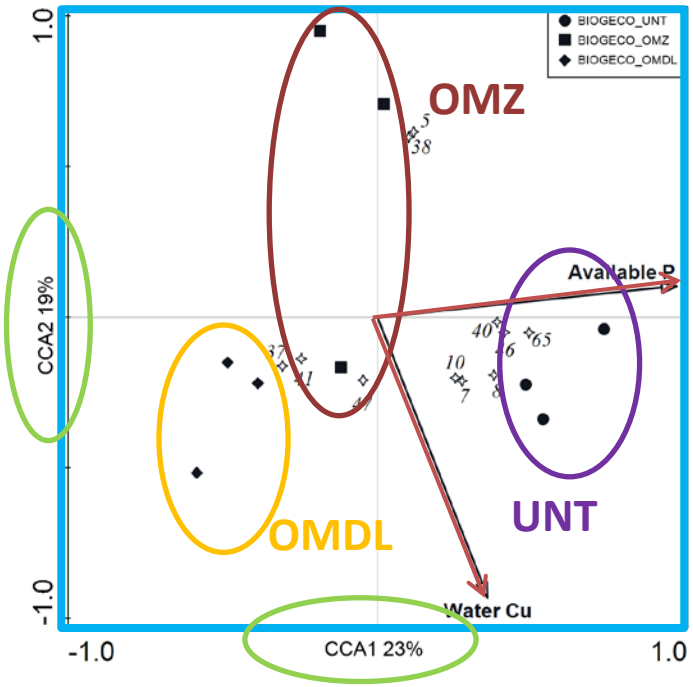
- Untreated & Treated
- Untreated cluster separately than treated with a similarity value of 10%.

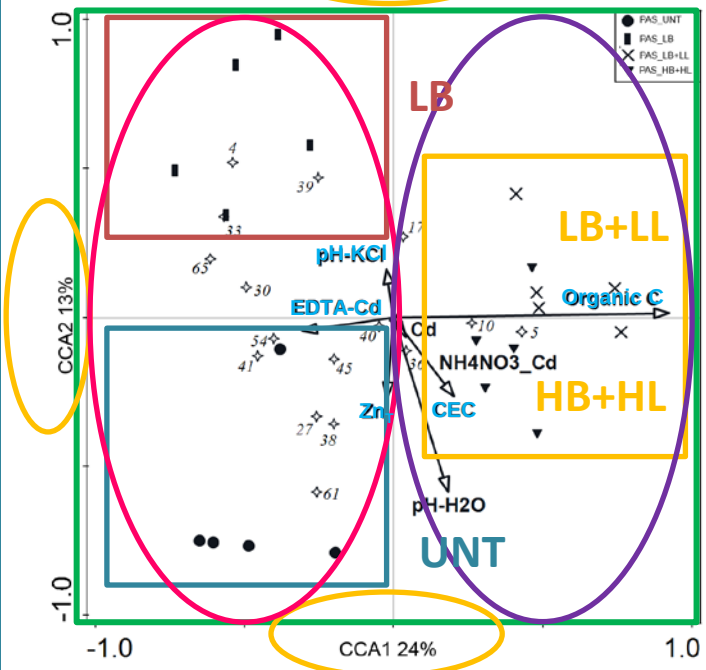
- Treated and untreated patterns of cluster separately (80% similarity)
- GS+E and LB cluster closely together at 85% of similarity.

Results and discussion

Canonical correspondence analysis (CCA)







Physico- and bio-chemical soil properties against bacterial community data

PAS: In situ stabilization & phytoexclusion

- Separates the four treatments
- Explained variation 37%
- Separates untreated soil from soil under Salix
- The pH KCl, total Zn and available Cd are related with the community of UNT and LB
- Separates the three different treatments.
- Explained variation 32%
- Explained variation 42%
- Acidophosphatase is the variable who explain the best the model.
- The CEC and organic C are the main variable which are related with combined treatments.
- Available P and soluble Cu are the variable who explain the best the model.

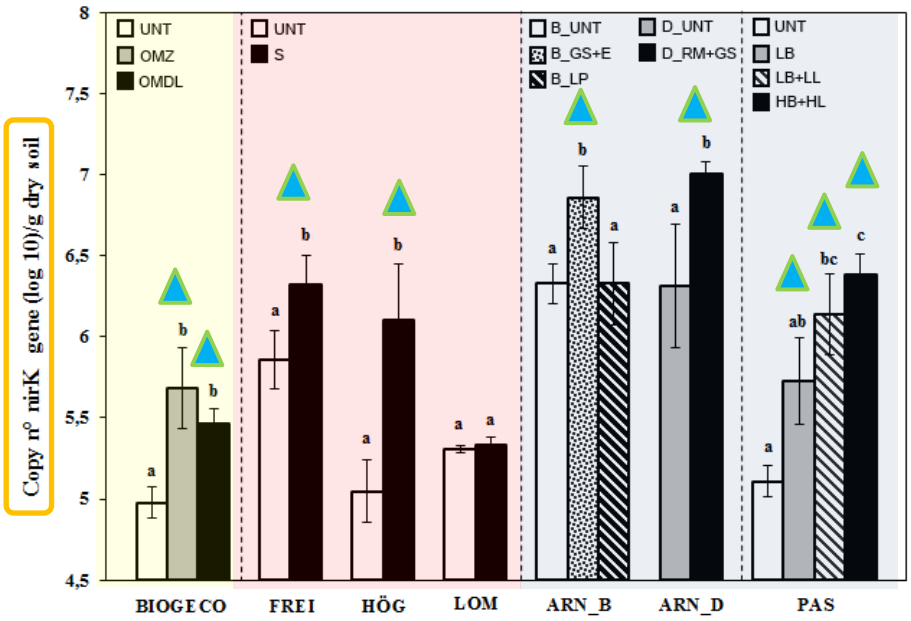
BIOGECO: (Aided)phytoextraction and (Aided)phytostabilization

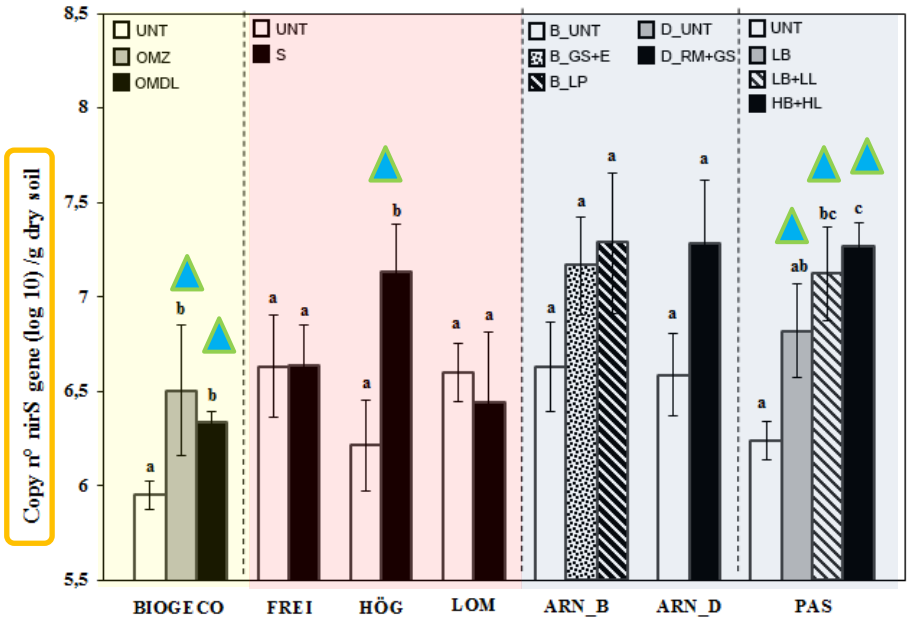
HÖG : Phytoextraction

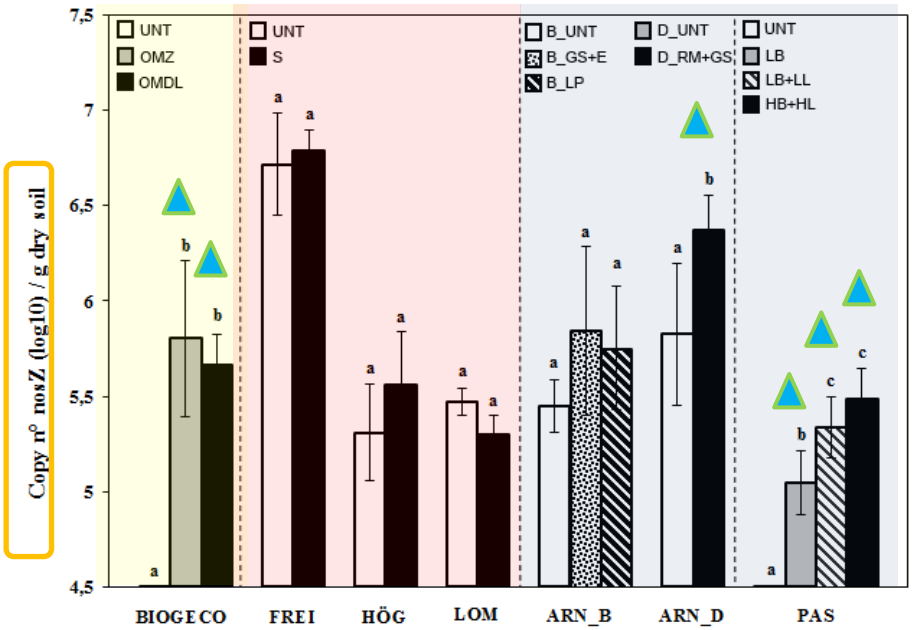
Results and discussion

qPCR: DENITRIFICATION GENES

Results and discussion



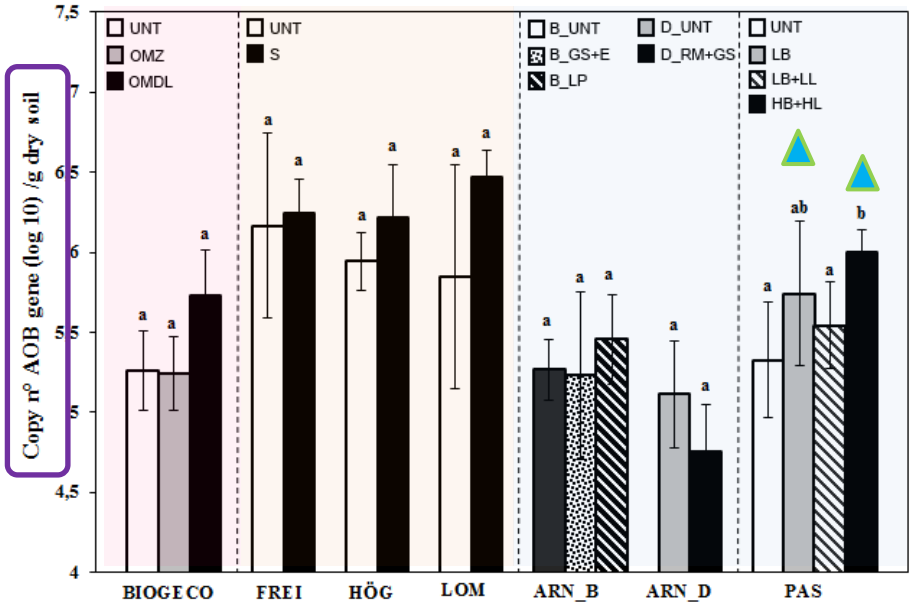


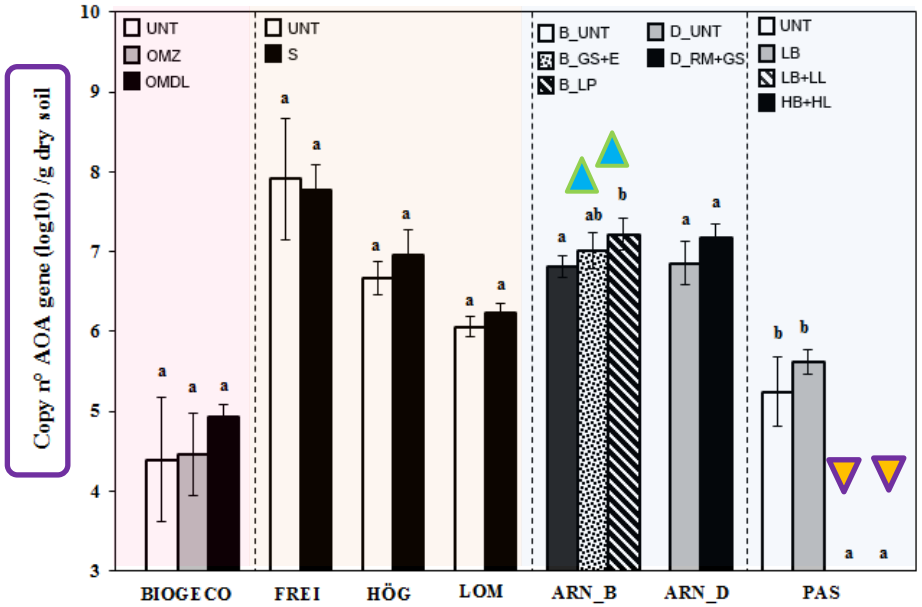


- nirK, nirS and nosZ

- In Biogeco, soil treatment (OMZ and OMDL) causes an increase in all denitrification genes.
- Phytoextraction: Increase in nirK and nirS genes in Freiberg and HÖG sites.
- Phytoexclusion: Generally the treatments lead to an increase of all studied denitrification genes.

qPCR: NITRIFICATION GENES





- amoA bacteria (AOB), amoA archaea (AOA)

- No changes in Biogeco plots

- Phytoextraction: no significant changes

- Phytoexclusion:
  - AOB: PAS plots copy number increase with the HB+HL amendment.
  - AOA: Increase with the amendment in ARN_B and under detection limit in treated of Poland

Results and discussion

Conclusions

- Depending on the site, the GRO applied induced an increase in microbial biomass and activity, as well as clear differences in bacterial community structure (at both the total community and group level)

- The number of gene copies (nirK, nirS, nosZ, amoA) in general increase when the treatment is applied.

- In the CCA the results were different depending on the studied site. Generally the available metal is the main factor who influence the bacterial community changes.

- GRO implementation can lead to shifts in the bacterial community and diversity.

Thank you for your attention!

This work was funded by the Spanish Research Council (CSIC)- JAE-Predoc 2009 and by the EU GREENLAND project (FP7-KBBE-266124)

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