MICROBIOLOGICAL DIVERSITY AND FUNCTIONALITY OF A CHRONICALLY HYDROCARBON CONTAMINATED SOIL POST CHEMISTRY OXIDATION

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Petrochemical area

La Plata,
Buenos Aires

Polyciclyc aromatic hydrocarbons (PAH)

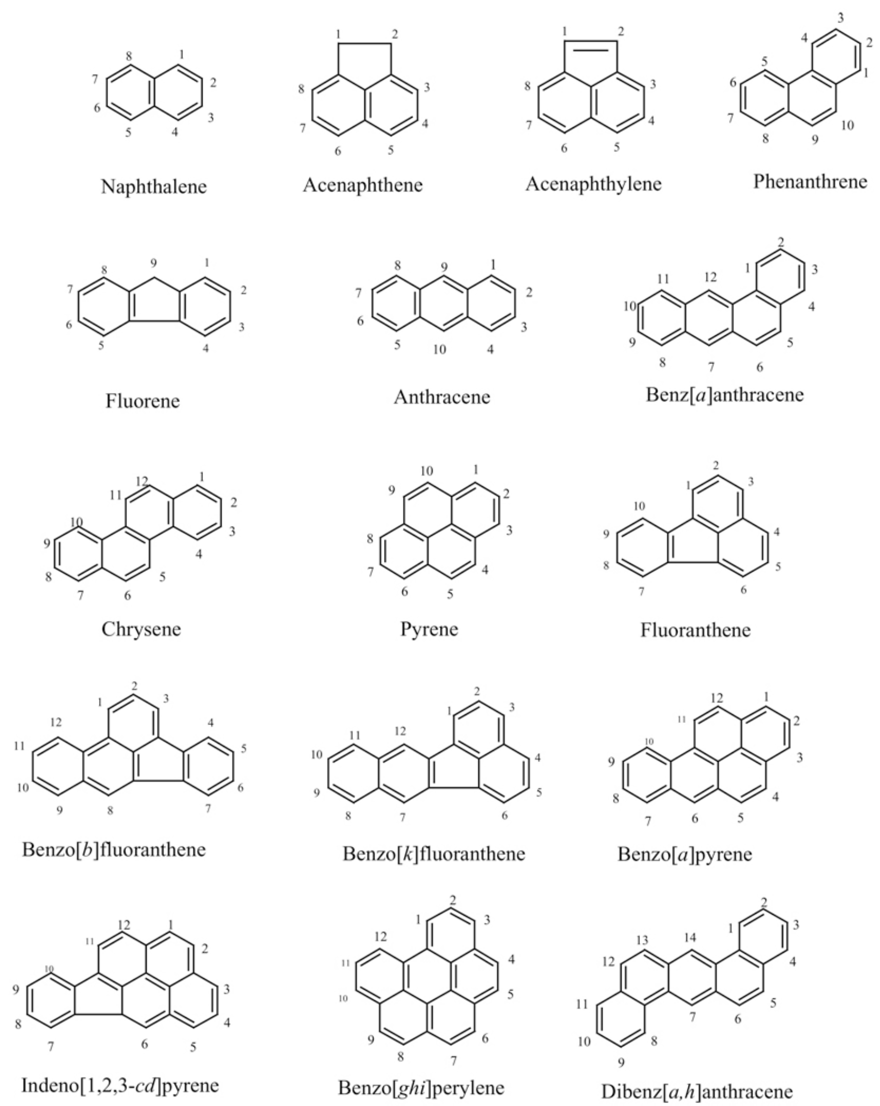
Polycyclic aromatic hydrocarbons (PAHs) are **highly recalcitrant** molecules that can persist in the environment due to their hydrophobicity and low water solubility.

PAHs are ubiquitous in the natural environment and originate from two main sources:

•***Natural*** (biogenic and geochemical) as volcanic eruptions and forest fire.
•***Anthropogenic***, are formed during the incomplete combustion of organic materials such as coal, diesel, wood and vegetation

Point sources of PAHs can originate from **petroleum** and **diesel** spills and from **industrial processes.**

The contamination with PAHs pose **potential risks** to human and ecological health



Priority PAHs as listed by US EPA

Remediation technologies

How to remove pollutants from the natural environment and/or convert them to less harmful products?

The process of remediation depends on environmental factors: soil pH, presence of nutrients, bioavailability of the contaminant.

Bioremediation strategies use the degradation potential of soil to decontaminate the impacted area.

Low cost and safely

Long time, slow degradation rate

Chemical Oxidations consists on the utilization of additives to induce chemical attack on the impacted area

Low cost and fast degradation rate

Impact on microbiological community and on soil

To address the limitations of individual remediation techniques and to achieve better **PAH** removal efficiencies, the combination of chemical and biological treatments can be used.

Chemical Oxidation

The most common oxidants are:

- Hydrogen peroxide (H_2O_2)
- Permanganate ion (MnO_4^-)
- Persulfate ion ($S_2O_8^{2-}$)

By activation generates sulfate radical anions ($SO4^{\bullet-}$, $E^\circ = 2.6$ V).

$$S_2O_8^{2-} \xrightarrow{h\nu} 2\ SO4^{\bullet-}$$

$$S_2O_8^{2-} \xrightarrow{Heat} 2\ SO4^{\bullet-}$$

$$S_2O_8^{2-} \xrightarrow{M^{n+}} 2\ SO4^{\bullet-}$$

Our study:
Ammonium persulfate (PS)

Aim of the work

To evaluate the effect of the ammonium persulfate application followed by a bioremediation process, on the soil matrix and on the microbial community.

Soil characteristics

Chronically contaminated soil, ***CS***.

Properties of CS	
pH	8.4 ±0.1
CE (mS/cm)	2.72 ±0.3
OPR (mV)	238 ± 13
WHC (%)	27.45
Organic carbon (%)	2.20 ± 0.88
Organic matter (%)	3.78
Total nitrogen mg/kg	0.2 ± 0.12
Available phosphorus (mg/kg)	8.33 ± 0.63
Total Fe (ppm)	$2.3\ 10^{4} \pm 4.7\ 10^{3}$
Available Fe (ppm)	12,57 ± 0.35
PS (g/ 100 g soil)	Nd
Total PAHs (ppm)	214 ± 24
Bioavailable PAH (%)	1%
AH (ppm)	2500 ± 500

Experimental design

CS

Bioremediation

BS

Moisture content 25%
Mixed once a week
Incubation at 25°C for 28 days

Additions of PS

Chemical Oxidation

OxS

Bioremediation

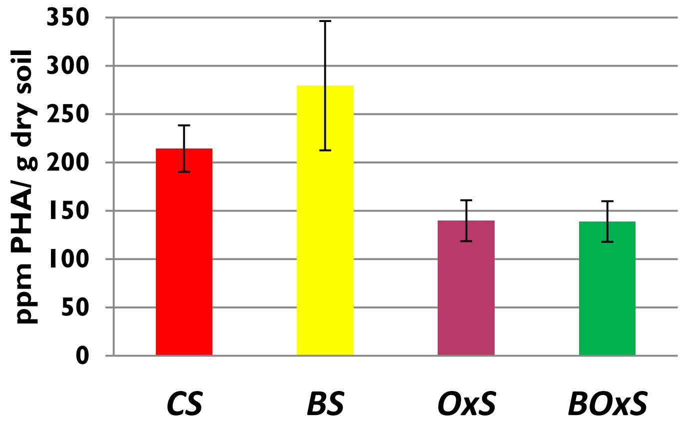
BOxS

- *[PS]*= 3.5 g/ 100 g dry soil
- 3 additions
- *Incubation at 30°C for 7 days*

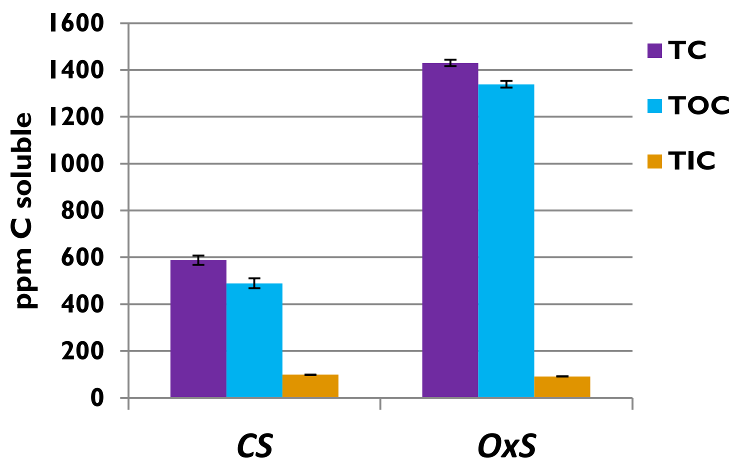
- *Total hydrocarbons*
- *Total Organic Carbon (TOC) , aqueous extract*
- *Soil matrix by Fluorescence Excitation Emission Matrixes (FEEM)*
- *Toxicity assay (seed germination test)*
- *Enzymatic assays (dehydrogenase, lipase, protease, arylsulfatase and urease activities)*
- *Cultivable community (bacteria and fungi)*
- Genetic analysis of *16S-rDNA, by PCR-DGGE* and Pyrosequencing

Results

- ***Hydrocarbon concentration***



- ***Total Organic Carbon (TOC)***



TC: Total carbon
TOC: Total Organic Carbon
TI: Total Inorganic Carbon

✓ No **PAH** elimination was detected in ***BS***.

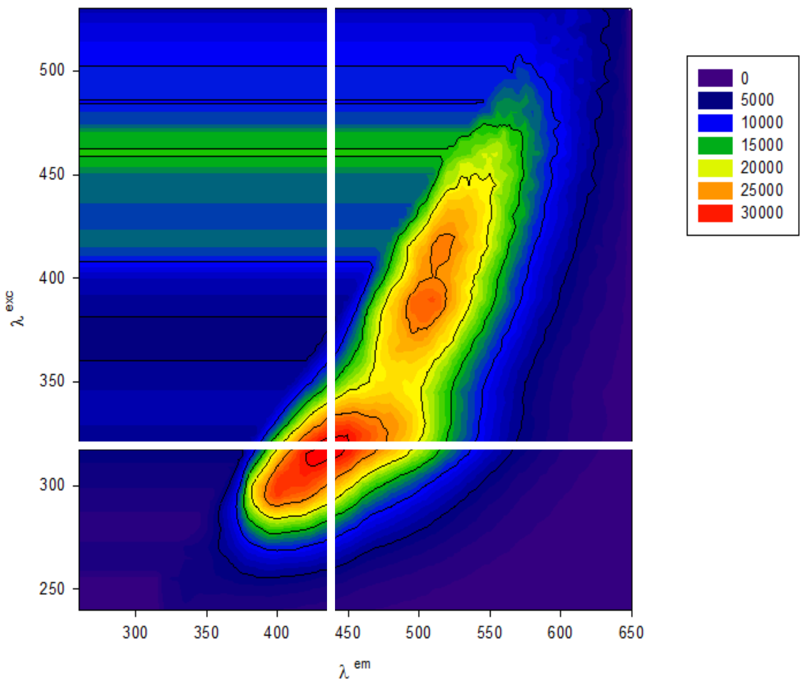
✓ A significant elimination (35%) was observed in ***OxS*** while no additional decrease was detected in ***BOxS***.

✓ No HA elimination was detected in 28 days.

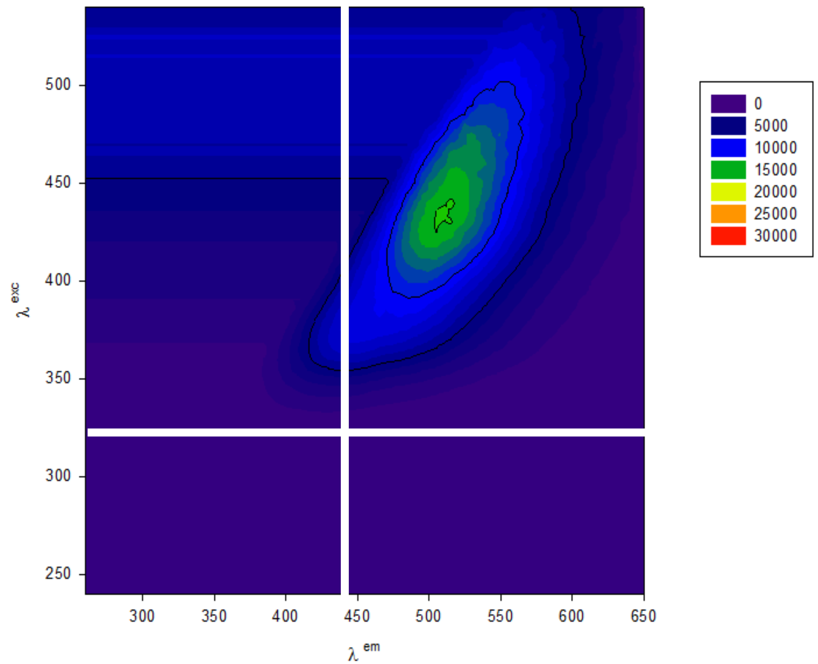
Alkaline extraction was performed to obtain an aqueous extract of natural organic matter of the soil.

✓ A significant increment on TOC values was detected post chemical oxidation.

- ***Fluorescence Excitation Emission Matrixes (FEEM)***



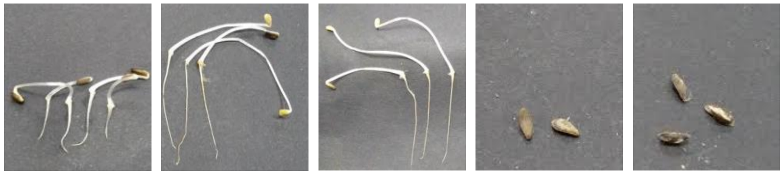
Contaminated soil (CS)



Oxidized soil (OxS)

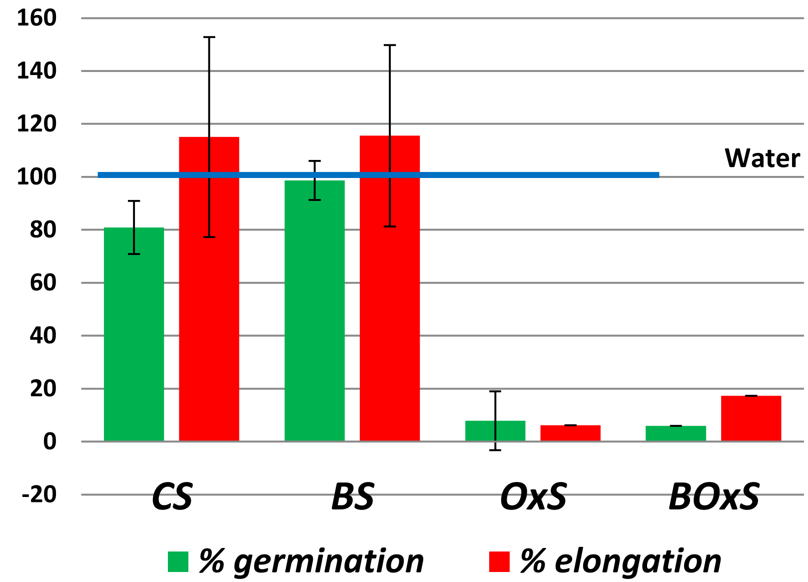
✓ FEEM of ***CS*** presents two zones of emission. The zone on λexc ~ 320 nm and λem ~ 440 nm could be assigned to the presence of PAH. These emissions were absent in ***OxS*** in line with the PAH elimination and increment on TOC value.

- ***Toxicity assay***



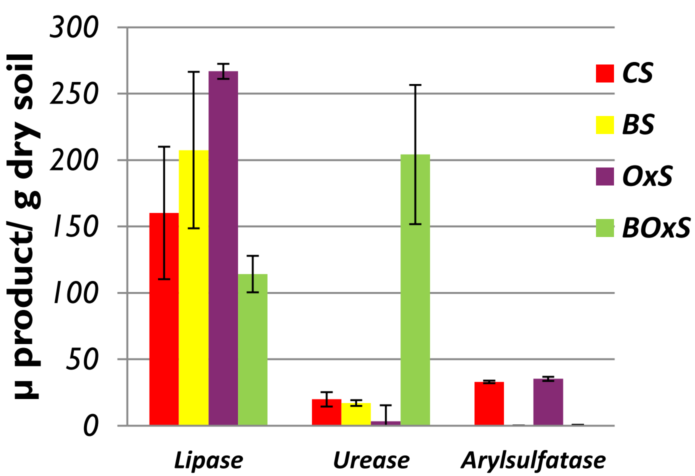
Water **CS** **BS** **OxS** **BOxS**

Seed germination test using *Lactuca Sativa* on water extracts was performed to evaluate the soil toxicity



✓ The toxicity detected in ***OxS*** was not reversed in ***BOxS***

- ***Enzymatic assays***



✓ Increased lipase activity was detected in ***OxS***, suggesting a potential HA elimination.

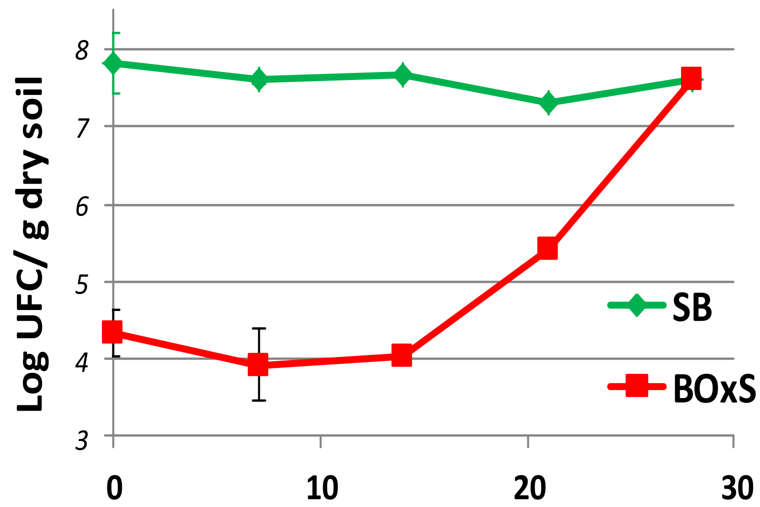
✓ Urease activity was increased in ***BOxS.***

✓ Arylsulfatase activity was not detected in the bioremediated microcosms.

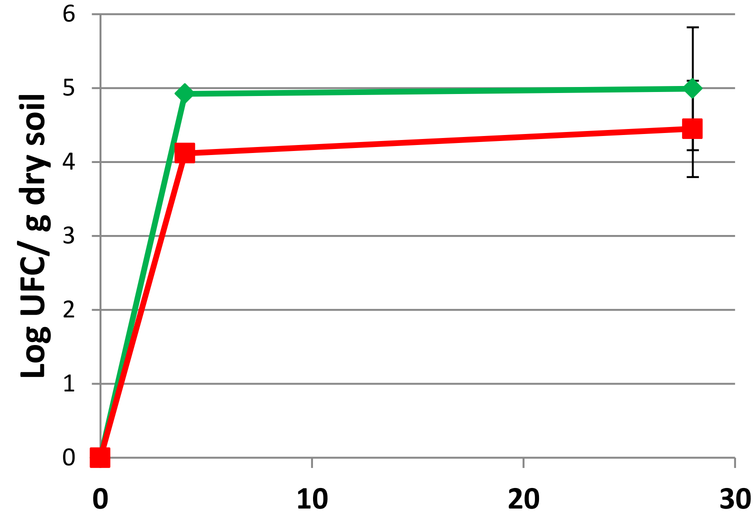
✓ No dehydrogenase and protease activity were detected

- ***Cultivable community***

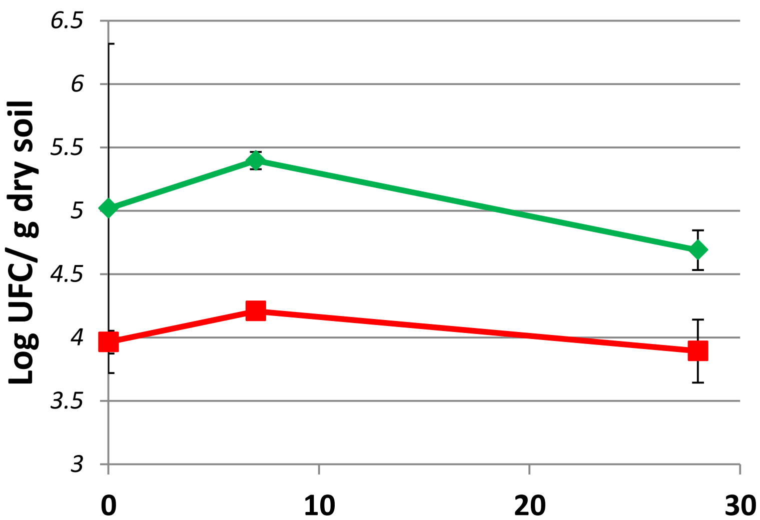
Heterotrophic bacterial population



Inorganic Phosphorus solubilizing bacteria

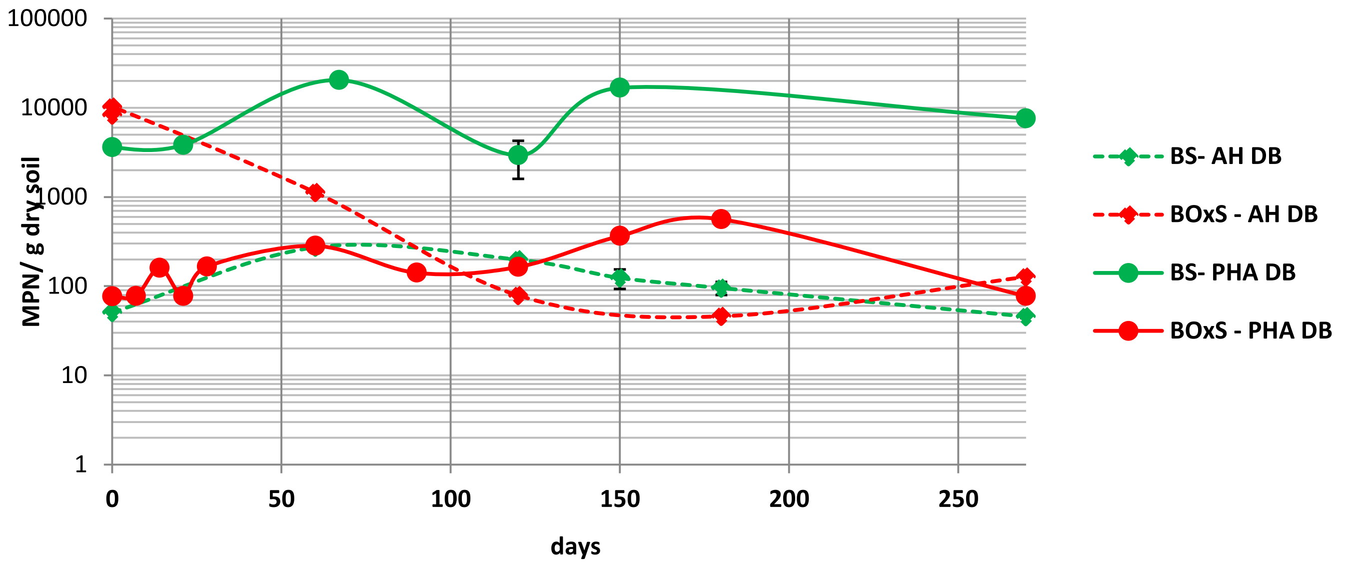


Fungi population



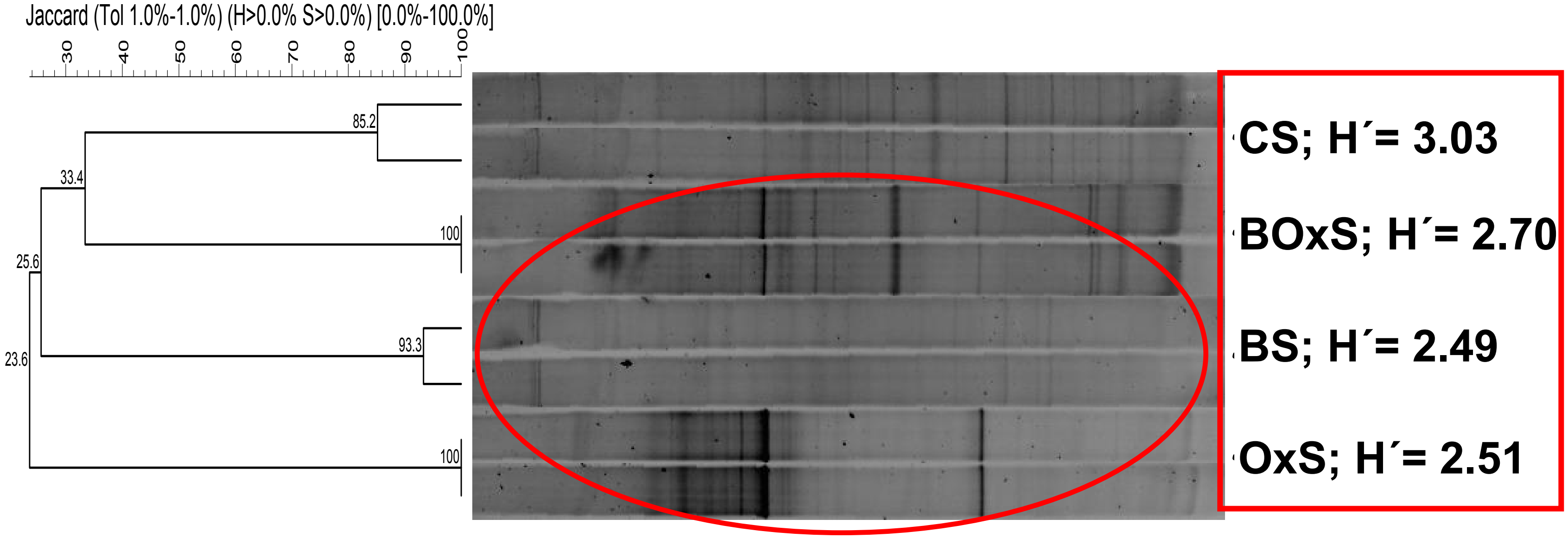
- ✓ PS produced a significant decrease in soil microbial populations.
- ✓ Resilience of heterotrophic and inorganic phosphorus solubilizing bacteria were observed after 28 days.

AH (n-hexane)- degrading bacteria **PAH (mix)- degrading bacteria**



- ✓ **PS produced a significant decrease in PAH- degrading bacteria population.**
- ✓ **PS produced a significant increment in AH- degrading bacteria population.**

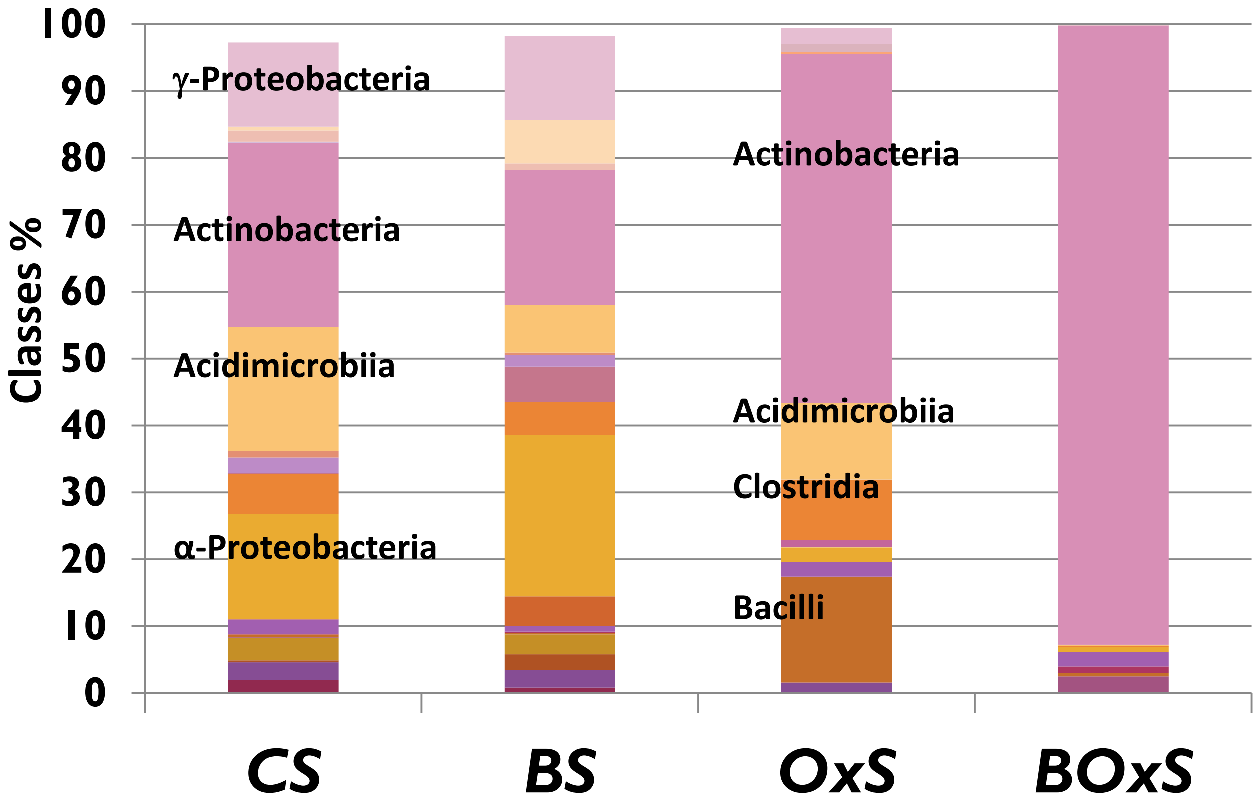
• **Genetic diversity by *16S-rDNA, PCR-DGGE***



H´: Shannon index

✓ All treatments produced a significant change in the bacterial community structure and a reduction in the diversity index.

- **Pyrosequence analysis of bacterial community structure (*16S-rDNA)***



- ✓ **The members of actinobacteria, clostridia, bacilli and acidimicrobiia classes were the predominant populations in OxS.**
- ✓ **Members of the actinobacteria became the dominant population after incubation, in BOxS.**

Conclusions

Effects of the chemical oxidation:

- Elimination 35% of PAH
- Increment in the soluble organic Carbon (TOC)
- Changes in the matrix soil (FEEM)
- Increment of the fitotoxicity
- The lipase activity was the only enzyme detected after oxidation
- Detriment on the cultivable populations
- Changes in the genetic diversity with an increase in the relative abundance of members of Actinobacteria, Acidimicrobiia, Clostridia and Bacilli classes

Effects of the bioremediation:

- **No additional hydrocarbon elimination nor any change in the phytotoxic effect were observed.**

- **Resilience of the heterotrophic bacterial population, with the decrease of the hydrocarbon degrading one suggested a better adaption of the first to the treated soil.**

- **Members of Actinobacteria, considered as k-strategist microorganisms were the major component in the later stage of successions in the bioremediated soil.**

- **The reduction of lipase activity could be associated with the assimilation of the available organic C during the bioremediation.**

Future perspective

- **The supply of available organic matter, by adding mature compost, or by in situ composting could support the specialist populations development.**

- **Monitoring through the quantification of specific genes, would bypass the limitation of culture dependent methods.**

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MUCHAS GRACIAS!