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## Introduction

Phytostabilisation is the most feasible technology for the recovery of large areas contaminated by trace elements [1]. Its advantages are multiple: *in situ* technique, cost effective, aesthetic value and restoration of ecosystem functions [2].

We aim to understand the species-specific effects on soil functionality, measured as microbial biomass and enzyme activities related to C, N and P cycling, as different tree species are expected to affect soil underneath differently due to the contrasted leaf litter quality and root exudates, among others.

We selected a trace element contaminated and remediated area (through phytoremediation strategy) in SW Spain where we studied long-term (15 years) effects of afforestation. As soil properties are essential for soil functionality we studied two areas with different soil characteristics (see [3]).

## Methodology

### Study Area

We selected two contaminated areas, North and South, along the restored Guadiamar Green Corridor (Figure 1).

### Tree species

Three tree species and adjacent treeless patches were selected with seven replicates per species and area:

- *Olea europaea* (OE) Wild olive
- *Populus alba* (PA) White poplar
- *Pinus pinea* (PP) Stone pine
- Treeless (T)

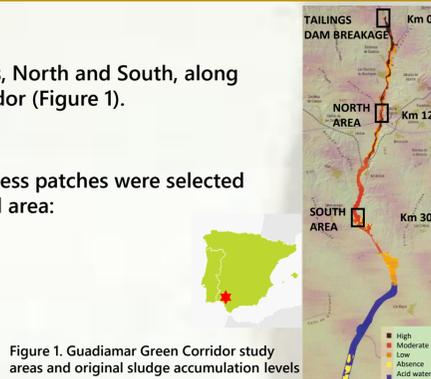


Figure 1. Guadiamar Green Corridor study areas and original sludge accumulation levels

### Soil characterisation

Top soil was sampled at 0-5 cm depth in March 2017.

#### Chemical analyses

We measured in air-dried soils: pH, total C and N, available P and trace elements.

#### Microbial analyses

We measured in fresh soils: microbial biomass C (MB\_C) and N (MB\_N) and enzyme activities (BGL, PO, NAG, LAP, ACP, DH, FDA) (Table 1).

Enzyme activity	Class	Degradation of	Cycle
BGL	Glycoside hydrolase	Cellulose	Carbon
β- glucosidase			
PO	Oxidoreductase	Lignin	Carbon
Phenol oxidase			
NAG	Glycoside hydrolase	Chitin	Carbon and nitrogen
N- acetyl glucosaminidase			
LAP	Peptide hydrolase	Peptides	Carbon and nitrogen
Leucyl aminopeptidase			
ACP	Phosphoric monoester hydrolase	P in SOM	Phosphorous
Acid phosphatase			
DH	Oxidoreductase	SOM	Soil microbial activity
Dehydrogenase			
FDA	FDA hydrolysable enzyme	SOM	Soil microbial activity
Fluorescein diacetate			

Table 1. Studied enzyme activities and their role in soil cycling

## Area and tree effect on soil chemistry

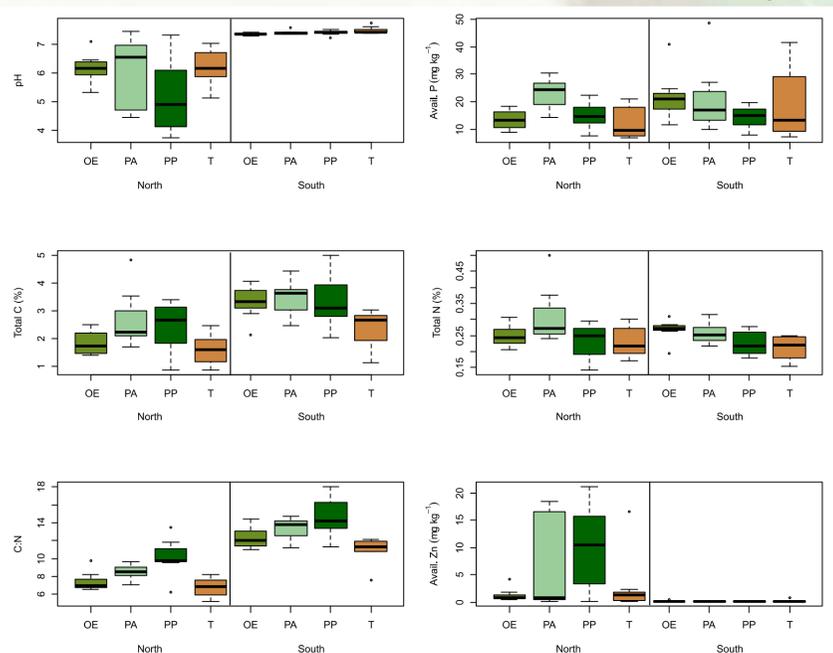


Figure 2. Soil chemistry underneath tree species (and treeless) in North and South areas. Median, upper and lower quartile (boxes) with maximum and minimum values (whiskers).

Area and species effects were analysed in soil chemical variables. Soil pH was neutral in South area but was acidic in North area. Stone pine (PP) presented an acidification effect on North area which explained high trace element availability (Zn). Total C and C:N ratio were higher in South area and under white poplar (PA) and stone pine (PP), completely opposite trend to treeless soils. Total N was found significantly higher under white poplar (PA). Available P was not significantly affected by neither field nor tree species.

## Area and tree effect on microbial activity

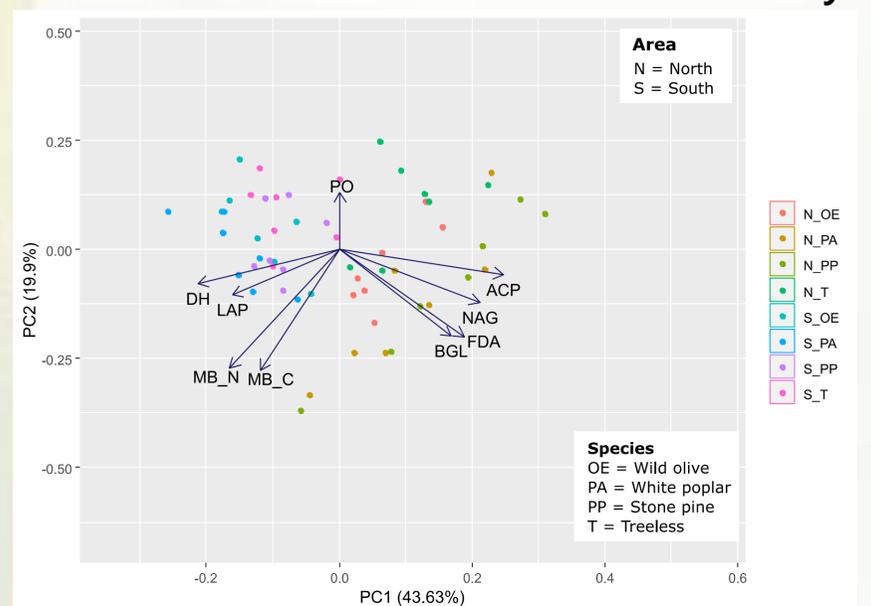


Figure 3. Principal Components Analysis (PCA) ordination of soils according to microbial biomass and enzyme activities (arrows) of soils underneath three tree species (and treeless soils) in two areas (dots).

PC1 axis explained most of the variation due to the strong differences among areas, but also among species. North area and PA and PP species presented higher ACP, NAG, FDA and BGL enzyme activities, while South area and PA recorded higher DH and LAP enzyme activities.

PC2 axis explained differences found among species, independent of area. Highest PO enzyme activity was recorded in treeless soils. Highest microbial biomass C and N were found in soils underneath white poplar (PA) and stone pine (PP).

## Soil chemistry and microbial activity relationship

Variable	MB_C	MB_N	BGL	PO	NAG	LAP	ACP	DH	FDA
pH	0.313*	0.508***	-0.449**	0.075	-0.684***	0.482***	-0.868***	0.695***	-0.504***
Avail. P	0.062	0.118	-0.090	0.005	-0.179	0.127	-0.204	0.150	-0.069
Total N	0.196	0.375*	0.304*	0.108	0.353*	-0.017	0.08	0.046	0.127
Total C	0.294*	0.465***	-0.024	0.131	-0.055	0.264	-0.386**	0.254	-0.350*
C:N	0.238	0.349*	-0.256	0.095	-0.349*	0.379*	-0.587***	0.309*	-0.559***
Avail. Zn	-0.316*	-0.432**	0.424**	0.011	0.573***	-0.377**	0.668***	-0.582***	0.396

Table 2. Pearson coefficient correlation and significance (\* p ≤ 0.05; \*\* p ≤ 0.01; \*\*\* p ≤ 0.001) between soil chemical and microbial properties.

Microbial biomass and enzyme activities were highly related to soil pH. BGL, NAG, ACP and FDA were optimum at acidic pH, while LAP, DH and microbial biomass C and N were enhanced at neutral soil pH. Trace element availability (Zn) is also highly explicative due to the negative correlation with pH. Total soil C and N enhanced the microbial growth in the soils. Nitrogen was key for BGL and NAG enzyme activities. A high C:N ratio was negative for NAG, ACP and FDA activities but positive for LAP and DH activities. Both FDA and DH are indicators of microbial activity but they presented opposite results.

## CONCLUSIONS

- We found a species-specific effect through a chemical footprint in topsoil: Afforestation of stone pine acidified the soil and increased the availability of trace elements.
- Increasing soil pH in the North area is recommended. Lime addition could be a feasible solution to neutralize pH and, consequently, reducing trace element availability.

- Tree afforestation increased carbon sequestration compared to treeless areas, which mitigates climate change and improves soil quality and structure.
- Tree afforestation had a positive effect on the recovery of soil functionality. White poplar (PA) and stone pine (PP) enhanced enzyme activity and nutrient mineralization, as well as microbial biomass growth. However, in trace element contaminated soils, afforestation with stone pine (PP) is not convenient.

### References:

- [1] Mendez, M.O. & Maier, R.M. (2008) 'Phytostabilization of mine tailings in arid and semiarid environments - an emerging remediation technology', Environmental Health Perspectives, Vol. 116, pp. 278-283.
- [2] Garbisu, C. et al. (2002) 'Phytoremediation: a technology using green plants to remove contaminants from polluted areas'. Rev. Environ. Health, vol. 17, no. 3, pp. 173-188.
- [3] Gil-Martínez M. et al. (2018) 'Long-term effects of trace elements contamination on soil microbial biomass and enzyme activities', Proceedings of the 12<sup>th</sup> International Conference on Mine Closure, Technical University Bergakademie Freiberg, Germany, pp. 633-644.

### Funding:

