SHIFTS IN THE STRUCTURE OF A MINE CONTAMINATED SOIL (Pb, Zn, Cu, As) FOLLOWING DIFERENT ORGANIC AND INORGANIC TREATMENTS

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1 INTRODUCTION

Soil microbial community structure is increasingly being marketed as ecologically-relevant endpoint and it can realistically be incorporated for assessing the potential risks associated with anthropogenic disturbances and soil amendment strategies on sustainability of soil ecosystems. In Portugal, additional research is needed if technologies based on the combined action of plants and the microbial communities they support within the rhizosphere are to be adopted in large-scale remediation actions (Nabais *et al.,* 2008). The information about this fact in mine soils is scarce and had focused on soil biochemical properties, producing no clear results. Furthermore, the effects of phytoremediation as soil remediation technique and metal contamination on microbial community structural would be achieved by PLFA studies. By phospholipid fatty acid analysis it is possible to examine broad scale patterns in microbial community structure (Bååth *et al.* 2005) and generally, after the application of multivariate statistical analyses, whole community fatty acids profiles indicate which communities are similar or different. Determination both microbial community composition and biomass size by this direct method gives results that very closely represent the *in situ* soil conditions and is currently used for soil monitoring purposes (Nielsen and Winding, 2002).

The present investigation studies the medium-term response in the soil microbial community structure after the application of different remediation technologies including several organic and inorganic treatments.

2 MATERIALS AND METHODS

A pot trial was conducted with a contaminated (Pb, Zn, Cu, As) sandy soil (Table 1) from the São Domingos mine (South of Portugal). Three replicates for each treatment (24 pots) were filled with 2 kg of the soil and four treatments were performed: inorganic fertilizer (I, 0.1 g N, 0.1 g P, 0.21 g K and 0.03 g Mg kg^{-1} soil); inorganic fertilizer and polyacrylate polymers, polímero with K^+ as counter ion and (P, 0.1% of polyacrylate polymer with 210 mg K^+ g^{-1} and 0.1% of polyacrylate polymer with 98 mg $N\text{-}NH_4^+$ g^{-1} as counter ions, 0.1 g P and 0.03 g Mg kg^{-1} soil); inorganic fertilizer municipal solid waste compost (MSWC) as organic amendment (O, 0.1 g N, 0.1 g P, 0.21 g K, 0.03 g Mg kg^{-1} and 30 g MSWC kg^{-1} soil); inorganic fertilizer, polymer and organic amendment (PO, 0.2% of polyacrylate polymer as before, 0.1 g P and 0.03 g Mg and 30 g MSWC kg^{-1} soil). All the pots were sown with perennial ryegrass. The measurements of PLFA pattern were made on soil samples collected at two different times (1 and 4 months) after plant cultivation and application of the treatments.

The microbial community structure was determined by phospholipid fatty acid (PLFA) analysis (Frostegard *et al*., 1993). Fatty acids (PLFA) are designated in terms of the total number of carbon atoms: number of double bounds, followed by the position of the double bound from the methyl end of the molecule. Cis and trans configurations are indicated by c and t, respectively. The prefixes a and i indicate anteiso- and iso-branching; br indicates unknown methyl branching position, 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule; and cy refers to cyclopropane fatty acids. The total microbial biomass (TotPLFAs) was estimated as the sum of all the extracted PLFAs. The sum of the PLFAs considered to be predominantly of bacterial origin was used as an index of the bacterial biomass (BactPLFAs), the quantity of the PLFA 18:2ω6,9 was used as an indicator of the fungal biomass (FungPLFA), the PLFAs i14:0, a15:0, i16:0 and 10Me18:0 as indicators of gram-positive (G^+) bacteria, and the PLFAs cy17:0, cy19:0, 16:1ω7c and 18:1ω7 as indicators of gram-negative (G^-) bacteria (Díaz-Raviña *et al.,* 2006). Concentration of all the individual PLFAs data, expressed as mole percentage and logarithmically transformed, was subjected to principal component analysis (PCA) to elucidate the main differences in the PLFA patterns. To compare treatments, data were tested by ANOVA and Tukey´s minimum significant difference test was used to differentiate the means.

TABLE 1 **Some physico-chemical characteristics of initial soil samples**

	Texture	pH H_2O	MO (%)	Cu mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}	As mg kg^{-1}
Initial soil	Sandy	5.0	1.0	583	7570	1230	2360

3 RESULTS AND DISCUSSION

Fig. 1 showed that the O and PO treatments showed higher microbial biomass values (total PLFA, fungal PLFA, bacterial PLFA, $Gram^+$ bacteria, $Gram^-$bacteria) than I and P treatments. The total microbial biomass and the biomass of specific groups were significantly higher in the treatments with MSWC than in the other treatments. No clear differences between samples collected at different sampling times were observed.

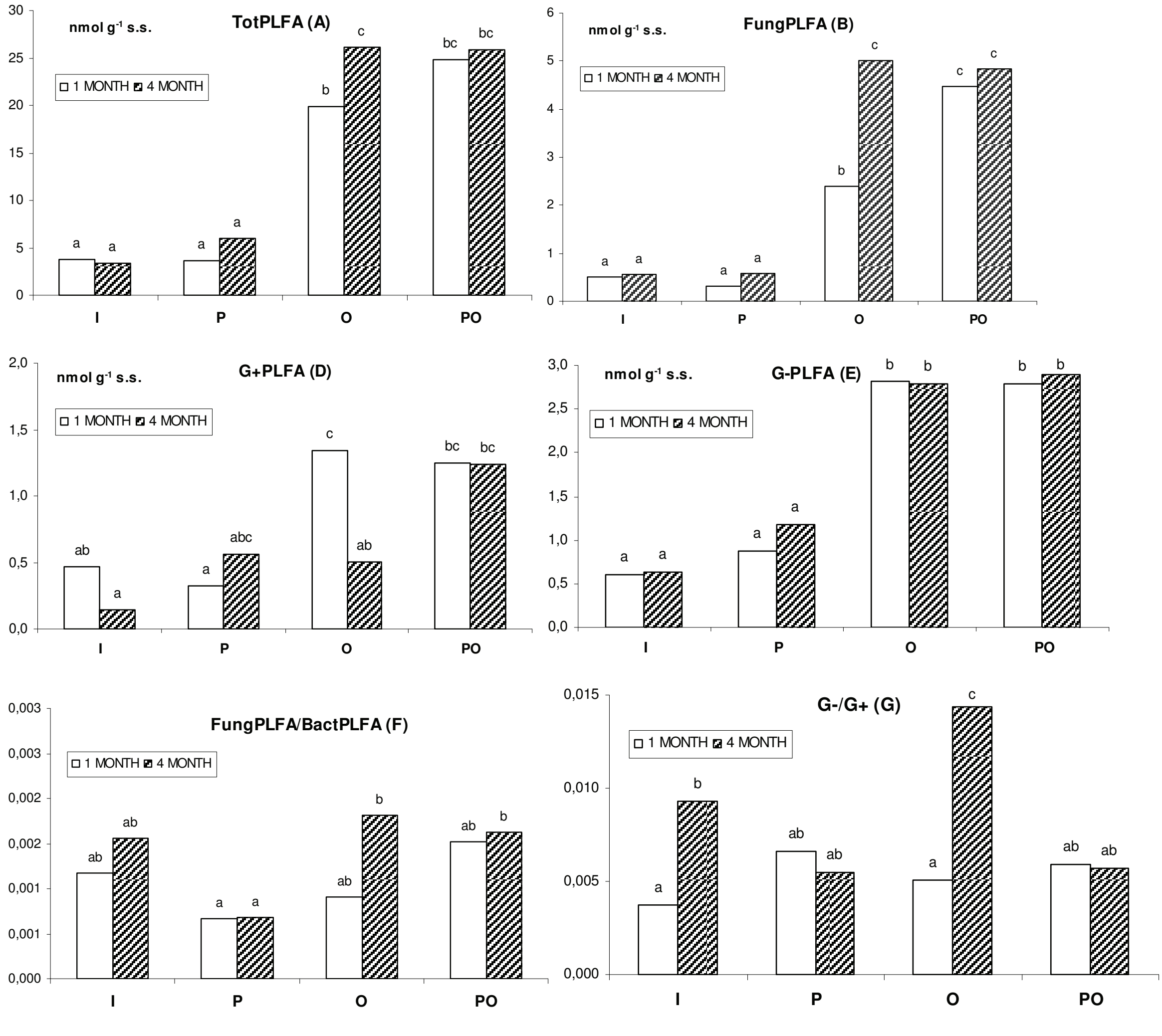

FIGURE 1 **Phospolipid fatty acid concentrations (mean values of three pot replicates) of mine soil with different reclamation treatments.** A, total PLFA (TotPLFA); B, fungal PLFA (FungPLFA); D, gram positive bacteria PLFA (G+ bactPLFA); E, gram negative bacteria PLFA (G- bactPLFA); F, fungal PLFA to bacteria PLFA ratio (FungPLFA/BactPLFA) and G, gram-negative to gram-positive

BactPLFA ratio (G^-/G^+). Treatments: I, inorganic fertilizer; P, polymer; O, MSCW; PO; polymer and MSCW; LI, liming plus inorganic fertilizer. Different letters indicate significant differences at the $P<0.05$ level.

The PCA obtained (Fig. 2) showed that the PC1 explained 34% of the total variance, thus, MSWC organic amendment had a more marked effect than inorganic and/or polymer, increasing 10Me16:0 and monounsaturated PLFAs 18:1w9, 16:1ω9, 16:1ω5, 17:1ω8, 19:1, was detected as consequence of organic fertilization.

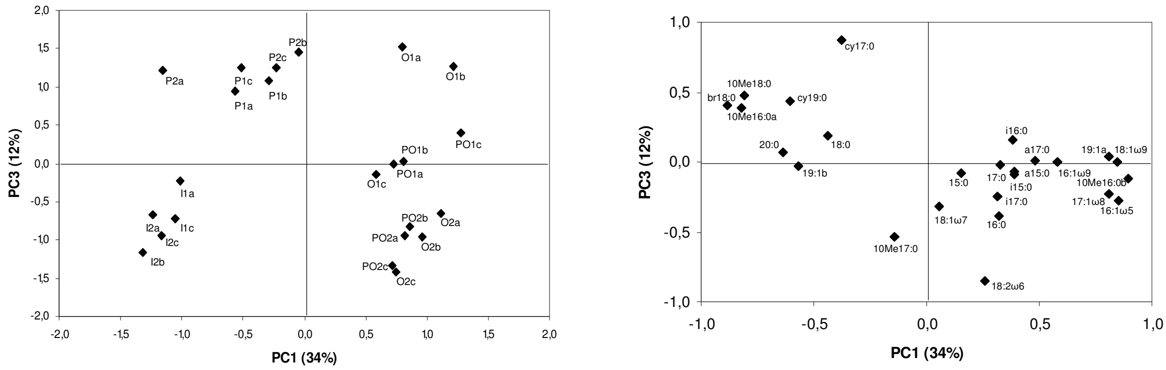

FIGURE 2 **Score and loading plots from PCA performed on the PLFAs of the mine soil. Treatment: I, inorganic fertilizer; P, polymer; O, MSWC; PO, polymer and MSWC; LI, liming plus inorganic fertilizer; LP, liming plus polymer; LO, liming plus MSWC; LPO, liming plus polymer and MSWC.**

4 CONCLUSIONS

The results clearly indicated that microbial community structure data, measured by PLFA analysis, allow us discriminate the different organic and inorganic treatments, the effect of organic treatments being more marked than those of inorganic and polymer treatments. No clear differences between samples collected at different samples times were observed. Our data showed the usefulness of phospholipid fatty acid analyses to detect the medium term impact of remediation technologies for restoration of mine contaminated soils.

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