



# Fire induced changes in soil microbial community estimated by BIOLOG and phospholipid fatty acid techniques

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## Abstract

The BIOLOG and Phospholipid Fatty Acid (PLFA) techniques were used to evaluate the short- and medium- term effects produced by an experimental fire and different stabilisation treatments (seeding, mulching) on soil microbial community. The study was performed in a shrubland ecosystem from Galicia (NW Spain) affected by an experimental fire of low-severity and two different post-fire treatments (seeding and mulching). Soil samples were collected from the top layer (0-5 cm) of the A horizon immediately and 90, 180 and 365 days after the fire and the microbial community was determined using both the BIOLOG and the PLFA methods. The results indicated that, independently of the method used, the experimental fire induced marked changes on the soil microbial community, which persisted even after 1 year

and that there was no evidence of noticeable microbial changes between the two different post-fire treatments and the corresponding burnt treatment. In addition, a clear significant effect of the sampling time on the soil microbial community structure was observed, the impact being even most important than that observed for the experimental fire.

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## 1 INTRODUCTION

Studies about microbial communities in burned soils have been focused on biochemical properties and little is known about microbial structural and functional diversity. The analysis of phospholipid fatty acids (PLFA pattern) provides information on microbial community structure of soils and therefore an approximation on taxonomic diversity (Frostegård et al., 2011). In order to complement the information provided by the PLFA, the microbial contribution to the biochemical transformations in soils can be determined as the capacity of carbon substrates degradation by the microbial communities (Garland & Mills, 1991). The Biolog Ecoplates® technique is frequently

used to this objective providing information on the functional diversity of the microbial community. The aim of the present study was to evaluate the short- and medium term impact of an experimental fire and different stabilization treatments on the soil microbial community of a shrubland in Galicia (N.W. Spain).

## 2 METHODS

The study was conducted in an experimental field located at an altitude of 660 a.s.l. in Cabalar (A Estrada, 42° 38' 58" N; 8° 29' 31" W; N.W. Spain). The soil, a Leptosol developed over granite and with a slope of 38-54%, has a vegetation cover dominated by *Ulex europaeus* L. and

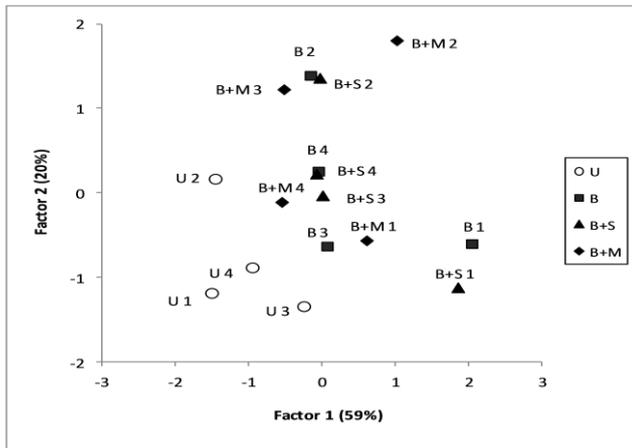


Figure 1. PCA results of the categorized substrates utilization pattern by the microbial communities from the different soil treatments at different times (1, 2, 3 and 4 denote samples collected 1, 90, 180 and 365 days after the fire and application of the stabilisation treatments, respectively). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus application of straw.

some *Pteridium aquilinum* (L.) Kuhn., *Ulex gallii* Planch., *Daboecia cantabrica* (Huds.) K. Koch and *Pseudoarrenhaterum longifolium* Rouy. The main characteristics of the A horizon (fraction < 2 mm) of this soil were acid pH (3.7) and high organic matter content ( $179 \text{ g C kg}^{-1}$ ,  $14.8 \text{ g N kg}^{-1}$ ). After the experimental fire four treatments were considered by quadruplicate (30 x 10 m plots): unburnt soil (C) as control; burnt soil (B); burnt soil with 232 g m<sup>-2</sup> of straw mulch (B+M); burnt soil with a mixture of seeds at a rate of 45 g m<sup>-2</sup> (*Lolium multiflorum*, 35%; *Trifolium repens*, 25%; *Dactylis glomerata*, 20%; *Festuca arundinacea*, 10%; *Festuca rubra*, 5%, *Agrostis tenuis*, 5%) (B+S). Soil samples were taken from the A horizon (0-5 cm depth) at different sampling times over one year (immediately and 90, 180 and 365 days) after the experimental fire.

The microbial community structure was determined by PLFA analysis using the procedure and nomenclature described by Frostegård et al. (1993). The data corresponding to the concentrations of all the individual PLFAs, expressed in mole percent and logarithmically transformed, were subjected to a principal component analysis (PCA) to elucidate the main differences in the PLFA patterns. The soil microbial functional diversity was assessed using Biolog substrate utilization EcoPlates (Biolog Inc., Hayward, CA, USA) (Garland & Mills, 1991). Differences in the well colour development were recorded as optical density (OD) at 590 nm at 24-h intervals for

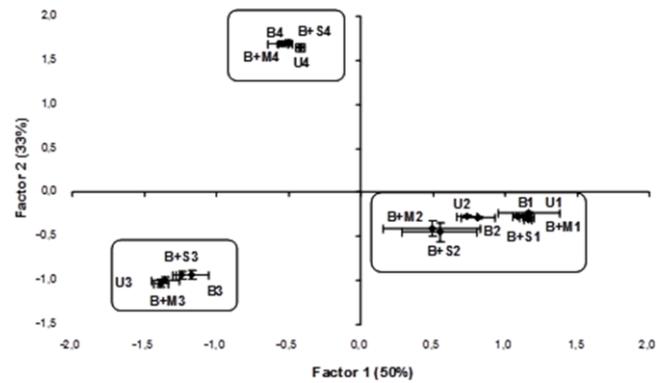


Figure 2. PCA results of the PLFAs of the whole data set of the different soil treatments at different times (1, 2, 3 and 4 denote samples collected 1, 90, 180 and 365 days after the fire and application of the stabilisation treatments, respectively). Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus application of straw.

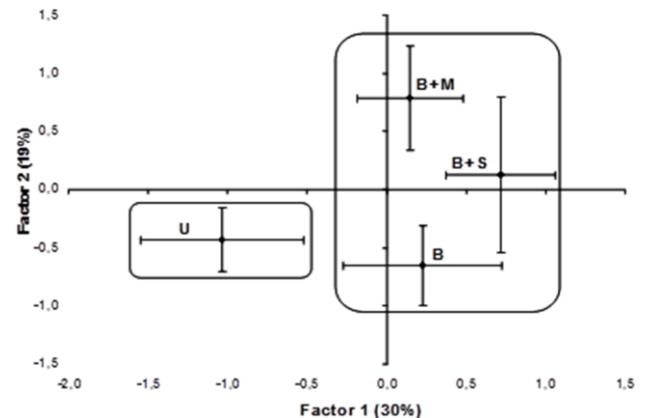


Figure 3. PCA results of the PLFAs of the different soil treatments 365 days after the fire and application of the stabilisation treatments. Treatments: U, unburned soil; B, burned soil; B+S, burned soil plus seeding; B+M, burned soil plus application of straw.

seven days of incubation and data from day 4 were used for analysis. A principal component analysis (PCA) was also carried out on the microbial use of the six groups of substrates (carbohydrates, carboxylic acids, amino acids, polymers, amines/amides and phenolic compounds) using the mean values for each treatment at each sampling time. All statistical analyses were made using the SPSS 15.0 statistical package.

### 3 RESULTS AND DISCUSSION

The principal component analysis (PCA) for the microbial use of substrates tended to separate the burned

treatments (positive values along factor 1) from the unburned treatments (negative values along factor 1) (Figure 1). The burned soils were associated with higher levels in the use of the most categorized substrates than those in the corresponding unburned soils. The factor 2 tended to separate samples collected at different sampling times, which were associated with level in the use of polymers and amines.

The principal component analysis (PCA) performed with the whole PLFA data set showed that the main differences in the PLFA pattern were due to the sampling time (Figure 2). A clear effect of the season on the microbial biomass and activity was also observed in previous studies performed in the same area (Díaz-Raviña et al., 1993, 1995). However, when separated PCAs were made for each sampling time a clear separation among unburned and burned soils were observed even 1 year after the fire (Figure 3). The data also indicated that bacteria and actinomycetes rather than fungi were favored by burnt soil conditions (data not shown), which is coincident with studies of other authors (Carballas et al., 2009; Bárcenas et al., 2011). The lack of any response of the microbial community to the mulching and seeding treatments is consistent with recent studies performed in the same experimental areas showing no changes in microbial biomass and activity following the application of these post-fire stabilisation treatments to control soil erosion (Díaz-Raviña et al., 2012; Fontúrbel et al., 2012).

**Conclusions**

The results showed a short- and medium-term effect of the sampling time and the prescribed fire on the soil microbial community on the basis of the Biolog and PLFA pattern data while, in contrast, the modifications in the microbial composition as consequence of the post-fire stabilization treatments (mulching, seeding) were of minor importance

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## REFERENCES

- Bárcenas-Moreno G, García-Orenes F, Mataix-Solera J, Mataix-Beneyto J, Bååth E. 2011. Soil microbial recolonization after a fire in Mediterranean Forest. *Biology and Fertility of Soils* 47:261-272.
- Carballas T, Martín A, Díaz-Raviña M. 2009. Efecto de los incendios forestales sobre los suelos de Galicia. In: Efecto de los incendios forestales sobre los suelos en España, Cerdá A & Mataix-Solera J (Ed.), Cátedra Divulgación de la Ciencia, Universitat de València, Ch. 3.6, pp. 269-301.
- Díaz-Raviña M, Acea MJ, Carballas T. 1993. Seasonal fluctuations in microbial populations and available nutrients in forest soils. *Biology & Fertility of Soils* 16:205-210.
- Díaz-Raviña M, Acea MJ, Carballas T. 1995. Seasonal changes of microbial biomass and nutrient flush in forest soils. *Biology & Fertility of Soils* 19:220-226.
- Díaz-Raviña M, Martín A, Barreiro A, Lombao A, Iglesias L, Díaz-Fierros F, Carballas T. 2012. Mulching and seeding treatments for post-fire soil stabilisation in NW Spain: short-term effects and effectiveness. *Geoderma* 191:31-39.
- Fontúrbel MT, Barreiro A, Vega JA, Lombao A, Martín A, Jiménez E, Carballas T, Fernández C. 2012. Effects of an experimental fire and post-fire stabilisation treatments on soil microbial communities. *Geoderma* 191:51-60.
- Frostegård Å, Tunlid A, Bååth E. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipids fatty acid analysis. *Soil Biology and Biochemistry* 25:723-730.
- Frostegård Å, Tunlid A, Bååth E. 2011. Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry* 43:1621-1625.
- Garland JL, Mills AL. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology* 57:2351-2359.