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Microbial biomass estimated by phospholipid fatty acids (PLFA pattern) in a soil with different post-fire treatments (seeding, mulching) one year after the experimental fire

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Abstract

The soil microbial community in a field experiment with different post-fire treatments (seeding, mulching) was characterized by means of phospholipids fatty acids analyses (PLFA). The soil was a Leptosol developed over granite with a slope of 38-54%, located in the N.W. (Spain), within the Atlantic humid temperate zone. The total biomass and the biomass of specific microbial groups (fungi, bacteria, actinomycetes, gram-positive bacteria, gram-negative-bacteria) was assessed in soil samples taken from the A horizon (0-5 cm depth) at different sampling times over one year. The results showed that the total microbial biomass and the biomass of specific microbial groups in burnt soils were slightly lower than in the corresponding unburnt control. The fire decreased significantly the fungal:bacterial and gram-negative:gram-positive ratios. Differences in physiological state of the microbial communities were observed as consequence of the medium-term prescribed fire impact; the higher values in the ratios of saturated to unsaturated fatty acids and cyclopropyl fatty acids to monoicoc precursors exhibited by the burnt soils as compared with the unburnt soil suggested that the microbial communities were stressed by the experimental fire. The data also showed the absence of any medium-term response of microbial community to the seeding and mulching treatments.

1 INTRODUCTION

Experimental and prescribed burning enable better quantification of the magnitude of the impact of fire on soil properties than that provided by opportunistic studies conducted after a wildfire, as pre-fire values of the relevant parameters can be measured and fire characteristics adequately assessed. Most investigations on experimental or prescribed fire effects in Galicia (NW Spain) have focused on soil physical and chemical properties. However, information concerning soil microorganisms, which are key components in maintaining soil fertility, in these perturbed ecosystems is very scarce (Fontúrbel et al., 2012).

2 OBJECTIVES

The aim of the present study is to characterize the soil microbial community in a field experiment with different post-fire treatments (seeding, mulching) under template conditions in the N.W. Spain by means of phospholipids fatty acid analyses (PLFA).

Table 1. Main soil properties for the control and the different soil treatments one year after the experimental fire (mean values ± SD of three field replicates). Treatments: C, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw addition.

Treatments	C	B	B+S	B+M
Moisture (%)	33.5 ± 3.1 a	35.9 ± 1.0 a	37.6 ± 2.2 a	38.2 ± 1,8 a
pH (water)	3.67 ± 0.06 a	3.94 ± 0.13 b	3.86 ± 0.10 b	3.92 ± 0.07 b
Electric conductivity (µS cm^{-1})	45 ± 10 a	43 ± 5 a	40 ± 5 a	50 ± 9 a
Water retention at field capacity (g water kg^{-1} soil)	758 ± 53 b	791 ± 53 a	745 ± 70 a	742 ± 56 a
Total C (g kg^{-1} soil)	182 ± 16 a	171 ± 4 a	177 ± 15 a	176 ± 10 a

3 METHODOLOGY

The study was conducted in an experimental field located at an altitude of 660 masl in Cabalar (A Estrada, 42° 38' 58'' N / 8° 29' 31'' W; NW Spain). The soil, a Leptosol developed over granite and with a slope of 38-54%, has a vegetation cover dominated by dominated by *Ulex europaeus* L. and some *Pteridium aquilinum* (L.) Kuhn., *Ulex gallii* Planch., *Daboecia cantabrica* (Huds.) K. Koch and *Pseudoarrenhaterum longifolium* Rouy. After the experimental fire four treatments were considered by triplicate (30 x 10 m plots): unburnt soil (C) as a control; b) burnt soil (B); c) burnt soil with 232 g m^{-2} of straw mulch (B+M); d) burnt soil with a mixture of seeds at a rate of 45 g m^{-2} (*Lolium multiflorum*, 35%; *Trifolium repens*, 25%; *Dactylis glomerata*, 20%; F*estuca arundinacea,* 10%; *Festuca rubra,* 5%, *Agrostis tenuis,* 5%) (B+S). Soil samples were taken from the A horizon (0-5 cm depth) 365 days after the prescribed fire. The main soil characteristics of the soil fraction < 2 mm are shown in Table 1.

The microbial biomass was determined by the PLFA analysis using the procedure and nomenclature described by Frostegård et al. (1993). The total microbial biomass was estimated as the sum of all the extracted PLFAs. Briefly, lipids were extracted from soil with a chloroform:methanol:citrate buffer mixture (1:2:0.8 v/v/v) and separated into neutral lipids, glycolipids and phospholipids using a pre-packed silica column. The phospholipids were subjected to a mild alkaline methanolysis and the fatty acid methyl esters were identified by gas chromatography (flame ionization detector) by the relative retention times of the fatty acids, using methyl nonadecanoate (19:0) as internal standard. The PLFAs were designated in terms of total number of carbon atoms, double bonding and position of the double bonds. Prefixes 'a', 'i', 'cy' and 'Me' refer to anteiso, iso, cyclopropyl and methyl branching. Non-specific branching was designed by 'br', whereas cis and trans configurations were indicated by c and t, respectively. The total microbial biomass was estimated as the sum of all the extracted PLFAs. The quantity of 10me18:0, 10me17:0 and 10me16:0 PLFAs was used as an indicator of actinomycetes biomass; the sum of the PLFAs considered to be predominantly of bacterial origin was used as an index of the bacterial biomass; and the quantity of the 18:2ω6 PLFA as an indicator of the fungal biomass (Frostegård & Bååth, 1996). The i14:0, i15:0, i16:0 and 10Me18:0 PLFAs are predominantly found in gram-positive (G+) bacteria, and the cy17:0, cy19:0, 16:1ω7c and 18:1ω7 PLFAs characterise gram-negative (G-) bacteria (Díaz-Raviña et al., 2006). The physiological state of the microbial communities was determined using the ratios: cyclopropyl fatty acids/monoenoic precursors (cy17:0 + cy19:0/16:1ω7c + 18:1ω7c) and total saturated/total monounsaturated fatty acids (Kaur et al., 2005).

In order to evaluate the effect of the different treatments on the microbiological properties analyzed, the values of three field replicates with the same treatment were averaged (mean value±SD). The data were analyzed by a standard analysis of variance (ANOVA1) and, in the cases of significant F statistics, the Tukey´s minimum significant difference test was used to separate the means.

4 RESULTS AND CONCLUSIONS

The microbial biomass values obtained for the 0-5 cm layer of the different soil treatments are shown in Table 2. In the unburned soil, the total microbial biomass, estimated as total PLFAs, was 479 nmol g^{-1} and the amount of PLFAs that were chosen to represent bacteria, fungi, actinomycetes, gram positive and gram negative bacteria represented 46, 14, 10, 27 and 10 mol% of the total amount of PLFAs. In the burnt soils, the total biomass ranged from 251 to 360 nmol g^{-1}. The amounts of PLFAs that were chosen to represent bacteria, fungi and actinomycetes PLFAs varied between 114 and 159 nmol g^{-1}; 28 and 44 nmol g^{-1}; and 27 and 37 nmol g^{-1}, respectively, and signified 43-45, 11-13 and 10-11 mol% of total amount

Table 2. Total biomass, biomass of specific microbial groups (bacteria, fungi, actinomycetes, gram-positive bacteria, gram-negative bacteria) and ratios between different biomass indices (fungal/bacterial; cyclopropyl fatty acids/monoenoic precursors; and saturated/monounsaturated) in the different soil treatments (mean values ± SE of three field replicates). Treatments: C, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw addition. Within a row different letters denote significant differences ($p < 0.05$).

Treatment	C	B	B+S	B+M
Total biomass PLFA (nmol g^{-1} soil)	479 ± 30 b	337 ± 40 a	360 ± 44 ab	251 ± 37 a
Bacterial PLFA (nmol g^{-1} soil)	218 ± 15 b	146 ± 23 ab	159 ± 18 ab	114 ± 18 a
Bacterial G^- PLFA (nmol g^{-1} soil)	129 ± 6 b	80 ± 13 a	81 ± 9 a	60 ± 21 a
Bacterial G^+ PLFA (nmol g^{-1} soil)	50 ± 5 a	38 ± 7 a	44 ± 6 a	31 ± 5 a
Fungal PLFA (nmol g^{-1} soil)	66 ± 3 b	44 ± 10 ab	44 ± 22 ab	28 ± 14 a
Actinomycetes (nmol g^{-1} soil)	48 ± 3 b	35 ± 5 ab	37 ± 5 ab	27 ± 4 a
Fungal:bacterial PLFA	0,30 ± 0.01 b	0,29 ± 0.02 b	0,28 ± 0.01 ab	0,25 ± 0.01 a
Bacterial G^-/G^+ PLFA	2,61 ± 0.17 b	2,13 ± 0.13 ab	1,89 ± 0.05 a	1,91 ± 0.14 a
Cyclopropyl/monoenoic precursors	0,49 ± 0.06 a	0,51 ± 0.02 a	0,52 ± 0.01 a	0,52 ± 0.02 a
Saturated/monounsaturated	0,84 ± 0.04 a	1,09 ± 0.08 b	1,11 ± 0.03 b	1,18 ± 0.06 b

PLFAs. The amounts of PLFAs representative of gram-negative and gram-positive bacteria ranged from 60-81 nmol g^{-1} and 31-38 nmol g^{-1}, respectively, and represented 23-24% and 12-14% of the total amount of PLFAs.The results showed that the microbial biomass in burnt soils was slightly lower than in the corresponding unburnt control. PLFAS of specific microbial groups (fungi, bacteria, actinomycetes, Gram-positive bacteria, Gram-negative bacteria) followed the same trend as that observed for the total amount of PLFA.

The fire decreased significantly the fungal:bacterial and gram-negative:gram-positive ratios, showing that the fungi and the gram-negative bacteria had a higher sensitivity to temperature (Vázquez et al., 1993; Carballas et al., 2009; Bárcenas-Moreno et al., 2011; Díaz-Raviña et al., 2012). The PLFA analysis can also be used to detect environmental stress in soils (Kaur et al., 2005). Differences in physiological state of the microbial communities were observed as consequence of the medium-term prescribed fire impact; the higher values in the ratios of saturated to unsaturated fatty acids and cyclopropyl fatty acids to monoenoic precursors exhibited by the burnt soils as compared with the unburnt soil suggest that the microbial communities were stressed by the experimental fire. These results are coincident with those reported in a previous study performed in the same experiment showing that a fire effect on several indices of microbial activity was still observed 1 year after the fire event (Fontúrbel et al., 2012). In general, the results also showed the absence of any medium-term response of the microbial community to the mulching and seeding treatments. It should be noticed, however, that the microbial biomass values in the mulching treatments were slightly lower than those exhibited by the rest of the burnt treatments (B, B+S).

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